Reviews of

97

Physiology, Biochemistry and Pharmacology

With 15 Figures

Springer-Verlag
Berlin Heidelberg New York Tokyo 1983

ISBN 3-540-12135-8 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-12135-8 Springer-Verlag New York Heidelberg Berlin

Library of Congress-Catalog-Card Number 74-3674

Offsetprinting and Binding: Konrad Triltsch, Würzburg
2127/3130-543210

Contents

Indexed in Current Contents

Rev. Physiol. Biochem. Pharmacol., Vol. 97

Tetraethylammonium Ions and the Potassium Permeability of Excitable Cells

PETER R. STANFIELD *

Contents

* Department of Physiology, University of Leicester, University Road, Leicester, LEI 7RH, United Kingdom

1 Introduction

1.1 Historical Introduction

This review concerns the actions of tetraethylammonium (TEA) ions on excitable cells. TEA^+ is a quaternary ammonium ion, belonging to a group of substances which began to interest physiologists from the time, in 1869, when *Crum-Brown* and *Fraser* showed that tetramethylammonium (TMA) salts possessed a curare-like action, destroying 'the conductivity of the motor nerves by an action on their peripheral terminations.'

TEA^+ appears to have been first used in the 1870s (*Jordan* 1877) and in experiments carried out in Ranvier's laboratory, where *Brunton* and *Cash* (1884) investigated the effects of injecting TEA-iodide (and many other substances) into frogs and rabbits. Injected into frogs, TEA^+ caused spasmodic twitchings of the trunk and leg musculature. Pinching the foot sometimes resulted in twitching of the whole body, even when the foot itself was no longer withdrawn reflexly. The motor responses to nerve stimulation were finally profoundly paralysed. However, skeletal muscle responded to direct stimulation, and the response was described as being lengthened on repeating the stimulus. *Marshall* (1914) too described such effects on nerve and muscle, but TEA^+ was also shown at this time to reduce vascular tone and have, in consequence, a hypotensive effect. *Burn* and *Dale* (1915) showed that TEA^+ was an effective blocker of autonomic ganglia.

By 1937, *Raventós* was able, in reviewing a wide variety of quaternary ammonium salts, to argue that most of the pharmacological actions were well established. However, since 1937 a substantial additional literature was developed concerning the effects of TEA^+ on the cation permeability of excitable cells. This line of research appears to have its roots in a number of findings. First, *Loeb* and *Ewald* (1916; see also *Cowan* and *Walter* 1937) found that TEA^+ could stimulate nerve fibres to conduct action potentials spontaneously, an effect which was antagonised by Ca^{2+} and which partly explains the tremor described by *Brunton* and *Cash* (1884) and *Marshall* (1914). Secondly, experimental results of *Lorente de Nó* (1949) seemed to suggest that TEA^+ could help to sustain electrical excitability of frog nerve when external Na^+ was reduced or even, in the case of 'Et-fibres', removed. *Crescitelli* (1952) showed, however, that excitability was lost in the absence of Na^+ if nerves were first completely desheathed. Thirdly, *Hodgkin* and *Huxley* (1952) elucidated the ionic basis of the action potential. It is primarily this element of the literature, concerning electrical excitability, that this review will discuss.

1.2 Effects of TEA^+

It should nonetheless be emphasised that TEA^+ has a variety of pharmacological actions. These actions may be divided into two classes. The first arises because, as a quaternary ammonium ion, TEA^+ may antagonize or mimic the actions of quaternary ammonium ions (particularly choline and acetylcholine) occurring physiologically. The second group of actions concerns the ability of TEA^+ to affect mechanisms of permeability or transport of small cations, particularly K^+.

The following properties are among the first group. First, TEA^+ combines with receptors for acetylcholine (ACh), particularly nicotinic receptors. The effect is seen to a degree at the neuromuscular junction (see, for example, *Stovner* 1957a, *Koketsu* 1958, *Barlow* et al. 1967, *Adler* et al. 1979) where however an anti-curare action, due to presynaptic effects (for example, *Ing* 1936, *Riker* and *Okamoto* 1969; Sect. 4.1.5) predominates at least in the short term. In the long term, block of transmission ensues owing to occupation and desensitization of ACh-receptors (*Parsons* 1969). At autonomic ganglia, TEA^+ is an effective blocker of transmission (*Burn* and *Dale* 1915, *Acheson* and *Pereira* 1946).

Secondly, while TEA^+ has no significant anticholinesterase activity at the neuromuscular junction (*Barlow* and *Ing* 1948), other effects predominating, it does appear, like other quaternary ammonium ions, to combine with the anionic, but not the esteratic, site at the active centre of acetylcholinesterase (*Froede* and *Wilson* 1971). In consequence, the

hydrolysis of poor substrates, such as acetylfluoride, may be enhanced (*Metzger* and *Wilson* 1967), perhaps because of a conformation change at the esteratic site (see, for example, *Wilson* 1967, *O'Brien* 1969). By the same token, the activities of other anticholinesterases may be either enhanced or reduced (*Andersen* et al. 1977).

Thirdly, TEA^+ may have a weak hemicholinium-3-like action, blocking uptake of choline at the terminals of cholinergic nerves (*Bhatnager* and *MacIntosh* 1967, *Chiou* 1970). Similarly in red cells (*Askari* 1966) and skeletal muscle (*Adamič* 1972) TEA^+ inhibits the uptake of choline by facilitated transfer.

Fourthly, in the renal tubule, TEA^+ is transported by a mechanism for the secretion of organic bases, including choline (*Rennick* et al. 1947, *Peters* 1960; see also *Berndt* 1981). The mechanism appears to be present in the proximal convoluted tubule (see, for example, *Holm* 1977), and renal clearance of TEA^+ may be complete enough to allow its use to measure renal plasma flow (*Maling* et al. 1979). TEA^+ also inhibits choline transfer in the small intestine (for example, *Sanford* and *Smyth* 1971).

Among the second group are the following properties. First, when present in the external solution at a concentration of 144 m*M*, TEA^+ can block the Na-pump of red blood cells (*Sach* and *Conrad* 1968). TEA^+ competitively inhibits ouabain-sensitive K^+ influx and Na^+ efflux. It will also inhibit partial reactions of the pump, such as the Na-Na exchange described by *Garrahan* and *Glynn* (1967), and may partially block ouabain binding to pump sites. Tetrapropylammonium ($TPrA^+$) is active at lower concentrations than is TEA^+ (*Kropp* and *Sachs* 1977).

Secondly, TEA^+ blocks the channel, permeable to both Na^+ and K^+, activated at the end plate of frog muscle by ACh (*Adler* et al. 1979). The block, which is separate from any reaction between TEA^+ and the ACh-receptor site itself, is potential-dependent, being enhanced by hyperpolarization. TEA^+ appears to have no such action on the glutamate-dependent increase in Na- and K-conductance of locust muscle (*Anwyl* 1977). In neither case is the equilibrium potential for the conductance change altered.

Thirdly, as will be discussed, TEA^+ blocks permeability sites underlying electrical excitability, blocking K-permeability in a rather specific way in nerve (*Hille* 1967a), muscle (*Stanfield* 1970a,b), sensory (*Katsuki* et al. 1966, *Cornwall* and *Gorman* 1979, *Fain* and *Quandt* 1980), epithelial (*Van Driessche* and *Gögelein* 1978) and other animal cells and in certan excitable plant cells (*Koppenhöfer* 1972b). Under certain conditions, however, TEA^+ can block Na-permeability in squid axon (*Rojas* and *Rudy* 1976).

1.3 Ionic Channels in Excitable Cells

As is well known, in the analysis of *Hodgkin* and *Huxley* (1952), electrical excitability of nerve fibres is attributed to potential-dependent permeability changes to Na^+ and K^+. Depolarization elicits first an increase in Na-permeability, which is then inactivated, and secondly an increase in K-permeability, which is sustained if depolarization is maintained.

The gating processes associated with these permeability changes were described by variables which changed with potential and time according to first order kinetics. Two kinds of gate exist for the Na-permeability, one (m) opening and one (h) closing with depolarization. Since the rise of Na-permeability under depolarization has an S-shaped time course, three m-gates were ascribed to each channel, while for the subsequent inactivation, which appeared to be a first order process, a single h-gate was ascribed.

For the K-permeability, a single kind of gate could account for K-permeability rising under depolarization with an S-shaped time course yet relaxing under repolarization with an exponential time course if four gates, each opening independently of the others under depolarization, were ascribed to each permeability site. For K-channels, then, gates exist either in a closed or an open state, moving between these as follows:

$$\underset{(1-n)}{\text{closed}} \underset{\beta_n}{\overset{\alpha_n}{\rightleftharpoons}} \underset{(n)}{\text{open}}$$

The rate constant α_n rises while β_n falls as membrane potential becomes more positive; n gives the fraction of gates in the open position. The K-conductance (g_K) may then be described by:

$$g_K = \bar{g}_K \, n^4$$

where $\bar{g}_K$ is the maximum K-conductance under the given experimental conditions.

As several authors have described (for example, *Armstrong* 1969) exactly the same result is achieved if K-permeability sites are treated as existing in states having four, three, two, one, or zero closed gates, and as moving between these states in a sequential fashion. The modelling in Figs. 2 and 4 of this review is based on such a scheme.

By implication, the model of *Hodgkin* and *Huxley* (1952) suggests that Na^+ and K^+ cross the membrane by way of separate permeability sites or channels, a notion which TEA^+ has, as a specific blocker of K-conductance in many preparations, played a substantial role in sustaining.

(For reviews of this general aspect, see *Narahashi* 1974, 1977, *Armstrong* 1975a, *Ulbricht* 1981).

In this review I shall attempt to discuss the effects of TEA^+ as a blocker of K-channels, dealing with a number of kinds of excitable cells. By and large, I shall not discuss in detail the use of TEA^+ as a blocker to reveal other mechanisms, though TEA^+ has had an important experimental role in a number of aspects. Examples are in measurement of asymmetrical displacement currents or charge movements (for reviews, see *Almers* 1978, *Armstrong* 1981, *Schneider* 1981); in measuring Na-channel current noise (for example, *Conti* et al. 1975); and in the uncovering of Ca-permeability mechanisms in a number of preparations (for review, see *Hagiwara* and *Byerly* 1981). Though other blockers of K-conductance, such as 4-aminopyridine (*Yeh* et al. 1976) have also found favour recently, TEA^+ remains very widely used.

1.4 Size of TEA^+ and of K^+

It is generally held that TEA^+ blocks K-channels because its diameter is much the same as that of the hydrated K-ion. Figure 1 shows silhouettes of a number of the quaternary ammonium ions which have been used experimentally. They were made using Corey-Pauling-Koltun (CPK)

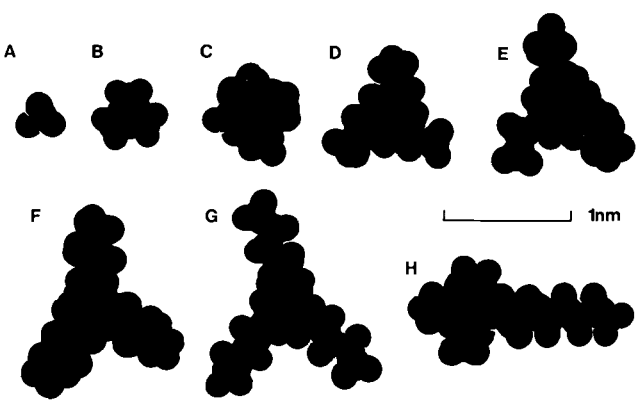


Fig. 1. Silhouettes of space-filling Corey-Pauling-Koltun (CPK) models of quaternary ammonium ions. **A**, NH_4^+; **B**, tetramethyl-(TMA^+); **C**, tetraethyl-(TEA^+); **D**, tetrapropyl-($TPrA^+$); **E**, tetrabutyl-(TBA^+); **F**, tetrapentyl-($TPeA^+$); **G**, tetrahexyl-ammonium; and **H**, nonyltriethylammonium (C9). NH_4^+ (**A**) permeates Na- and K-channels. Applied outside at frog node, TEA^+ (**C**) is the most effective blocker of K-conductance (*Hille* 1967b). Applied inside C9 (**H**) is a more potent blocker than is TEA^+ (**C**) (*Armstrong* and *Hille* 1972). In squid axon increasing alkyl chain length (**B through G**) appears to enhance blocking potency, and block by tetrahexylammonium (**G**) is nearly irreversible (*French* and *Shoukimas* 1981)

models. The maximum diameter of TEA^+ (Fig. 1C), measured from such models, is about 0.8 nm. A similar diameter may be computed from the anticipated molal volume of TEA^+ (*Robinson* and *Stokes* 1965). The diameter, predicted from Stokes' law, which gives the size of ions in solution on the assumption that their mobilities are determined by the frictional resistance to their movement resulting from their dimensions and the viscosity of the medium through which they move, is smaller (about 0.56 nm). But Stokes' law is known to underestimate the size of small ions. Quaternary ammonium ions lack hydration shells in solution (*Diamond* 1963).

Armstrong (1966b) has argued that the maximum *radius* of a K-ion with a full hydration shell – he assumed that six water molecules surround each K^+ in solution, as in crystals of $KAl(SO_4)_2 \cdot 12\,H_2O$ – was 0.46 nm. The considerations used were the crystalline radius of K^+ and the size of water molecules. Other considerations yield smaller estimates. First, K^+ has a larger limiting molar conductivity (mobility) than does TEA^+ (73.5 versus 32.6 $cm^2 \cdot \Omega^{-1} \cdot mol^{-1}$ at 25°C [*Robinson* and *Stokes* 1965]) suggesting that it has a smaller size. The approach suggested by *Bockris* and *Reddy* (1970) using a simple geometrical approximation which, however, assumes the hydration shell is constrained within a perfect sphere, suggests that the hydrated K^+, the most probable hydration number being three (their Table 2.20), has a hydrated diameter of 0.46 nm. This value is similar to that given by *Weingart* (1974; 0.40 nm) but larger than that given by *Adrian* (1956; 0.30 nm).

It may be pointed out here that TEA^+ is able to mimic K^+ with liquid ion exchangers such as that (Corning K^+-ion exchanger 477317) used in the manufacture of K-sensitive microelectrodes (*Neher* and *Lux* 1973). These electrodes are, in fact, much more sensitive to TEA^+ than to K^+, and to the larger $TPrA^+$ (Fig. 1D) than to TEA^+. Since its concentration may be measured by such electrodes, TEA^+ has been used to investigate diffusion of small cations in the extracellular space of the mammalian brain (*Nicholson* et al. 1979).

2 Invertebrate Giant Axons

2.1 TEA^+ Block of K-Current in Squid Axon and in Other Invertebrate Giant Axons

In squid axon, TEA^+ blocks K-conductance, but does so only when applied inside, external concentrations of up to 100 m*M* being ineffective (*Tasaki* and *Hagiwara* 1957, *Armstrong* and *Binstock* 1965). This finding is not a universal one even among invertebrate giant axons, but

the absence of an effect of external TEA^+, at concentrations up to 40 m*M*, is also seen in giant axons of the crayfish *(Procambarus),* where internal TEA^+ does block K-conductance (*Shrager* et al. 1969). Among other large invertebrate nerve fibres, TEA^+ blocks from outside in *Carcinus* nerve (*Burke* et al. 1953), while in *Myxicola* giant axons, TEA^+ half-blocks K-conductance at 24 m*M* when applied outside, the block being readily reversible (*Wong* and *Binstock* 1980). Internal TEA^+ blocks in *Myxicola* giant axons (*Schauf* et al. 1976).

2.2 Experiments in Squid Axons

Tasaki and *Hagiwara* (1957) first injected TEA^+ into squid axon to a final concentration of 40–60 m*M* and measured the action potential and the concomitant changes in membrane impedance. Action potentials were prolonged and possessed a marked plateau. During this plateau, membrane impedance, measured with an AC method, fell during the upswing of the action potential, but then increased towards its resting level during the plateau, sometimes rising to have a slightly higher than resting value at the shoulder of the action potential, just as rapid repolarization recommenced. These findings appeared to the authors to challenge the Hodgkin-Huxley explanation of the ionic basis of the action potential, a challenge taken up by *FitzHugh* (1960) and by *George* and *Johnson* (1961) who both adopted a similar adaptation of the Hodgkin-Huxley equations. These equations (see Sect. 1.3) were modified to allow the K-conductance to be activated about 100 times more slowly. *FitzHugh* (1960) also speeded sodium inactivation threefold, a solution not adopted by *George* and *Johnson* (1961). Though these formulations were successful in describing action potentials with a plateau, both failed to explain the high and increasing membrane impedance during the plateau.

As the experiments of *Armstrong* and *Binstock* (1965) showed, 40 m*M* TEA^+ inside squid axons has the effect of blocking outward movement of K^+, while permitting K^+ to move in, particularly when axons are immersed in high K^+ (440 m*M*) solutions. Thus, internal TEA^+ induces in squid axon a kind of anomalous or inward rectification, behaviour very like anomalous or inward rectification of muscle fibres (*Katz* 1949, *Hodgkin* and *Horowicz* 1959).

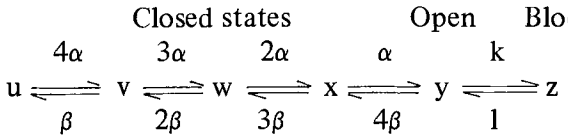
This inward rectification explains immediately why the plateau of the action potential had a high impedance rather than a low one: the block of K-conductance by TEA^+ was enhanced by depolarization as K^+, moving outwards, swept TEA^+ into the now open K-channels. This surmise was shown to be correct by further experiments by *Armstrong* (1966a, 1969, 1971) who injected TEA^+ at lower concentrations and who also

investigated a number of related quaternary ammonium ions. This important line of research has recently been extended by *Swenson* (1981) and by *French* and *Shoukimas* (1981).

With small concentrations of TEA^+ (in the millimolar range) inside the axon, K-currents obtained under depolarization were not slowed as *Fitz-Hugh* (1960) and *George* and *Johnson* (1961) had supposed in their modelling but increased along an initially normal S-shaped time course. The K-currents then inactivated again, apparently as the block by TEA^+ developed. So K-channels appeared to move from a closed to an open state and then to an inactivated state – a state where the K-channel is open, but is blocked by TEA^+ which has moved into it. Repolarization results in current tails which initially increase, as TEA^+ leaves the K-channels, giving the tails a 'hooked' appearance. These then shut down again, with a slower than usual time course, as had also been reported by *Armstrong* and *Binstock* (1965).

2.2.1 Modelling the Block by TEA^+

Such behaviour was modelled according to the supposition that K-channels exist in a number of closed states (a method of allowing K-currents to turn on with an S-shaped time course), an open state, and an inactivated state (*Armstrong* 1969), according to:



The rate constants for the reactions allowing K-currents to run through their closed states (u through x) to the open state (y) are those expected from Hodgkin-Huxley kinetics. As described above (Sect. 1.3), the results of this part of the scheme are identical with the results of the equations of *Hodgkin* and *Huxley* (1952). States u, v, w, and x represent channels with four, three, two, and one closed gates respectively. It should be mentioned that *Conti* and *Neher* (1980) have recently made single channal recordings in squid axon suggesting that K-channels do have more than one closed state but that the ratio of the rate constants between one closed state and the next is not that given above from *Hodgkin* and *Huxley* (1952).

The fraction of K-channels in a TEA^+-blocked condition is given by z, which rises under depolarization. It will do this whether or not the rate constants k and l are made to change with depolarization: the onset of inactivation can acquire an apparent voltage dependence because its onset

is a consequence of preceding voltage-dependent steps. *French* and *Shoukimas* (1981) have also emphasised this argument (see also Fig. 4A). *Armstrong* (1981) has applied it to the physiological inactivation of Na-channels.

So far as K-channels are concerned, *Armstrong* (1966a, 1971) has shown that, to a first approximation, k depends on the concentration of blocking quaternary ammonium ion in the axoplasm, while l depends on $[K]_o$ and potential.

The rate of change with time of the fraction y of K-channels in an open, conducting state is given by:

$$\frac{dy}{dt} = \alpha x + lz - (k + 4\beta)y$$

Similar differential equations describe the way occupancy of other states changes with time. The scheme may be solved for y, which will be proportional to K-conductance, using Runge-Kutta methods to solve these differential equations simultaneously. The results of some of these computations are given in Fig. 2, where (Fig. 2A) the time course of state y is plotted for a depolarization from – 70 to + 50 mV with $l = 0.025\ ms^{-1}$ and k varying between zero and $1.0\ ms^{-1}$. Fig. 2B shows the relaxation of y if the membrane potential is returned to – 70 mV both in the case where no block has occurred and in the case where the block has run to about 90% completion. In this case, k at – 70 mV was kept at zero and l was changed between 10 and $0.1\ ms^{-1}$. The so-called hook tails are reproduced, as is the slowing of the subsequent relaxation of y, caused because recovery from the block occurs by way of the open state y: inward current will increase before falling as K-channels close.

Some of this behaviour may be mimicked by supposing that K-channels and the onset of block by TEA^+ are independent parallel potential-dependent processes (Fig. 2C,D). It is actually surprisingly hard experimentally to distinguish these two possibilities: first a link between activation and incactivation as in Fig. 2A,B, and secondly independence of these processes as is assumed in Fig. 2C,D. Both schemes generate conductances which increase and then fall under depolarization, and both predict a hooked appearance to tails as the conductance relaxes under repolarization. One difference is that only the sequential scheme predicts the slowing of the current tail which is observed experimentally.

Meves (1978) has reviewed the issue of linked and independent activation and inactivation in sodium channels, where a similar experimental difficulty arises (see also *Horn* et al. 1981).

Part of the evidence that TEA^+ blocks open channels comes from the appearance of a delay in the onset of the block when that is measured with two pulse experiments (a conditioning pulse of variable duration,

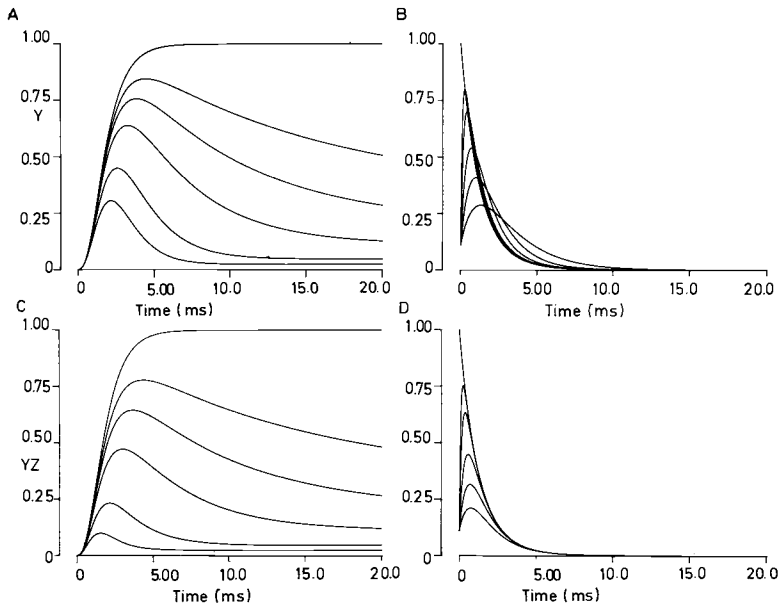


Fig. 2 A–D. Modelling of the rise and fall of K-conductance in a squid axon with the assumptions that, in **A** and **B**, block by TEA^+ occurs as a consequence of channel opening (the sequential scheme of Sect. 2.2.1) while, in **C** and **D**, block by TEA^+ occurs in parallel with (but independently of) channel opening. In both cases, the rate constants for channel opening, α and β, are taken from *Hodgkin* and *Huxley* (1952) (see text), so that:

$$\alpha = \frac{-0.01\,(V + 50)}{\exp\{-[(V + 50)/10] - 1\}} \quad ms^{-1}$$

$$\beta = 0.125 \cdot \exp\{-(V + 60)/20\} \quad ms^{-1}$$

where V = membrane potential.

A The fraction of K-channels open (y, *ordinate*) plotted against time (ms, *abscissa*) for a depolarization from −70 to +50 mV at t = 0. l = 0.025 ms^{-1} and, from above to below, k = 0; 0.05; 0.10; 0.20; 0.50; and 1.00 ms^{-1}.

B The fraction of K-channels open during a repolarization from +50 to −70 mV either after no block has developed (curve starting from 1.00) or after a block during the step to +50 mV has run to completion with k = 0.5 and l = 0.025 ms^{-1}; and then recovers at −70 mV with k = 0 and l = 10, 5, 2, 1, and 0.5 ms^{-1} from above downwards.

C The same depolarization as in **A**, but where the inactivation of K-channels runs as a parallel process independent of channel opening, given by

$$\underset{z}{\text{open}} \underset{l}{\overset{k}{\rightleftharpoons}} \underset{(1-z)}{\text{closed}}$$

The fraction of K-channels open is here given by yz *(ordinate)*. The rate constants k and l have the same numerical values as in **A**.

D Repolarization from +50 to −70 mV after a block by TEA^+ (curve starting at 1.00) and after block with k = 0.5, l = 0.025 ms^{-1} has run to completion. Rate constants k and l have the same numerical values as in **B**.

Armstrong's experiments (1966a, 1969, 1975a) favour the scheme schown in **A** and **B**, as do those of *French* and *Shoukimas* (1981) and of *Swenson* (1981). In *Armstrong*'s (1966a) experiments l was approximately 1 ms^{-1} and k was, at an axoplasmic TEA^+-concentration of 1 m*M*, approximately 3 ms^{-1} at +100 mV and at 6° to 10°C

say to -10 mV, followed immediately by a test pulse to a positive potential, say $+90$ mV, to measure how much current has been inactivated [*Armstrong* 1969]). The decay of the peak current reached during the test pulse associated with increasing conditioning pulse duration is sigmoid, implying that a delay occurs in the onset of inactivation. However, as *Meves* (1978) has pointed out in connexion with Na-inactivation, the presence of a delay in onset, measured in this way, is not a perfect test for a link between activation and inactivation. This is because an element of apparent delay can be introduced by the state of the activation process being less advanced at the moment the test pulse begins after shorter conditioning pulses. As *Gillespie* and *Meves* (1980) point out, pulse protocols for such experiments ideally have a gap at the holding potential between conditioning and test pulses, whose function is to return activation kinetics to a constant state by the time the test pulse starts. Nonetheless, the delays measured by *Armstrong* (1969) were shown to fit well to the scheme of block being a sequel of channel opening, being more substantial then those predicted by a scheme of independent channel opening and block.

2.2.2 Block by Other Quaternary Ammonium Ions

TEA^+ is not the only quaternary ammonium ion to block K-channels of squid axon from the inside. *Armstrong* (1966b, 1975a) has argued that TEA^+ might be the most potent blocker among symmetrical tetraethylammonium ions because its diameter (~ 0.8 nm) is nearest to that of the hydrated K^+. One can envisage K-channels as having wide inner mouths, just the right size to admit a hydrated K^+ which might then lose its water of hydration to proceed further through the channel. TEA^+ may then be thought to enter this wide inner mouth but to be unable to proceed further into the narrower portion of the channel which confers its selectivity. Further, if channels have to be open before block can develop, it seems reasonable to suppose that the gating mechanism of the channel might lie further toward the inside of the membrane than the site which the blocking TEA^+ occupies.

Experiments with other quaternary ammonium ions seem to confirm the idea of the channel gating mechanism lying close to the inside of the membrane, and of the wide inner mouth, but have raised a doubt as to the size of that wide inner mouth (*French* and *Shoukimas* 1981, *Swenson* 1981). The most recent experiments suggest that it is larger than just large enough to admit one TEA^+ or one hydrated K^+.

Experiments of *Armstrong* (1969, 1971) and of *Swenson* (1981) centred around increasing the length of one alkyl chain. *French* and *Shoukimas* (1981) have used symmetrical tetraalkylammonium ions. Some of the results are summarised in Fig. 3A.

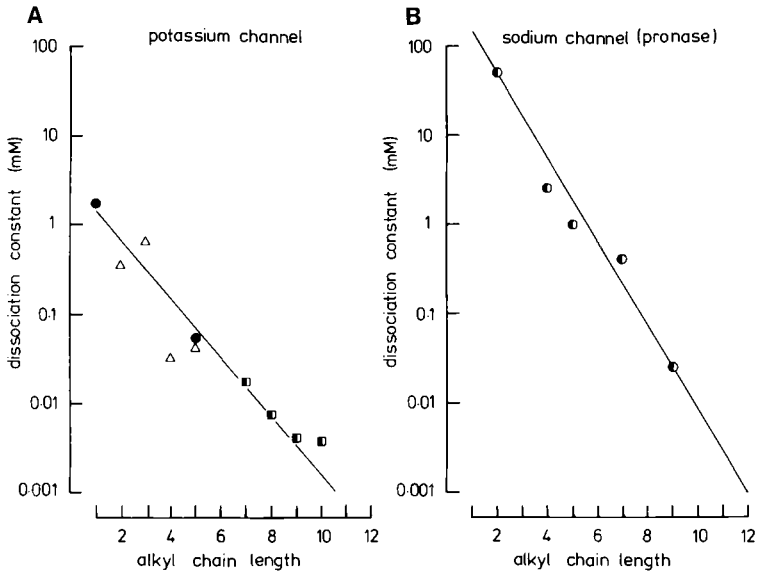


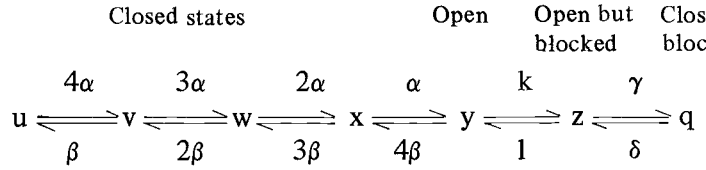
Fig. 3. A,B. Dissociation constants for the block, measured by electrophysiological means, of **A** K-channels and **B** Na-channels modified by pronase treatment, by a number of quaternary ammoium ions. For the filled (●) and half-filled symbols (◧), (◐), the quaternary ammonium ions are triethylalkylammonium ions, while the open symbols (△) denote block by symmetrical quaternary ammonium ions. **A** ●, results from *Armstrong* (1969), measured at + 50 mV; ◧, results from *Swenson* (1981), measured at + 100 mV; and △, results from *French* and *Shoukimas* (1981), measured at 0 mV. In spite of the differences in membrane potential, the dissociation constants found are similar in the three groups of experiments. Further, the results are open to the conclusion that the increase in affinity with increasing alkyl chain length is similar whether one or four alkyl chains are increased. **B** ◐, results from *Rojas* and *Rudy* (1976) on Na-channel. Here the dissociation constants are apparently independent of membrane potential

In *Armstrong*'s experiments, one ethyl group of TEA^+ was reduced in length to give methyltriethylammonium (C1) or was increased to give propyl-(C3), butyl-(C4), pentyl-(C5), nonyl-(C9, illustrated in Fig. 1H), or dodecyltriethylammonium (C12). In each case increasing alkyl chain length increased blocking potency, with affinity increasing approximately twice for each CH_2 group added. This change is not substantially different from that expected (4.1 times for each CH_2 group) from the consideration of the energy change (205 J/mol) required to move one CH_2 group from the non-polar lipid into the aqueous solution. Replacing one ethyl group by a phenyl group (phenyltriethylammonium) reduced potency very substantially, but blocking effectiveness was restored by adding CH_2 groups to connect the aromatic ring to the quaternary nitrogen, the subsequent order of potency being phenylmethyl- < phenylethyl-

< phenylpropyltriethylammonium. Of the tetraalkylammonium ions, C9 and C12 could block from the outside, but by virtue only of their lipid solubility, which allowed them to permeate the axon membrane.

Nonyltriethylammonium was a most potent blocker and its action was virtually irreversible if external [K^+] was 10 m*M*. Raising external K^+ to 440 m*M* enhanced reversal, as did hyperpolarization, which drove K^+ inwards through the K-channel, flushing out the blocking C9. But hyperpolarization, though it speeded reversal of block could also prevent reversal going as far toward completion. The simplest explanation of this observation is that the channel gates are able to close in spite of the foot-in-the-door effect that C9, like TEA^+ (Fig. 2B) has. They close more quickly at negative membrane potentials and so trap the blocking ion in the channel.

Armstrong (1971, 1975a) has modelled this process by proposing an additional closed and blocked state for K-channels. Thus with internal C9, K-channels exist in the following states:



The rate constant γ increases with hyperpolarization and δ with depolarization. Modelling this process with a computer could satisfactorily predict the experimental finding that recovery is enhanced in speed but reduced in degree by hyperpolarization (*Armstrong* 1971, 1975a): C9 can be trapped in the K-channel by the gates closing behind it. Or perhaps, more mundanely, one might argue that the rate constant l may fall when the channel closes if it (l) depends on external K^+ (see below, Sect. 2.2.3).

Armstrong (1971) argued that the triethyl head group of C9 was important for block and showed that if the head group of C9 were reduced in size (he used octyltrimethylammonium) blocking potency was lost. Recently *Swenson* (1981) has shown that a number of agents with head groups *larger* than triethyl could also block, though the blocking potency of decyltripropylammonium, for example, was about 25 times less than that of decyltriethylammonium (C10). Nonetheless, the inner mouth of the K-channel had to be larger than the 0.8 nm diameter originally proposed by *Armstrong* (1971).

French and *Shoukimas* (1981) found that symmetrical tetraalkylammoniums larger than TEA^+ also blocked K-channels very effectively. Further, just as nonyltriethylammonium is more effective than tetraethylammonium, so with symmetrical ions blocking potency increased with increasing size. Block by tetrahexylammonium was virtually irreversible.

As Fig. 3A shows, the affinities for the symmetrical tetraalkylammonium ions are actually little different from those found by *Armstrong* (1969) and *Swenson* (1981) for quaternary ammonium ions where only one alkyl group was changed from ethyl. Since the change in affinity with changing chain length was also remarkably similar, the argument is weakened that it is that expected from the need to move one additional CH_2 group from the hydrophobic environment of the membrane lipid to aqueous solution to reverse block. Nonetheless, *French* and *Shoukimas* (1981) attributed their result to 'stronger interactions of the longer alkyl side chains with the hydrophobic regions of the membrane near the channel.' In support of that, *Swenson* (1981) found that adding hydroxyl groups to the alkyl chains substantially reduced blocking potency.

2.2.3 Dependence of Block on Voltage

If the development of block depends on K-channels first opening, then the block will acquire an apparent potential-dependence because channel opening, which must precede it, is potential-dependent. This point is illustrated in Fig. 4A, where it may be seen that the rate of decline of y, the fraction of channels in an open state, is faster at positive potentials even though the rate constants k and l assumed in the computation are independent of membrane potential.

The rate constants are thus best estimated experimentally at very positive potentials, where channel opening is rapid compared with block. *Armstrong* (1969, 1971) showed that to a first approximation, the rate constant k in the scheme outlined above depended on the concentration of blocking quaternary ammonium ion in the axoplasm. The dependence is of the form

$$k = b \cdot [TEA]_i$$

consistent with one TEA^+ blocking one K-channel (see also *Swenson* 1981, *French* and *Shoukimas* 1981). The rate constant l was independent of $[TEA^+]$ but appeared to depend on $[K]_o$ and membrane potential, hyperpolarization driving K^+ in to flush out the blocking ion. *Swenson* (1981) and *French* and *Shoukimas* (1981) argued for an element of voltage-dependence for both rate constants, as might be expected if TEA^+, or one of the other blocking quaternary ammonium ions, occupies a site within the membrane voltage field (see also *Hille* 1975).

Swenson (1981) made estimates of b and l which suggested that the blocking site lay about 20% of the way through the membrane from the inside. *French* and *Shoukimas* (1981) found a potential-dependence for the equilibrium constant which suggested that the blocking site lay about 15% of the way out through the channel. However, estimating the

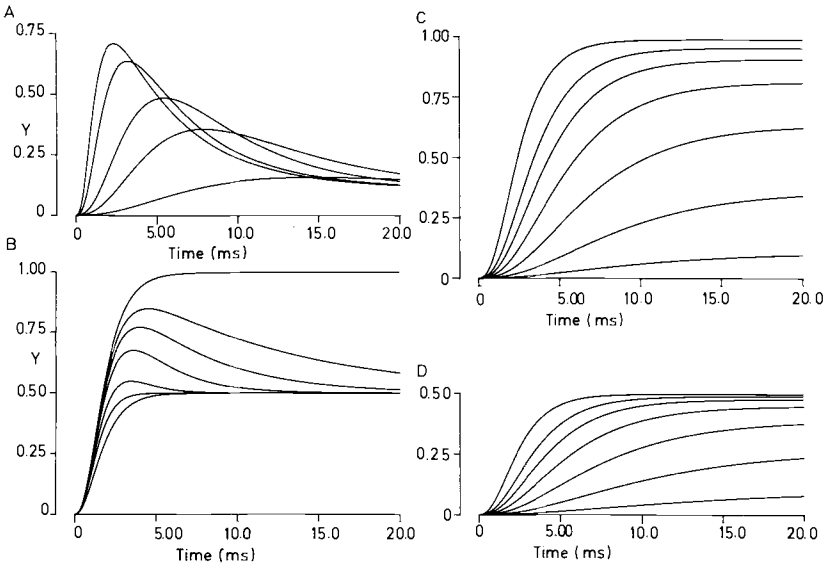


Fig. 4. **A** Modelling of K-channel opening and inactivation, using the sequential scheme of Sect. 2.2, with α and β having the same potential-dependence as in *Fig. 2;* k and l have the same values (0.20 and 0.025 ms^{-1}, respectively) during each of the depolarizations, which are to +100, +50, 0, −20 and −40 mV from −70 mV. Although the rate constants are the same for each depolarization the apparent rate of block is higher at more positive potentials where channel opening is faster. **B** If the onset of K-channel block is fast compared with K-channel opening, the time course of the rise of the fraction of K-channels open may be apparently normal even though fewer K-channels are open at any given time. The sequential scheme of Sect. 2.2.1 is used in this calculation. The depolarization represented is from −70 to +50 mV and k = l at all potentials with values from above down of 0; 0.05; 0.10; 0.20; 0.50; 1.00; and 5.00 ms^{-1}. At +50 mV, α = 1.00 ms^{-1}, β = 0.0005 ms^{-1}. **C** Rise of the fraction of K-channels open, without TEA^+ block, under depolarization to +20; 0; −20; −30; and −40 mV. **D** Rise of the fraction of K-channels open, with TEA^+ block occurring with rate constants k = l = 5.00 ms^{-1} at potentials of +20; 0; −10; −20; −30; and −40 mV. K-channel opening appears undistorted by the sequential block by TEA^+. See *Armstrong* and *Hille* (1972)

potential-dependence of the rate constants by measuring the time constant for the decline of the K-current either when the axoplasmic concentration of quaternary ammonium ion was very high, which gave the forward rate constant, or when the concentration was very low, which gave the back rate constant, suggests that these have a potential-dependence substantially steeper than that of the equilibrium constant. This finding is consistent with block depending upon some preceding process which is itself dependent on potential. Since channel gating is the most likely such process, these experiments provide elegant confirmation of the idea that block occurs only when K-channels open.

2.2.4 Tetramethylammonium and Ammonium

Tetramethylammonium (TMA^+) produced a block of slightly different quality from that of other quaternary ammonium ions: its block had a steeper dependence on potential and a lower affinity. Approximately 100 m*M* TMA^+ half-blocked K-conductance at 0 mV, while TMA^+, moving to its blocking site, experienced about 37% of the potential difference across the membrane (*French* et al. 1979). This degree of potential-dependence is similar to that shown by a number of other blocking ions: Li^+, 37%; Na^+ 47%; Cs^+, 48%; glucosamine, 24%; and TRIS, 32%. These last two are quite large compounds (TRIS has a maximum diameter from CPK model building of 0.66 nm) suggesting that the K-channel may be quite large in size some way into the membrane. Further, both glucosamine and TRIS have -OH groups, which *Swenson* (1981) suggested inhibited block at the inner site occupied by larger quaternary ammonium ions. This line of approach seems to offer the hope of extending a knowledge of the size and chemistry of the K-channel.

NH_4^+ is known to permeate K-channels of squid axons (*Binstock* and *Lecar* 1969), with the permeability ratio P_{NH_4}/P_K being measured from steady-state current-voltage relations as 0.26. *Binstock* and *Lecar* (1969) showed that TEA^+ blocked NH_4-current through K-channels, but not through Na-channels which NH_4^+ also permeated. TEA^+ also blocks the weak permeance of Na^+ through K-channels in squid axons perfused with Na-solutions, a permeance occurring only at positive membrane potentials (*French* and *Wells* 1977).

2.2.5 Attempts to Predict K-Channel Density

Arguing that TEA^+ and the hydrated K^+ were about the same size, and that in consequence the probability that a K or a TEA ion would enter a K-channel would be given simply by the ratio of the K^+ and TEA^+ concentrations in axoplasm, *Armstrong* (1966a) made the first attempt to predict the K-channel density in squid axon. The assumption was also made that once TEA^+ has entered a K-channel it does not leave during the time the onset of block of K-current is being estimated. Were it to do so, channel conductance would be underestimated. On these kinds of assumptions, K-channels had a predicted density of 67 channels/μm^2. Later, using the rate of entry of nonyltriethylammonium (C9) and similar assumptions, *Armstrong* (1975a,b) produced a slightly lower channel density, with a single channel conductance, under the conditions of the experiment, of 2–3 pS. From the estimate using TEA^+, ions were predicted to flow through the K-channel at 600/ms and using C9 at 2000–3000/ms, under a similar driving force of 150 mV.

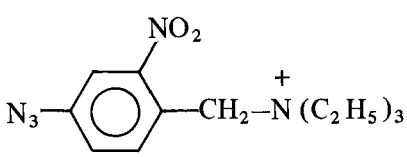
In fact, the estimate using C9 is likely to be the more accurate because of the condition that the blocking ion should not leave the channel once it has entered. Both *Armstrong* (1969) and *Shoukimas* and *French* (1981) found that the forward rate constants for blocking were very similar for a range of quaternary ammonium ions, but the back rate constant was much lower for the larger ions.

Armstrong's estimate of 2–3 pS is in reasonably close agreement with those obtained from noise analysis (12 pS; *Conti* et al. 1975) and from single channel recording (9–11 pS; *Conti* and *Neher* 1980) in squid axon.

Moore et al. (1979) found that TEA^+ induced noise in squid axon. This noise was associated with K-channels, with fluctuations caused by TEA^+ going onto and coming off open channels. Thus TEA^+ may actually enter and leave K-channels quite readily. This blocking and unblocking was rapid compared with channel gating if TEA^+ was present at 5–50 m*M* in axoplasm. Their result was consistent with TEA^+ completely blocking channels once they had opened: the single channel conductance estimated from the noise due to block by TEA^+ (or by decyltriethylammonium) was similar to that estimated from current noise associated with channel gating in the absence of TEA^+. The values obtained were lower (of the order of 2 pS) than those found by *Conti* et al. (1975) and by *Conti* and *Neher* (1980).

2.2.6 Attemps to Develop Photo-Affinity Label for K-Channels

Lazdunsky et al. (1979) have developed derivatives of tetrodotoxin (TTX) which act as photo-affinity labels of Na-channels, their binding being rendered irreversible upon exposure to light. *Hucho* et al. (1976) developed a derivative of TEA^+ (4-azido, 2-nitrobenzyltriethylammonium) which reversibly blocked K-conductance after exposure to u/v light of wavelength 254 nm (at intensities too low to destroy ionic permeation: see, for example, *Fox* 1974).



Hucho (1977) used the substance in an attempt to extract K-channels from crayfish axons homogenised before treatment with the label. However, the labelled extract from the membrane appeared to be a phospholipid or a small proteolipid, and *Hucho* argued that the substance reacted with 'the lipid rim of the funnel-like entry to the K-channels'. Crayfish

axons had about 354 binding sites · μm^{-2}. This element of binding was said to be absent from *Torpedo* electric organ membranes, thought to have no voltage-dependent K-channels, though the label bound to ACh receptors in this tissue (see also *Hucho* and *Schiebler* 1977). A specific high affinity label for K-channels seems much needed.

2.2.7 Quaternary Ammonium Ions May Block Na-Channels

Although *Tasaki* and *Hagiwara* (1957) found that TEA^+ reduced action potential height by about 5%–10%, *Armstrong* and *Binstock* (1965), who made the same finding, also showed that Na-currents were unaltered by TEA^+ injection.

Armstrong (1966b) found that TEA^+ did not affect Na-current in squid axon, but that tetrapropylammonium ($TPrA^+$) did, blocking Na-channels when they were opened by depolarization. The axoplasmic concentration used was 8 m*M*. *Armstrong* argued that Na-channels had a wide inner mouth, larger than that of K-channels, sufficient in size to admit the larger hydrated Na^+, or the TPrA ion, but too large to accommodate a TEA ion comfortably.

However, *Rojas* and *Rudy* (1976) have shown that quaternary ammonium ions readily block Na-channels if Na-channels are first treated with proteolytic enzyme – with pronase or certain fractions of pronase – a process which removes the inactivation of Na-conductance under depolarization (*Armstrong* et al. 1973). Such a procedure had no effect on the ability of Na-channels to select between alkali metal ions. But TEA^+ now blocked Na-current, even if with an affinity somewhat lower than its affinity for K-channels (Fig. 3B). With Na-channels, as with K-channels, increasing the length of one alkyl chain increased blocking effectiveness. Unlike the block of K-current, both inward and outward currents seem to be blocked (though most of the experiments were carried out in Na-perfused axons at potentials where Na-currents were outwards). Neither affinity nor onset of block seemed to change with voltage over the range of voltages where channels are open.

Thus, in general, it may be said that quaternary ammonium ions are not absolutely specific for K-channels when they block from the inside.

2.2.8 TEA^+ and Late Ca-Entry

Baker et al. (1971) used the Ca-dependent luminescent protein aequorin to show that Ca^{2+} entered the squid axon under depolarization. This entry occurred in two phases: an early phase, blocked by TTX, through Na-channels and a later phase whose time course was similar to that of the increase in K-conductance but which was not blocked by internal TEA^+ (see also *Baker* et al. 1973; *Baker* and *Glitsch* 1975) indicating that entry occurred by a separate route.

Nonetheless, *Inoue* (1980) has recently shown that Ca^{2+} may enter through K-channels in squid axon, under conditions where axons are perfused with 20 m*M* NaF and immersed in 100 m*M* $CaCl_2$ solution, with both internal and external solutions having a lower than physiological ionic strength. This Ca-entry was sufficient to generate a 'K-channel spike' in the presence of TTX. The spike was so denoted because it was blocked by internal TEA^+, a block which was particularly intense in the absence of permeating K^+. *Inoue* (1980) argued that the slow Ca-channel of *Baker* et al. (1971) did not contribute, and *Meves* and *Vogel* (1973) did not record current through this Ca-channel in F^--perfused axons: in other preparations at least, internal F^- blocks Ca-channels (*Kostyuk* 1980).

TEA^+ does not block the Mg^{2+} entry which occurs under depolarization (*Rojas* and *Taylor* 1975).

3 Node of Ranvier

3.1 Block of K-Currents in Frog Node of Ranvier

In the frog node, TEA^+ is able to block K-channels both from the inside (*Koppenhöfer* and *Vogel* 1969, *Armstrong* and *Hille* 1972) and from the outside (*Bockendahl* and *Meves* 1960, *Lüttgau* 1960, *Schmidt* 1965, *Schmidt* and *Stämpfli* 1966, *Koppenhöfer* 1967, *Hille* 1967a).

When applied inside, by being allowed to diffuse from a cut end of a myelinated nerve fibre under voltage-clamp, TEA^+ blocks in a fashion identical with that believed to occur in squid axon. Outward currents are blocked much more effectively than inward ones and channels are blocked only once they have opened. As with squid axon, substitution of one ethyl group by nonyl (C9) enhances blocking potency (*Armstrong* and *Hille* 1972).

Applied outside, TEA^+ is the most effective blocking quaternary ammonium ion (*Hille* 1967a,b) and substitution of even one ethyl group reduces potency (Table 1). Affinity for the blocking site is reduced five-fold by replacing ethyl by propyl and 40-fold by substituting with methyl. TMA^+ is virtually without effect on K-conductance and is probably a better substitute for Na^+ in external solutions than is the frequently used choline, which half blocks K-currents at 240 m*M* (*Hille* 1967a,b).

Measuring the fraction of K-conductance blocked as a function of TEA^+ concentration yields a concentration-effect relation which is best fitted by the assumption that TEA^+ binds to its blocking site according to a first order reaction:

$$TEA^+ + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} TEAR$$

Table 1. Blocking effectiveness of certain quaternary ammonium ions, applied externally

	Frog node K_i (m*M*) *Hille* (1967b)	Molluscan neurone K_i (m*M*) *Hermann* and *Gorman (1981)*
Tetramethylammonium	500	> 500
Trimethylethylammonium	300	> 50
Dimethyldiethylammonium	60	–
Methyltriethylammonium	15	14
Tetraethylammonium	0.4	0.4
Triethylpropylammonium	2	–
Tetrapropylammonium	60	> 50
Tetrabutylammonium	–	> 50

Since the effect on K-conductance will be proportional to the fraction of sites filled if each site represents one channel, *Hille* (1967a) argued that the blocking site was part of the K-channel. This notion is now a commonplace of ionic channel pharmacology.

In node of *Rana pipiens* the dissociation constant for the reaction between TEA^+ and its receptor was 0.4 m*M* and was independent of membrane potential. In nodes of the frog *R. ridibunda,* the dissociation constant was 0.52 m*M* (*Mozhayeva* and *Naumov* 1972). It was again independent of membrane potential, but was increased by increasing either $[Ca^{2+}]_o$ or $[H^+]_o$, perhaps because resulting alterations in membrane surface charge repelled TEA^+ from its binding site. *Mozhayeva* and *Naumov* (1972) used high external [K]. So far as I am aware no competition between external K^+ and external TEA^+ for the blocking site has been reported. In nodes of *Xenopus laevis* (*Koppenhöfer* 1967), 0.3 m*M* TEA^+ reduced P_K by 32% suggesting a dissociation constant of about 0.6 m*M*.

Hille's (1967a) experiments suggested that in a frog node where a fraction of the K-conductance is blocked, the remaining K-channels open under depolarization and close under repolarization at the same rate as in the absence of TEA^+ so that TEA^+ simply blocks channels from the outside without affecting channel gating. The absence of an effect of TEA^+ on the kinetic behaviour of K-channels further suggests (but does not prove) that TEA^+ binds to K-channels whether or not they are open, in contrast with internal block where opening is a prerequisite of binding. This conclusion is not absolutely certain, however, since, if the rate of binding of TEA^+ is higher than the rate of channel opening, little change in the time-dependence of K-current would be seen. The computations of Fig. 4 demonstrate this point. Fig. 4B repeats calculations of *Armstrong* and *Hille* (1972, their Fig. 8) solving the sequential scheme of channel

blocking described above (Sect. 2.2) and showing that, as the rate constants k and l are increased in value (but held at a constant ratio), the predicted fraction of K-channels open (y) shows less sign of time-dependent inactivation. Using high rate constants for block (Fig. 4C,D) shows that K-conductance can be reduced in size with little apparent effect on its time-dependence.

Given these considerations, it is useful to have an estimate of the rate constant for the association and dissociation of TEA^+ and its receptor. This estimate is difficult to make with conventional recording methods, but *Vierhaus* and *Ulbricht* (1971b) attempted to measure the onset of the prolongation by TEA^+ of the nodal action potential in *Rana esculenta* and compared it with the rate with which Na^+ affected action potential height and duration (*Vierhaus* and *Ulbricht* 1971a). Their analysis was complicated by a delay in the onset of the action of the drug, but washout of TEA^+ was very rapid. They estimated a backward rate constant (k_{-1}) of 0.2 ms^{-1} and a dissociation constant of 1 m*M*. Thus the reaction between TEA^+ and its site of action seems to be very rapid, but perhaps slow enough to exclude the notion that it binds only to open channels when applied outside (see, however, *Moore* et al. 1979).

The experiments of *Koppenhöfer* (1967) suggested that in *Xenopus* node TEA^+ did alter the gating of K-channels, currents being slowed by TEA^+. Such a slowing cannot be explained as a consequence of the consecutive scheme of channel opening and block. Rather it was shown to be consistent with an effect of TEA^+ on the rate constants (α_n and β_n) of the Hodgkin-Huxley equations. In these terms *Koppenhöfer* (1967) found that τ_n was increased by 70% in 0.3 m*M* TEA^+.

Further, although *Hille* (1967a) emphasised that TEA^+ was without effect on Na-channels, *Schmidt* and *Stämpfli* (1966) and *Koppenhöfer* (1967) found an effect on inactivation of Na-current. In *Koppenhöfer*'s (1967) experiments, the time constant for inactivation was increased 40% in 5 m*M* TEA^+. Similar results have been reported by *Bromm* et al. (1978) for node of Ranvier of *Rana esculenta* and more recently by *Schönle* and *Koppenhöfer* (1981) for the same species. These last workers found that 10 m*M* TEA^+ slowed Na-inactivation by about 19%. It also increased peak Na-currents by about 5%, the onsent of this effect after TEA^+ application being slower than the onset of the effect in blocking K-currents. They argued that TEA^+ was not perfectly selective for K-channels. However, the effects were small and occurred at concentrations which were very high for K-channel blockage.

3.2 Fast and Slow K-Channels in Frog Node

Recently it has seemed possible that there may be more than one kind of Na-channel or of K-channel in axon membranes. *Matteson* and *Armstrong* (1981), for example, have reported slowly activating Na-channels in squid axon membrane. Their description implies one kind of Na-channel which can change its kinetic behaviour with changing experimental conditions.

In frog node of Ranvier, *Ilyin* et al. (1980) have reported the existence of a group of K-channels which open some ten times more slowly under depolarization than is conventionally described for K-channels. This kind of kinetic behaviour was thought to represent a different set of K-channels because of their different distribution among different nerve fibres, because of their different voltage-dependences, as measured by n_∞ curves, and because the slower K-channels were much less sensitive to TEA^+. *Dubois* (1981) and *Conti* et al. (1982) have also recently argued for more than one kind of K-channel in frog node.

Ilyin et al. (1980) suggested that *Koppenhöfer*'s (1967) observation of a slowing of the increase in K-conductance under depolarization might be explained by this finding. The relatively larger number of rapidly activating K-channels which would be blocked by TEA^+ would produce an apparent slowing of K-conductance, even if neither fast nor slow K-channels have their gating affected by TEA^+. These slow K-channels, possibly with slow inactivation of Na-conductance (*Chiu* 1977), might affect adaptation of the firing of action potentials in response to prolonged stimuli.

Differences in respect of action potential and of accommodation are known to exist between motor and sensory nerve fibres (*Schmidt* and *Stämpfli* 1964, *Frankenhaeuser* and *Vallbo* 1965, *Bergmann* and *Stämpfli* 1966, *Bretag* and *Stämpfli* 1975). In sensory fibres, the action potential is prolonged only a little by 5 m*M* TEA^+ (*Schmidt* and *Stämpfli* 1964).

3.3 TEA^+ and Localisation of K-Channels in Myelinated Nerve Fibres

While Na-channels appear to be limited to the nodal membrane of myelinated axons (*Ritchie* and *Rogart* 1977a, *Chiu* and *Ritchie* 1982), K-channels appear, in *mammalian* myelinated nerves, to be largely absent from nodes of Ranvier. This arrangement has been demonstrated by the absence of an effect of TEA^+ (*Bostock* et al. 1978) or of 4-aminopyridine (*Sherratt* et al. 1980, *Ritchie* et al. 1981) on action potentials of myelinated nerve fibres and on the failure to record K-currents under voltage clamp in rabbit (*Horackova* et al. 1968, *Chiu* et al. 1979) and rat (*Brismar* 1980) node. Such outward current as exists in rat fibres shows little delay

and is largely insensitive to external TEA^+ (6 m*M*), which blocks only slowly and incompletely (*Brismar* 1980).

Nonetheless, K-channels, activated by depolarization, are present in myelinated nerve fibres. These appear to lie at least partly in the paranodal membrane since they are revealed during partial demyelination, either by diphtheria toxin (*Sherratt* et al. 1980, *Bostock* et al. 1981), by lysolecithin (*Chiu* and *Ritchie* 1981, *Ritchie* et al. 1981), or by collagenase treatment or osmotic shock (*Chiu* and *Ritchie* 1980, 1981). Under these circumstances, K-currents may be recorded under voltage clamp (*Chiu* and *Ritchie* 1980, 1981). Further 4-aminopyridine and TEA^+ will each prolong action potentials (*Ritchie* et al. 1981) with the result that both these substances may be used to facilitate conduction in fibres blocked by demyelination (*Sherratt* et al. 1980, *Bostock* et al. 1981). *Chiu* and *Ritchie* (1980, 1981) found internal TEA^+ to be better than external at blocking paranodal K-currents. External application of TEA^+ at 20–60 m*M* depressed the late current by only about 30%.

From the previous account of the action of TEA^+ on frog nerve it is clear that frog nodes of Ranvier do have K-channels (see also *Chiu* et al. 1979, *Ritchie* et al. 1981). *Chiu* and *Ritchie* (1982) have recently shown that K-currents (but not Na-currents) may be recorded from internodes of the bullfrog which have been acutely demyelinated by treatment with lysolecithin. External application of TEA^+ at 10 m*M* blocked about 80% of this outward K-current in about 30 s. Whether these internodal K-channels are actually less sensitive to TEA^+ than nodal channels has yet to be tested, but from *Hille*'s (1967a) result 10 m*M* TEA^+ should block about 96% of K-currents in the node of *Rana pipiens*. The results of *Bostock* et al. (1981) also suggest the presence of TEA^+-sensitive K-channels in the internodes of rat nerve.

3.4 Gallamine and K-Currents

Since TEA^+ has a weak curariform action, it seems reasonable to ask whether other curare-like agents block K-conductance. Surprisingly perhaps, this question has only recently been asked and *Schauf* and *Smith* (1981) have shown that the neuromuscular blocker gallamine triethiodide (1,2,3-tris[2-triethylammonium ethoxy] benzene triiodide) also blocks K-currents. Concentrations of between 0.1 and 10 m*M* were used. K-conductance was blocked in frog node and in paranodal K-channels of rat nerve fibres. External gallamine triethiodide did not affect Na-currents. Internal gallamine triethiodide (10 m*M*) slowed Na-activation and eliminated outward Na-currents, perhaps rather as internal TEA^+ blocks outward K-current. It is possible that some of these effects (for example,

on channel gating) might be due to an effect on surface charge of the high concentration of I^-. Gallamine does not block K-conductance of *Myxicola* giant axons whether it is applied from the inside or the outside (*Schauf* and *Smith* 1981).

3.5 TEA^+ and Single Channel Conductance in Frog Node

Siebenga et al. (1974) measured a TEA^+-sensitive element of voltage noise, which they attributed to K-channels in frog node. *Begenisich* and *Stevens* (1975) used voltage clamp to measure current fluctuations which were blocked by TEA^+ and were thus associated with K-channels. Under the experimental conditions used, these channels, when open, had a unitary conductance of 4 pS, which was independent of membrane potential. Na-channels of frog node have a conductance of about 8 pS (*Conti* et al. 1976).

4 Skeletal Muscle

4.1 Permeability Mechanisms in Skeletal Muscle Fibres of Vertebrates

Electrophysiological studies, including those using voltage clamp methods depending on impaling microelectrodes (*Adrian* and *Freygang* 1962, *Adrian* et al. 1970a) or the vaseline gap (*Hille* and *Campbell* 1976), have identified a number of ionic channels in skeletal muscle fibres of the vertebrates, with twitch muscle fibres of the frog having been the most extensively studied.

First, and of particular relevance here, the K-conductance is complex in muscle, and at least three, and possibly four, kinds of K-channels exist. A delayed K-conductance, activated by depolarization like that of nerve fibres, but then completely inactivated under maintained depolarization, is responsible (with inactivation of the Na-conductance) for the repolarization of the action potential (*Adrian* et al. 1970a, *Stanfield* 1970a). *Bernard* et al. (1972) drew an analogy between the inactivation of delayed rectification in muscle and the inactivation of delayed K-currents in squid axon produced by TEA^+. The resting potential is determined in the long-term by a separate K-conductance, which shows inwardly rectifying properties as first described by *Katz* (1949; see also *Hodgkin* and *Horowicz* 1959). A third K-conductance, slowly activated by depolarization (*Adrian* et al. 1970b; see also *Stanfield* 1970a), may itself be composed of two separate elements. First, an element is present which depends on membrane potential, being seen also in fibres perfused

with EGTA-containing solutions under vaseline gap voltage clamp (*Almers* and *Palade* 1981). However, since muscle fibres release Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm under depolarization, it is attractive to suppose that an element of the slow K-conductance might be activated by Ca^{2+}, much as is the case in many other cells (see, for example, *Meech* 1978). In support of this notion, *Fink* and *Lüttgau* (1976) have shown that metabolically exhausted fibres, which may be presumed to have a high sarcoplasmic free Ca^{2+}, have a very high K-conductance. Further, recent experiments, using patch recording (*Neher* et al. 1978), have shown that a K-permeability, activated by intracellular Ca^{2+} in the micromolar concentration range, exists in the membrane of rat muscle cells in culture (*Pallota* et al. 1981). A similar channel has been described by *Latorre* et al. (1982), extracted from rabbit muscle and incorporated into artificial lipid bilayers. These channels have a high unitary conductance (100–200 pS).

Also present in twitch muscle fibres are a sodium permeability whose function and kinetic and selectivity properties are similar to those of nerve fibres (*Adrian* et al. 1970a, *Campbell* 1976); a Cl-permeability, which is generally rather high under resting conditions, particularly in mammalian muscle (*Hodgkin* and *Horowicz* 1959, *Palade* and *Barchi* 1977); and a Ca-permeability activated with an extremely slow time course by depolarization (*Beaty* and *Stefani* 1976, *Stanfield* 1977, *Sanchez* and *Stefani* 1978) and best seen in the presence of TEA^+.

Certain variations exist in this general scheme, both between muscle fibres from different orders of vertebrates and also between twitch and tonic muscle fibres of frog. Most of these differences lie beyond the scope of this review.

4.1.1 TEA^+ and Delayed Rectification

Hagiwara and *Watanabe* (1955) first applied TEA^+ to toad muscle and measured the action potential with an impaling microelectrode. They found that the quaternary ammonium ion prolonged the action potential and tended to lead to repetitive firing of impulses. The overshoot of the action potential was reduced less then might be expected from the Nernst equation when part of the external Na^+ was replaced by TEA^+. However, the evidence seems quite firm against the idea that this effect is due to TEA^+ permeating Na-channels. *Hagiwara* and *Watanabe* (1955) themselves showed that the Na-concentration (13 m*M*) at which propagation of the action potential failed was the same whether or not TEA^+ was present in the external solution. *Campbell* (1976) has shown that TEA^+ does not permeate the Na-channel of frog muscle ($P_{TEA}/P_{Na} < 0.008$). The effect of TEA^+ on overshoot must be due to a block of K-permeability, which a number of workers have shown to occur.

Muscle delayed rectification, the K-conductance activated by depolarization and involved in repolarization of the action potential, is blocked by TEA^+ present in the external solution (*Ildefonse* et al. 1969, *Kao* and *Stanfield* 1970, *Stanfield* 1970a, *Ildefonse* and *Rougier* 1971). Internal application of TEA^+ also produces block as *Audibert-Benoit* (1972) and *Fink* and *Wettwer* (1978) have shown. *Audibert-Benoit* (1972) argued that TEA^+ might exert its blocking action only from the inside, by analogy with squid axon and also on the grounds that the space occupied by externally applied ^{14}C-TEA had been shown in skeletal muscle by *Volle* et al. (1972) to be about twice the space occupied by markers such as inulin and sodium.

However, *Stanfield* (1973) showed that rapid external application of TEA^+ to single dissected muscle fibres produced a rapid effect in prolonging the action potential, the effect turning on along an approximately exponential time course with a time constant of 3–4 s. There was no subsequent long-term effect that might have been attributed to TEA^+ entry into the muscle fibres. *Audibert-Benoit* (1972) herself showed that the action of externally applied TEA^+ was rapidly reversible while the effects of injected TEA^+ were unmodified after 30–90 min.

TEA^+, applied externally, reduced delayed K-conductance by 50% at 8 m*M* (*Stanfield* 1970a). The concentration-effect relation was consistent with the block being produced by TEA^+ binding to the site (presumably the K-channel) through which it acts in a first-order, 1:1 fashion. As well as reducing K-conductance, TEA^+ also slowed its activation and in 58 m*M* TEA^+, the rate constants, computed according to a Hodgkin-Huxley scheme, appeared to be slowed about fivefold. The mechanism of this slowing is not clear, though it was thought *not* to be due to the presence in skeletal muscle fibres of a slow K-conductance system less vulnerable to the action of quaternary ammonium ions (see also *Stanfield* 1975).

Since TEA^+ prolongs the action potential of skeletal muscle fibres, it potentiates the twitch without affecting tetanic tension or the tension developed during K- or caffeine contractures (see, for example, *Washio* and *Mashima* 1963, *Sandow* 1964, *Henderson* and *Volle* 1971, *Stanfield* 1973, *Foulks* et al. 1971, 1973). *Henderson* and *Volle* (1971) showed that tetrapropylammonium ions did not produce twitch potentiation. However, the longer chain quaternary ammonium, TBA^+, produced a use-dependent potentiation of the twitch, related to a prolongation of the action potential (see also *Kao* and *Stanfield* 1970).

4.1.2 TEA^+ and Inward Rectification

As well as blocking delayed rectification, TEA^+ also blocks inward rectification, but it does so at higher concentrations, supporting the argument that inward rectification is a quite separate permeability mechanism. Inward rectification, whether measured at low (2.5 m*M*) or at high (80 or 100 m*M*) potassium concentrations, is half-blocked by 20 m*M* TEA^+ (*Stanfield* 1970b). Again, the block of inward rectification occurs through a 1:1 interaction between TEA^+ and the K-channel. Measurement of K-efflux in skeletal muscle also yields a value for the dissociation constant for the reaction between TEA^+ and this K-channel of 20 m*M* (*Volle* et al. 1972), demonstrated to be virtually independent of $[K]_o$. This last property of TEA^+ block contrasts with block by Rb^+ (see also *Adrian* 1964, *Standen* and *Stanfield* 1980), where K^+ and Rb^+ do compete for the blocking site of Rb^+. [TEA^+ blocks the weak permeance of Rb^+ (*Standen* and *Stanfield* 1980).] In support of the notion that delayed and inward rectification are pharmacologically separate, it may be mentioned that Rb^+ permeates and does not block delayed rectification (*Gay* and *Stanfield* 1978), and that 4-aminopyridine blocks delayed (*Gillespie* 1977) but not inward rectification (*Fink* and *Wettwer* 1978) in skeletal muscle. As long ago as 1955, *Hagiwara* and *Watanabe* had shown that TEA^+ increased the resting membrane resistance of toad muscle, but that the effect was less marked than the effect on action potential duration.

Recently it has become clear that an inwardly rectifying K-conductance, very similar to that present in skeletal muscle fibres, is present in cells as different egg cells of starfish (*Hagiwara* et al. 1976) and tunicate (*Ohmori* 1978) and macrophages from mouse spleen (*Gallin* 1981). But in egg cell membranes, inward rectification is unaffected even by prolonged external exposure to 100 m*M* TEA^+ (*Hagiwara* et al. 1976).

4.1.3 TEA^+ and Slowly Activating K-Conductance of Muscle

The K-conductance of frog skeletal muscle which slowly activates under depolarization (over hundreds of milliseconds at room temperature) is relatively unaffected by external TEA^+ (*Stanfield* 1970a), which argues that it is a separate mechanism from delayed and inward rectification. *Almers* and *Palade* (1981) have confirmed that the slow K-conductance they find in EGTA-perfused fibres is immune to the action of TEA^+ applied outside.

However, as *Fink* and *Lüttgau* (1976) have shown, muscle fibres that are exhausted by treatment with cyanide and iodoacetate, followed by repetitive stimulation, develop a high membrane K-conductance, whose size (14 mS · cm^{-2}) is similar to the maximum K-conductance associated with delayed rectification at room temperature (23 mS · cm^{-2}; *Stanfield*

1970a). Further the K-conductance that develops is blocked by TEA^+ whether applied at high concentration (115 m*M*) outside (though the block is incomplete) or applied inside (*Fink* and *Wettwer* 1978). However, this conductance has different pharmacological properties from delayed rectification as *Fink* and *Lüttgau* (1976) and *Fink* and *Wettwer* (1978) have shown, since it is unaffected by Zn^{2+} (which alters delayed rectification; *Stanfield* 1975); is blocked by Rb^+ (which permeates delayed rectifier); and is little affected by external 4-AP (which blocks delayed rectification). The K-conductance seems to increase under depolarization (*Fink* and *Lüttgau* 1976) and may also be activated, at least to a degree, by injection of Ca^{2+}; by application of caffeine, which releases Ca^{2+} from sarcoplasmic reticulum; or by electrical stimulation. Conversely, in partly exhausted fibres, the increase in g_K could be prevented by iontophoretic injection of EGTA (*Fink* and *Lüttgau* 1976, *Wettwer* et al. 1981). It seems likely that these observations are due to a separate Ca-activated K-conductance. The channel with this property, extracted from rabbit skeletal muscle by *Latorre* et al. (1982), *is* blocked by TEA^+.

4.1.4 Tonic Muscle Fibres of Vertebrates

Tonic muscle fibres of amphibians possess delayed rectification, but not inward rectification (see, for example, *Adrian* and *Peachey* 1965, *Stefani* and *Steinbach* 1969). TEA^+ blocks delayed rectification in toad tonic fibres, when applied outside at 60 m*M* (*Stefani* and *Uchitel* 1976). In mammals tonic muscle fibres are present in extraocular muscles (see, for example, *Bach-y-Rita* and *Ito* 1966) and delayed rectification is blocked by 25 m*M* external TEA^+ (*Bondi* and *Chiarandini* 1979).

4.1.5 Spontaneous Twitching of Skeletal Muscle Induced by TEA^+

The earliest reports of the effects of TEA^+ describe spontaneous twitching of muscle after TEA^+ injection (*Brunton* and *Cash* 1884, *Marshall* 1914, *Hagiwara* and *Watanabe* 1955). The evidence is that this effect is produced by an action at the fine unmyelinated endings of motor nerves, rather than in the myelinated parts of the axon or in the muscle fibres themselves. So far as the nodes of Ranvier are concerned, *Bergmann* et al. (1968) showed that spontaneous activity could be produced by TEA^+ in *Xenopus* or frog node only when $[Ca]_o$ was reduced too. The voltage dependence of Na-conductance had to be shifted to more negative membrane potentials by lowering $[Ca]_o$ (see also *Frankenhaeuser* and *Hodgkin* 1957, *Huxley* 1959, *Armstrong* 1981), and K-conductance had to be suppressed by TEA^+ at 5 m*M* to achieve the condition of spontaneous firing: a net inward current in the region of the normal resting potential. However, *Beaulieu* and *Frank* (1967a,b, *Beaulieu* et al. 1967) showed in

frog muscle that this effect *was* due to electrical activity set up initially in nerve rather than in muscle, since twitching of muscle was prevented by treatment with *d*-tubocurarine, by allowing depletion of acetylcholine from nerve terminals as a consequence of blocking choline reuptake by hemicholinium-3, or by substantial reduction of external $[Ca^{2+}]$ to reduce transmitter output. Small reductions in $[Ca^{2+}]_o$ enhanced the spontaneous activity. Further, the response did not occur in chronically denervated muscles (see also *Frank* 1961).

The action on nerve fibres appeared to occur at the fine unmyelinated terminal portions of the motor nerves. Action potentials set up at this site were generally not propagated antidromically to the ventral root containing the fibres innervating the muscle. TEA^+ appeared not to produce long-term depolarization of nerve terminals (*Beaulieu* et al. 1967). At sites other than skeletal muscle, low $[Ca^{2+}]$ and TEA^+ may also set up spontaneous firing in noradrenergic nerves (*Wakade* and *Wakade* 1981).

Because TEA^+ will also prolong the action potential of nerve terminals, owing to its action in blocking K-permeability, it enhances the release of acetylcholine from those terminals, rather as the prolongation of the action potential of muscle results in potentiation of the twitch. This action permits TEA^+ to overcome neuromuscular block produced either by low concentrations of *d*-tubocurarine (see, for example, *Rothberger* 1902), by low $[Ca^{2+}]_o$ (*Stovner* 1957b, *Beaulieu* and *Frank* 1967b) or by Mg^{2+} (*Stovner* 1957c). TEA^+, at concentrations of the order of 1 m*M*, will also counteract the action of botulinus toxin (*Cull-Candy* et al. 1976, see also *Thesleff* and *Lundh* 1979) which inhibits the release of acetylcholine. TEA^+ enhances transmitter release in a similar way in avian muscle (*Harvey* and *Marshall* 1977), in the mammalian autonomic nervous system (*Thoenen* et al. 1967, *Kirpekar* et al. 1972), and in invertebrate muscle (*Zucker* 1974). In invertebrate nervous systems (see below) this action has been used to help characterise the monosynaptic connexions formed by a given neurone. In squid giant synapse, and at the frog neuromuscular junction TEA^+ can permit the setting up of local Ca-dependent responses in the presynaptic terminals (*Katz* and *Miledi* 1969a,b) in the presence of high $[Ca]_o$ and TTX.

4.2 Invertebrate Skeletal Muscle

Among muscle fibres of invertebrates, those from arthropods (specifically crustaceans and insects) have been most intensively studied. Like vertebrate skeletal muscle, invertebrate fibres possess a number of ionic permeability mechanisms, but few of these have exact counterparts in skeletal muscle fibres of vertebrates. Perhaps there are parallels with vertebrate

smooth muscle. In particular, most invertebrate fibres respond to nerve stimulation with graded rather than all-or-nothing depolarizations. But, as *Fatt* and *Katz* (1953) first showed, this graded activity can, in crab muscle fibres, be converted to all-or-nothing activity by TEA^+, present in the external solution, even at quite low concentrations (1.7 m*M*). Choline produced a similar effect, but at higher concentrations, while tetrabutylammonium (TBA^+) was substantially more potent (and was largely irreversible in its effect), producing single electrical responses up to 18 s in duration at 13°C.

Similar effects of quaternary ammonium ions were also demonstrated in crayfish muscle by *Fatt* and *Ginsborg* (1958) who found benzyltrimethylammonium to be active too. In this preparation, it was shown that the electrical activity depended upon Ca^{2+} (or Sr^{2+} or Ba^{2+}) carrying current across the cell membrane – the first demonstration of Ca-spike (see *Hagiwara* 1973, *Hagiwara* and *Byerly* 1981). In lobster abdominal muscle, TEA^+-induced action potentials are longer in fast (lasting 4–10 s) than in slow (0.2–1 s) muscle fibres (*Jarohmi* and *Atwood* 1969). A similar requirement for TEA^+ exists for the production of action potentials in insect muscle fibres (*Washio* 1972, *Patlak* 1976, *Fukuda* et al. 1977, *Suzuki* and *Kano* 1977, *Ashcroft* 1981).

It seems clear that this influence of TEA^+ on electrical activity is due to its block of K-conductance (for example, *Mounier* and *Vassort* 1973) and is *not* due to any permeance of TEA^+ (*Werman* and *Grundfest* 1961). However, the order of effectiveness ($TBA^+ > TEA^+ >$ choline) found among the quaternary ammonium ions used by *Fatt* and *Katz* (1953) is different from that found by *Hille* (1967b) in frog node or by *Hermann* and *Gorman* (1981) for Ca-activated K-conductance of molluscan neurones (see Table 1 and below), a point which may deserve further study (see also Sect. 4.1.1; *Henderson* and *Volle* 1971).

The K-conductance of arthropod muscle is complex and a number of ionic channels may be identified. Those activated under depolarization consist of an early outward current, activated at the same time as the Ca-permeability and effectively preventing the Ca-current from producing action potentials, and a delayed K-current activated more slowly by deplarization (*Keynes* et al. 1973, *Hagiwara* et al. 1974, *Mounier* and *Vassort* 1975a,b, *Hencek* and *Zachar* 1977, *Zachar* 1981, *Salkoff* and *Wyman* 1981, *Ashcroft* and *Stanfield* 1982). These conductances are separable on kinetic grounds, and both are blocked by TEA^+ in the external solution (see also *Suarez-Kurtz* and *Sorenson* 1977) or, in barnacle muscle, in the internal solution (*Keynes* et al. 1973) when TEA^+ is added to the perfusate at 60 or 100 m*M*. Little attempt appears to have been made to separate these conductances on the grounds of different affinities for TEA^+, as has been done for other preparations (*Nakajima* 1966,

Stanfield 1970a,b, *Neher* and *Lux* 1972, *Vassort* 1975, *Thompson* 1977, *Ilyin* et al. 1980). However, early outward current may be more vulnerable to TEA^+ in *Carausius* (insect) muscle (*Ashcroft* and *Stanfield* 1982), and it is block of this conductance that is mainly responsible for the action of TEA^+ in permitting Ca-permeability to generate action potentials.

Mounier and *Vassort* (1975b) have argued that in crab muscle fibres, this early outward current is actually activated by Ca-entry. However, in insect muscle early outward current may develop (in ontogeny) before Ca-entry (*Salkoff* and *Wyman* 1981) while La^{3+} blocks Ca-entry without blocking this K-current (*Ashcroft* and *Stanfield* 1982). Probably both early outward and delayed currents in arthropod muscle are activated by depolarization.

Inward rectification is present in some arthropod muscle fibres, such as locust (*Usherwood* 1967) and crab (*Haudecoeur* and *Guilbault* 1974b), where it is blocked by TEA^+. But inward rectification is absent from *Carausius* muscle, among the insects (*Ashcroft* and *Stanfield* 1982), where the resting K-conductance rectifies in the outward direction and is blocked by TEA^+.

In crab muscle, certain of the TEA^+-sensitive conductances have been described as Cl-conductances (*Haudecoeur* and *Gilbault* 1974a–c, *Brûlé* et al. 1976). There is, however, no evidence from other sources to support the view that Cl-channels may be sensitive to TEA^+. Indeed, others have properly used TEA^+ to separate on pharmacological grounds K- and Cl-permeability mechanisms (see, for example, *Stanfield* 1970b; and below, *Kenyon* and *Gibbons* 1979a).

5 Cardiac Muscle

5.1 Vertebrate Heart

It seems inappropriate to attempt to list here the ionic conductances held to underlie electrical activity in heart muscle of vertebrates. The ionic permeability of cardiac muscle is complex, and remains a matter of controversy and reinterpretation. Certain aspects of this area have recently been reviewed by *Boyett* and *Jewell* (1980). Further, TEA^+ has been used relatively little in the investigation of cardiac action potentials. This is mainly because it has little effect on K-conductance when applied outside (see below) and perhaps partly because it may exert effects associated with the autonomic innervation of the heart, antagonising, for example the ACh-induced hyperpolarization of atrial muscle (see, for example, *Freeman* 1979, *Garnier* and *Nargeot* 1979). Certainly, TEA^+ has not been used to separate on pharmacological grounds the K-channels

believed to be present in the membrane of myocardial cells, though it has played a part in the reinterpretation of the nature of one ionic current in Purkinje fibres of mammalian heart.

Carmeliet (1961) and *Hutter* and *Noble* (1961) found that the chloride permeability of Purkinje fibres affected the duration of the plateau of the action potential, which lengthened in low Cl^- solutions. *Dudel* et al. (1967) found a transient outward current in sheep Purkinje fibres, activated at potentials positive to -20 mV under voltage clamp and markedly reduced by removal of external Cl^-; these authors therefore attributed the current to Cl^-. The current was called i_{qr} in the model of the Purkinje fibre action potential developed by *McAllister* et al. (1975), the subscript denoting first-order activation (q) and inactivation (r) processes which depend on membrane potential.

However, *Kenyon* and *Gibbons* (1979a) showed that the transient or early outward current was affected only a little by reducing $[Cl]_o$ to 8% of its control value. Further, 20 m*M* TEA^+ reduced the early outward current to 35% of its normal amplitude. Both these observations suggest that the largest part of the early outward current is K- and not Cl-current. The majority of the early outward current is also blocked by 4-AP (*Kenyon* and *Gibbons* 1979b). The element not blocked by 4-AP has been attributed to a Ca-activation of current by *Boyett* (1981; see also *Siegelbaum* and *Tsien* 1980). *Hart* et al. (1982) state that they can find no evidence for these early outward currents, gated by voltage or activated by Ca-entry.

However, the block by TEA^+ of much of the early outward current can account for the observations of *Haldimann* (1963) that 20 m*M* TEA^+ prolongs the action potential of both Purkinje and ventricular fibres of sheep heart, an action which he attributed to block of K-conductance. *Kenyon* and *Gibbons* (1979a) also describe in Purkinje fibres a slowing, particularly of phase I repolarization of the action potential, the phase leading into the plateau, which was also prolonged. Such an effect has been described as well in monkey heart by *Walden* et al. (1969, 1970).

Haldimann (1963) and *Kenyon* and *Gibbons* (1979a) both show that the effect of TEA^+ has a slow onset, taking (*Haldimann* 1963) 2 h to come to its maximum when applied at 20 m*M* to Purkinje fibres or to small bundles of ventricular fibres. Further, the effect was not easily reversed and was unaltered 4 h after returning normal (TEA^+-free) Tyrode to the experimental chamber. Since the TEA^+ permeability of the surface membrane of calf and sheep ventricular trabeculae has been measured as 6×10^{-8} cm $\cdot$ s^{-1} with 5.4 m*M* $[K]_o$ by *Weingart* (1974), using ^{14}C-TEA efflux measurements, it seems possible that a part of the effect is exerted from the inside.

Such is the interpretation of *Ochi* and *Nishiye* (1974) who found that external TEA^+ (20 m*M*) had little effect on papillary muscles from

guinea pig heart. But when TEA^+ was applied internally, by being allowed to diffuse from the cut end of a papillary muscle (the cut end being held in Na-, Ca-free, high K-solution to slow sealing of gap junctions between adjacent heart cells), it produces substantial prolongation, to 1.5 times control, of the action potential. This effect was half-complete at 2.4 m*M* TEA^+.

Ito and *Surawicz* (1981) have used the same technique to load TEA^+ (20 m*M*) into dog Purkinje fibres, where it prolongs the action potential, with a greater effect at lower than at higher rates of stimulation. Further, they found no effect on maximum diastolic membrane potential, on overshoot, or on maximum rate of rise of the action potential. They argued that TEA^+ might block only the plateau potassium current i_{x1} of *McAllister* et al. (1975) without affecting the background currents, such as i_{K1}. They had earlier shown (*Ito* and *Surawicz* 1977) internal TEA^+ had the effect of blocking the hyperpolarization, which in perfused heart may lead to the cessation of beating, occurring when the perfusing solution is returned from low $[K]_o$ to normal.

The technique of applying TEA^+ inside cardiac muscle depends on its permeating gap junctions between myocardial cells. Such permeation has been measured, by estimating the diffusion coefficient of TEA^+ inside myocardium, by *Weingart* (1974; see also *Weingart* et al. 1975). *Weingart* allowed ^{14}C-TEA either to diffuse into a myocardial trabecula from a cut end, or to diffuse from a region loaded with TEA^+ which had entered across the cell membrane. Both methods gave a diffusion coefficient of 2×10^{-6} cm$^2 \cdot$ s^{-1}, about a quarter the value expected in aqeuous solution. *Ochi* and *Nishiye* (1974) obtained a diffusion coefficient of 1×10^{-6} cm$^2 \cdot$ s^{-1} from the onset of the effect of TEA^+ in prolonging the action potential.

Like *Ochi* and *Nishiye* (1974), other workers have shown TEA^+ to have little effect when applied outside (for example, *Chang* and *Schmidt* 1960), while some (*Aronson* and *Cranefield* 1973, *Rosen* et al. 1978, *Lin* and *Vassalle* 1979) have used TEA^+ simply as a substitute for external Na^+, TEA^+ affecting the action potential only after long-term exposure.

Coraboeuf and *Vassort* (1967a,b) described a prolongation of the action potential of the perfused guinea pig heart by external TEA^+, attributing to a block of K-conductance. This K-conductance (or its sensitivity) appears to increase with age (*Bernard* and *Gargouïl* 1968) since the effect of 20 m*M* TEA^+ is greater on 20-day foetal rat hearts than on 16- or 13-day hearts, the heartbeat starting at about 9–10 days and term being at 21 days post coitum.

In frog heart, both *Rougier* et al. (1968) and *de Hemptinne* (1978) showed that 20 m*M* TEA^+ does not block outward K-currents, but blocks

inward K-currents obtained under hyperpolarization. However, in axolotl heart, outward current appeared to be blocked (*Bernard* et al. 1968).

In chick heart, TEA^+ increases action potential amplitude and duration (*Pappano* 1976), especially when that duration has been shortened by exposure to valinomycin, as a K-ionophore (*Shigenobu* and *Sperelakis* 1975), or when the heart has been rested for a period of 15 s (*Mackenzie* and *Standen* 1982). *Mackenzie* and *Standen* (1982) found steady-state action potentials of their cultured cell aggregates (1 Hz stimulation) appeared little affected by external TEA^+, using relatively brief exposures to the quaternary ammonium ion, even though the rested action potential was substantially prolonged. This result perhaps bears some similarity to that of *Ito* and *Surawicz* (1981; above). *Maruyama* et al. (1980) found that spontaneously beating cultured aggregates of chick heart are unaffected by 20 m*M* TEA^+ added to the culture medium except after exposures longer than 1 h. Injected TEA^+ was, however, quickly effective at prolonging action potentials.

5.2 Invertebrate Heart

The effects of TEA^+ have been examined on the hearts of a number of arthropods, hearts which are normally paced by the cardic ganglia which supply their innervation. TEA^+ enhances action potential amplitude and duration at 5 m*M* in the heart of the woodlouse (*Porcellio; Holley* and *Delaleu* 1972, *Delaleu* et al. 1972) and of crab (*Lassalle* and *Guilbault* 1972), and at 20 m*M* in the heart of the arachnid *Limulus* (*Tanaka* et al. 1966).

6 Smooth Muscle of Vertebrates

A number of workers have carried out voltage clamp analyses of smooth muscle cells, using a double sucrose gap technique to control membrane potential and measure membrane current. Such a technique was first applied to myometrium by *Anderson* (1969), whose work has been extended by *Anderson* et al. (1971), *Mironneau* et al. (1971), *Mironneau* (1974), *Kao* and *McCollough* (1975), *Vassort* (1975, 1976), and *Muramatsu* et al. (1978). Voltage clamp methods have also been used with vascular smooth muscle (*Daemers-Lambert* 1976; for review, see *Keatinge* and *Harman* 1980) and smooth muscle of the gastrointestinal tract (for example, *Inomata* and *Kao* 1976a,b, *Weigel* et al. 1979; for recent review, see *Suzuki* and *Inomato* 1981).

Under voltage clamp, depolarization yields an inward current, possibly carried virtually entirely by Ca^{2+} (*Weigel* et al. 1979, *Suzuki* and *Inomata* 1981, *Osa* 1974, *Harder* and *Sperelakis* 1979), being insensitive to TTX, but blocked by divalent cations such as Mn^{2+} and Co^{2+} and by La^{3+}, D600, and verapamil. Though some voltage clamp accounts describe only one element of outward current (*Anderson* 1969, *Mironneau* et al. 1971, *Mironneau* 1974, *Kao* and *McCullough* 1975, *Daemers-Lambert* 1976), others describe more than one element. Both in myometrium (*Vassort* 1975) and in gastrointestinal smooth muscle (*Weigel* et al. 1979, *Suzuki* and *Inomato* 1981), a transient outward current is present, activated nearly synchronously with the Ca-current, and carried by K^+. This current has been ascribed a dependence on Ca-entry in myometrium (*Vassort* 1975, 1981), in gastrointestinal tract (*Weigel* et al. 1979), and possibly in ureter where it is blocked by Mn^{2+} (*Shuba* 1981). It is not activated when Ba^{2+}, rather than Ca^{2+}, carries inward current (*Weigel* et al. 1979; compare *Gorman* and *Hermann* 1979). However, like the transient outward current of invertebrate neurones (see Sect. 7.1, below), which is not Ca-activated, this current is partly inactivated at the resting potential but may be reprimed by hyperpolarization (*Vassort* 1981). *Suzuki* and *Inomata* (1981) suggest that in smooth muscle of the gastrointestinal tract, at least, the issue of whether this current is activated by Ca^{2+} or by depolarization remains unresolved.

In addition to this transient outward current, *Suzuki* and *Inomata* (1981) describe a late outward current in intestinal muscle which is carried both by Na^+ and K^+, with P_{Na}/P_K (the permeability ratio Na^+:K^+) being 0.6. In myometrium, *Vassort* (1975) described a similar non-selective current, which he called slow current, while in ureter, *Shuba* (1977) showed that removal of Na^+ from the external solution suppressed the plateau of the action potential induced by TEA^+. *Vassort* (1975, 1981) has also described a late current, carried by K^+ and activated over hundreds of milliseconds at 32°C.

TEA^+ provides a means of dissecting these currents pharmacologically. Thus it has a powerful blocking action on the transient outward current, 15 m*M* TEA^+ suppressing this current in myometrium (*Vassort* 1975, 1981). A similar result was obtained by *Weigel* et al. (1979) in small intestine. The late K-current of myometrium is incompletely blocked by TEA^+ at 15 m*M*, while the non-specific slow current of *Vassort* (1975) and the Na-, K-carried late outward current of *Suzuki* and *Inomata* (1981) is quite altered by TEA^+.

TEA^+ has also been shown to block K-current in smooth muscle by *Moronneau* et al. (1971), *Inomata* and *Kao* (1976), and *Muramatsu* et al. (1978). *Cooper* et al. (1975) and *Casteels* et al. (1977) found TEA^+ at 10 m*M* blocked ^{42}K-efflux from the smooth muscle cells of aterial wall into high K^+ external solutions.

Since the presence of the transient outward current will, rather as in invertebrate skeletal muscle, prevent Ca-entry from readily producing regenerative changes in membrane potential, its block by TEA^+ will either enhance electrical activity in spontaneously active smooth muscle cells, permit the production of action potentials in response to electrical stimulation, or induce spontaneous firing of action potentials or oscillations of membrane potential in quiescent smooth muscle cells. A large number of electrophysiological studies of smooth muscle have described such effects. Among these accounts are those by *Suzuki* et al. (1963), *Ito* et al. (1970), *Osa* and *Kuriyama* (1970), *Ito* and *Kuriyama* (1971), *Sakamoto* (1971), *Fujii* (1971) and *Szurszewski* (1975) in smooth muscle of the gastrointestinal tract, where the effect may be shown not to be due to any agonistic action of TEA^+ on muscarinic receptors (*Golenhofen* et al. 1978, *Bolton* 1981); by *Creed* and *Kuriyama* (1971) in bile duct; by *Osa* (1974) in myometrium; by *Washizu* (1967) and *Shuba* (1977) in ureter; by *Steedman* (1966), *Mekata* (1971), *Droogmans* et al. (1977), *Ross* and *Belsky* (1978), *Holman* and *Surprenant* (1979) and *Harder* and *Sperelakis* (1979) in vascular smooth muscle; and *Kirkpatrick* (1975) and *Stephens* et al. (1975) in tracheal and airway smooth muscle.

In many of these cases, TEA^+ was shown to increase resting membrane resistance by blocking resting K-conductance. But in pregnant mouse myometrium, *Osa* (1974) found that 3 m*M* TEA^+ increased the height and rate of rise of action potentials *without* any increase in resting membrane resistance or depolarization of the resting potential. Conversely in carotid artery, TEA^+ raises membrane resistance and depolarizes after short exposures, but permits the generation of phasic contractions only after long exposures (*Haeusler* and *Thorens* 1980). Such observations imply that resting K-conductance may be separable pharmacologically from other K-conductances blocked by TEA^+.

7 Neuronal Somata

The cell bodies of neurones have been subject to intensive study, partly to elucidate synaptic mechanisms in the nervous system and partly because they form an ideal experimental preparation in which to study Ca-currents under voltage clamp (for review, see *Kostyuk* 1980). In terms of their ionic permeability properties, neurones of invertebrates have perhaps been studied more intensively and I shall deal with them first. Cell bodies of both vertebrate and invertebrate neurones have, in general, more complex permeability properties then do axons and TEA^+ has been widely used, since *Nakajima* (1966; see also *Washizu* 1959) first did so, to separate on pharmacological grounds the different K-permeability mechanisms that are present.

7.1 Invertebrate Nerve Cells

TEA^+ has been used in the study of neurones from three invertebrate groups: annelids, arthropods, and molluscs. Two orders from the gastropod molluscs are particularly widely studied: these are pulmonates such as *Helix* and as *Limnaea* and *Planorbis* and the opisthobranchs such as *Aplysia* and as *Archidoris, Anisodoris,* and *Tritonia.* Neurones from each of these groups have similar permeability properties, but the relative sensitivities to TEA^+ may be quite different from one sub-order to another. In all cases, TEA^+ blocks at least certain elements of K-conductance from the external solution and was first shown to do so in *Onchidium* neurones by *Hagiwara* and *Saito* (1959).

Generally, the neuronal somata have both Na- and Ca-permeability mechanisms. These are separable by means of TTX, or removal of external Na^+, and by means of divalent transition metal ions such as Co^{2+} or Mn^{2+}, which block Ca-current (see, for example, *Geduldig* and *Gruener* 1970, *Standen* 1975a,b, *Adams* and *Gage* 1979a,b). Somata generally have three elements of K-conductance. These are separable on kinetic grounds. The first is a transient or early outward current described in *Aplysia* by *Connor* and *Stevens* (1971) and in *Helix* by *Neher* (1971). This current activates and inactivates under depolarization, much like the transient or early outward current of invertebrate skeletal muscle (and perhaps of vertebrate smooth muscle; see Sects. 4.2 and 6). Further, as in some smooth muscles, it appears to be substantially inactivated at the normal resting potential and must be reprimed by hyperpolarizing. As a consequence hyperpolarizing prepulses may lead to an apparent reduction in the size of the Ca-current elicited by a depolarization because the depolarization now activates the nearly synchronous outward current also (*Geduldig* and *Gruener* 1970, *Neher* 1971, *Connor* and *Stevens* 1971, *Standen* 1974, *Kostyuk* et al. 1975). This transient outward current is potential- and *not* Ca-dependent. *Connor* and *Stevens* (1971) refer to it as I_A or A-current (see also *Adams* et al. 1980, who have reviewed the currents of nerve cells).

The second K-conductance mechanism is the delayed outward current, which is slowly activated by depolarization: it is also comparable with the delayed current of invertebrate skeletal muscle (see Sect. 4.2). This current was called I_K or K-current by *Connor* and *Stevens* (1971).

The third K-conductance is dependent on Ca^{2+}. It may be activated by injections of Ca^{2+} into nerve cells (*Meech* 1972), though it has recently been shown to depend on membrane potential as well as on $[Ca]_i$. *Gorman* and *Thomas* (1980) found that Ca-activated K-conductance increased e-fold for a 25 mV depolarization. The conductance may also be activated by Ca^{2+} entering by way of Ca-channels under depolarization as

Meech and *Standen* (1975) first showed. The current is sometimes called I_C or C-current following the nomenclature of *Connor* and *Stevens* (1971; *Thompson* 1977). *Gorman* and *Thomas* (1980), for example, call it $I_{K(Ca)}$.

7.1.1 TEA^+ Dissection of K-Conductances

Neher and *Lux* (1972) identified in neurones of *Helix pomatia* two of the three components of K-conductance listed above – the transient and delayed outward currents – and showed that the second of these was the more sensitive to TEA^+ applied externally, being blocked by 5–10 m*M* TEA^+. The transient current was not altered by TEA^+ at this concentration but was blocked by 20–90 m*M*. Much the same result had been found in supramedullary neurones of a vertebrate (puffer fish) by *Nakajima* (1966), and a nearly identical result was obtained by *Kostyuk* et al. (1975).

This distinction, based on affinity for TEA^+, breaks down when internal TEA^+ is used. Iontophoresis of TEA^+ into cells to achieve a final concentration of 17 m*M* (*Neher* and *Lux* 1972) blocks both currents in like manner. *Kostyuk* et al. (1975) found transient outward current was the more sensitive to internal TEA^+. Unlike the situation first described by *Armstrong* and *Binstock* (1965), both inward and outward currents are blocked by internal TEA^+, even when $[K]_o$ was increased to 35 m*M* to enhance inward current tails (*Neher* and *Lux* 1972).

Meech and *Standen* (1975), who first described the Ca-activated K-current, attempted to separate it from the voltage-dependent delayed current in neurones of *Helix aspersa* on grounds of TEA^+ sensitivity. However, they found the two elements of current equally sensitive to TEA^+, being half-blocked by about 10 m*M* in both cases. In neurones of *Tritonia*, *Thompson* (1977) has found that TEA^+ sensitivity occurs in the order delayed (K-) current > transient outward (A-) current $\gg$ Ca-activated (C-) current. To enhance the pharmacological distinction, the A-current was, in this species, more sensitive to 4-AP, while the Ca-dependent K-conductance was, not unexpectedly, blocked by agents which block the entry of Ca^{2+} such as Co^{2+} and Mn^{2+}. The distinction based on TEA^+ affinity appears to hold for neurones of *Archidoris* and *Anisodoris* where TEA^+ has been used at 100 m*M* to separate delayed and Ca-activated K-currents (*Aldrich* et al. 1979). In many vertebrate nerve cells also (see below) TEA^+ does not block well the Ca-activated element of K-conductance.

However, the distinction between delayed and Ca-activated K-current made on the basis of TEA^+ block clearly *cannot* be applied universally, as the result of *Meech* and *Standen* (1975) for *Helix aspersa* already shows.

Further, in a recent study of TEA^+ block in neurones of *Aplysia, Hermann* and *Gorman* (1981) found that the Ca-activated conductance was actually the *more* sensitive to TEA^+. *Hermann* and *Hartung* (1981) found a similar result for neurones of *Helix pomatia.* In the experiments of *Hermann* and *Gorman* (1981) K-conductance was activated both by iontophoresing Ca^{2+} into the neurones and by permitting Ca-entry to occur under depolarization.

Their experiments also introduce a new principle to the study of block by external TEA^+, since the block shows a weak potential-dependence such as might be expected if TEA^+ binds to a site in the K-channel and is in consequence of its positive charge expelled from the channel by depolarization.

The delayed K-current was blocked by TEA^+ in a concentration-dependent way consistent with a single molecule of TEA^+ combining with a single channel and a dissociation constant which was 6 m*M* at + 20 mV, higher at more positive and lower at more negative membrane potentials. In the same neurones (R15) of *Aplysia, Adams* and *Gage* (1979a) found this current to be half-blocked by 5–10 m*M* TEA^+.

In the case of the more sensitive Ca-activated K-conductance, TEA^+ blocked with a dissociation constant of 0.4 m*M* at − 30 mV, increasing to 0.5 m*M* at + 20 mV, indicating a weak potential-dependence with the affinity falling at more positive voltages. Table 1 also gives the results of experiments in which some or all of the ethyl groups of TEA^+ were substituted by other alkyl groups, experiments of the kind that *Hille* (1967b) and *Armstrong* (1969, 1971) have performed on nerve fibres. The result that TEA^+ is the most potent blocker of Ca-activated K-conductance in *Aplysia* neurones and that the affinity falls as ethyl groups are substituted by either longer or shorter alkyl groups is identical with that found by *Hille* (1967b) for frog node as Table 1 shows. *Hermann* and *Gorman* (1981) have suggested that there might also be a sign of voltage-dependence of TEA^+ block in frog node (*Hille* 1967a, *Koppenhöfer* 1967, *Mozhayeva* and *Naumov* 1972).

Conversely, with *internal* TEA^+, delayed outward current is much more effectively blocked than is Ca-activated K-current. Further, channels are more likely to be blocked at positive membrane potentials (*Hermann* and *Gorman* 1979, 1981). Indeed in the case of Ca-activated K-conductance, channels are blocked by TEA^+ only at potentials positive to zero. *Heyer* and *Lux* (1976) also found an element of K-conductance which was not blocked by internal TEA^+.

Hermann and *Gorman* (1981) actually found a small increase in Ca-activated K-current at negative potentials, comparable to the apparent enhancement of Ca-entry, as measured by aequorin light emission, found in nerve cells immersed in solutions containing TEA^+ at high concentration

(*Zucker* and *Smith* 1979). This effect is exerted, not by TEA^+ itself, but by an impurity of commercial TEA^+, triethylamine, which alkalinizes cell cytoplasm, affecting intracellular buffering of Ca^{2+} (*Zucker* 1981).

Anomalous rectification is present in at least some molluscan neurones (*Gola* and *Romey* 1971) but is not blocked by external TEA^+ (*Gola* 1972) even at concentrations of 200 m*M* (*Eaton* and *Brodwick* 1976).

7.1.2 TEA^+ and Function of Nerve Cells

Klee (1978) has argued that different sensitivities to TEA^+ occur in cells of *Aplysia* with different functions. So-called S cells, which include pace-making neurones, are more sensitive to TEA^+ (and less sensitive to 4-AP) than are F cells, which have fast rising and fast repolarizing action potentials. In *Aplysia* also, certain nerve cells (R2) are light sensitive and have K-currents which are activated by illumination of the soma (*Brown* and *Brown* 1973). This current appears to be independent of $[Ca]_o$, but is blocked by 100 m*M* TEA^+ (*Brown* et al. 1977), as is the K-current activated by Ca-entry.

In leech segmental ganglia, *Kleinhaus* and *Prichard* (1975, 1977) have also investigated different sensitivities to TEA^+ among cells of different functions. Retzius cells were most sensitive among the cells investigated while among sensory cells, the order of sensitivity was nociceptive > pressure > touch. A similar order of ability to generate Ca-dependent action potentials was found. Variations in sensitivity were likewise found in insect (locust metathoracic ganglia) by *Goodman* and *Heitler* (1979). TEA^+ sensitivity has also been examined in cockroach (*Periplaneta; Pitman* 1975, 1979) and, among crustacea, in crayfish (*Iwasaki* and *Ono* 1979) and crab neurones (*Tazaki* and *Cooke* 1979).

7.1.3 TEA^+ and Monosynaptic Connexions of Neurones

If TEA^+ is injected by iontophoretic or other means into the soma of a nerve cell, it will diffuse into the axon, blocking its K-permeability. *Horn* (1977) has in this way shown that axons of certain cells of *Aplysia* also have a potential-dependent Ca-permeability which is sufficient to produce Ca-dependent action potentials in the presence of TTX or after Na-removal, activity which may be blocked by Co^{2+}.

TEA^+ has also been used in this way in invertebrate nervous systems to establish the synaptic connexions formed by a particular cell. The argument is that TEA^+, injected into a cell will diffuse to the nerve terminals, prolonging the action potential there, so enhancing the output of transmitter, with a resulting increase in the size of postsynaptic potentials. *Kehoe* (1969, 1972) showed that the output of ACh from a neurone of *Aplysia* could be so affected and established that two cholinergic phases

of inhibition were produced by the same presynaptic cell. Several workers have used this criterion (usually with others, concerning latency, constancy of synaptic transmission, and effects of presynaptic polarization) to identify monosynaptic connexions. Among those using this method have been *Gerschenfeld* and *Paupardin-Tritsch* (1973), *Bryant* and *Weinreich* (1975), *Shimahara* and *Tauc* (1975), *Winlow* and *Benjamin* (1977), *Hinzen* and *Davies* (1978), and *Berry* and *Cottrell* (1979). A reservation with the method is that cells may be electrically coupled so that, as *Weingart* (1974) showed for heart muscle, TEA^+ may spread from cell to cell. This issue is discussed by *Deschênes* and *Bennett* (1974) and reviewed by *Berry* and *Pentreath* (1976).

7.2 Vertebrate Nerve Cells

A number of vertebrate neurones have been studied with TEA^+. Among these are the supramedullary neurones of the puffer, *Spheroides* (*Nakajima* 1966); dorsal root ganglion cells (*Koketsu* et al. 1959); motoneurones (*Washizu* 1959, *Barrett* and *Barrett* 1976) and sympathetic ganglion cells (*Minota* 1974, *Koketsu* and *Nakamura* 1976, *Kuba* and *Koketsu* 1977, *Brown* and *Adams* 1980) of the frog; sympathetic ganglion cells of mammals (*Christ* and *Nishi* 1973, *McAfee* and *Yarowsky* 1979), sometimes in culture (*O'Lague* et al. 1978a,b) and cat spinal motoneurones (*Schwindt* and *Crill* 1981); and cortical neurones (*Krnjevic* et al. 1971) and hippocampal neurones (*Schwartzkroin* and *Prince* 1980) of the guinea-pig. *Moolenaar* and *Spector* (1977, 1978, 1979) have investigated the actions of TEA^+ on mouse neuroblastoma cells in culture.

Both externally and internally (*Koketsu* et al. 1959, *Krnjevic* et al. 1971, *Schwindt* and *Crill* 1980, 1981), TEA^+ blocks K-conductance, prolonging the action potential, while, as with invertebrate neurones, electrical excitability can be sustained in Na-free solutions if TEA^+ is present, owing to the presence of a potential-dependent Ca-permeability (*McAfee* and *Yarowsky* 1979, *Moolenaar* and *Spector* 1977). In most cells, a late after-hyperpolarization follows the action potential of the soma, believed to be generated by a Ca-dependent increase in K-conductance. This after-hyperpolarization is either unaltered or enhanced by TEA^+ (*Barrett* and *Barrett* 1976, *Minota* 1974, *McAfee* and *Yarowsky* 1979, *O'Lague* et al. 1978a, *Schwartzkroin* and *Prince* 1980, *Moolenaar* and *Spector* 1979). In these cases the potential-dependent K-conductance involved in repolarizing the action potential is blocked by the quaternary ammonium ion.

Under voltage clamp conditions, *Nakajima* (1966) found a slowly activating and inactivating element of K-conductance more susceptible to TEA^+ than a rapidly activating one. Conversely, in cat motoneurones,

Schwindt and *Crill* (1981) found a rapidly activating component of K–conductance which was more susceptible to TEA^+, iontophoresed extracellularly, than the slowly activating one, which may underlie the TEA^+-sensitive after-hyperpolarization.

TEA^+ blocks neither the anomalous K-rectification of mammalian sympathetic ganglion cells (*Christ* and *Nishi* 1973), nor the hyperpolarization of bullfrog sympathetic ganglion cells produced by adrenaline (*Koketsu* and *Nakamura* 1976). Nor does it block the acetylcholine-dependent M-current (*Brown* and *Adams* 1980), which is carried by K^+ in bullfrog sympathetic neurones.

8 Secretory Cells

Electrophysiological methods are increasingly being applied to both exocrine and endocrine gland cells. These developments have been reviewed recently by *Petersen* (1980). TEA^+ has been used in some of these studies, though in the cases of salivary (*Petersen* 1970, *Fritz* 1972) and pancreatic acinar cells (*Matthews* and *Petersen* 1972), simply as a Na-substitute, with effects apparently little different from those obtained on replacing Na^+ with TRIS.

On other cells, such as the β-cells of pancreatic islets (*Henquin* 1977) and on a growth hormone and prolactin secreting cell line (GH3 cells) developed from rat adenohypophysis and grown in culture (*Biales* et al. 1977), TEA^+ does act as a blocker of K-permeability. Since such cells have voltage-dependent conductances and develop action potentials in response to their physiological stimuli (*Dean* and *Matthews* 1968, *Kidokoro* 1975), *Petersen* (1980) has termed them electrically excitable gland cells.

In GH3 cells, 10 m*M* TEA^+, added to the external medium, prolongs the action potential to around 300 ms (*Biales* et al. 1977). In perifused pancreatic islets, TEA^+ has been used at concentrations up to 20 m*M* (*Henquin* 1977, *Henquin* et al. 1979, *Atwater* et al. 1979b, *Carpinelli* and *Malaisse* 1980, *Herchuelz* et al. 1980). Two methods have been used to assess its effects on K-permeability: electrophysiological recording (*Atwater* et al. 1979b, *Henquin* et al. 1979) and measurement of ^{86}Rb efflux from β-cells (*Henquin* 1977, *Henquin* et al. 1979, *Herchuelz* et al. 1980), Rb^+ being assumed to permeate K-channels (*Sehlin* and *Taljedal* 1975).

The electrophysiological experiments of *Atwater* et al. (1979b) drew a distinction between resting K-conductance, which appears to be unaltered by TEA^+, and the increase in K-conductance, occurring on depolarization,

which is blocked with the result that action potentials are increased in amplitude and duration. TEA^+, in the perifusate, inhibits ^{86}Rb-efflux and enhances the secretion of insulin in response to glucose, which is itself believed to initiate depolarization of β-cells by reducing K-permeability (*Sehlin* and *Taljedal* 1975, *Henquin* 1977, *Henquin* et al. 1979, *Atwater* et al. 1979b). The response to physiologically high concentrations of glucose (20 m*M*) is not enhanced, however; rather the response to glucose is shifted to lower glucose concentrations.

Indeed, TEA^+ does not cause insulin secretion in the absence of glucose (*Henquin* 1977, *Henquin* et al. 1979) and the exact nature of the interaction between glucose and TEA^+ remains a matter of speculation (*Herchuelz* et al. 1980). Nonetheless, TEA^+ substantially alters the pattern of spike activity produced by β-cells in response to glucose. This pattern normally consists of bursts of action potentials separated by intervals of hyperpolarization (*Henquin* et al. 1979, *Atwater* et al. 1979b) which are believed to be generated by a Ca-dependent increase in K-permeability (*Atwater* et al. 1979a). *Atwater* et al. (1979c) have correlated insulin secretion with these bursts of action potentials. TEA^+ not only prolongs the action potentials and enhances their size but also abolishes the hyperpolarizing intervals (*Atwater* et al. 1979b, *Henquin* et al. 1979), though it has less effect on a quinine-sensitive element of ^{86}Rb-efflux (*Carpinelli* and *Malaisse* 1980). This element is unaffected by glucose, probably being Ca-activated, by analogy with red cells where quinine blocks the Ca-dependent K-permeability (*Armando-Hardy* et al. 1975).

9 Protozoa

Deitmer (1982) has made a pharmacological dissection of potential-dependent and mechanoreceptor K-currents in the ciliate *Stylonichia* on the grounds of different sensitivities to TEA^+: the mechanoreceptor current was blocked by lower concentrations of TEA^+. *Naitoh* and *Eckert* (1973) had found a similar permeability mechanism (recorded as a hyperpolarizing response to mechanical stimulation) was blocked by TEA^+ in *Paramecium*, while *Friedman* and *Eckert* (1973) had found that TEA^+ blocked resting K-conductance and might also block voltage-sensitive K-conductance ('late repolarizing current'). *Brehm* et al. (1978) showed that injection of TEA^+ linearized current-voltage relations measured with constant current methods.

A large number of behavioural mutants of *Paramecium* exist with different deficits in the ionic permeabilities of their surface membranes (*Kung* et al. 1975). Among these is a 'TEA^+-insensitive' mutant, described

by *Chang* and *Kung* (1976) where 4 m*M* TEA^+, applied externally, failed to set up spontaneous Ca-dependent action potentials as it was found to do in the wild-type ciliate. This TEA^+ insensitivity is, however, due to more K-channels being open at rest than is the case for the wild type, rather than to K-channels losing their sensitivity to TEA^+. Indeed, injection of TEA^+ raised the membrane resistance to the same level as that found in the wild type after injection of TEA^+. In both cases, internal was more effective than external TEA^+ at blocking K-permeability.

These and other permeability properties of *Paramecium* have been reviewed by *Eckert* and *Brehm* (1979).

10 Excitable Plant Cells

Certain plant cells, such as those of *Nitella,* show electrical activity (*Osterhout* and *Hill* 1930). These cells produce action potentials believed to be caused by an increase in permeability of Cl^- (*Mailman* and *Mullins* 1966, *Koppenhöfer* 1972a) followed by an increase in K-permeability. This K-permeability is blocked by TEA^+ at concentrations lower than 10 m*M* (*Belton* and *van Netten* 1971, 1978, *Koppenhöfer* 1972b).

11 Summary and Conclusions

TEA^+ blocks K-channels in a wide variety of cells, but with the dissociation constant for the reaction between the quaternary ammonium ion and its receptor, presumably the K-channel, varying substantially between K-channels of different kinds (Table 2). This variability allows TEA^+ to contribute to a pharmacological separation of K-channel types, yet few general rules emerge as to TEA^+ affinity. Certainly the high degree of specificity of block and the apparent near-constancy of affinity shown by tetrodotoxin (TTX) in its block of Na-channels (*Kao* 1966, *Ritchie* and *Rogart* 1977b, their Table 1) is, perhaps not surprisingly, not shown by TEA^+. As an example, the affinity of K-channels of frog nerve for TEA^+ (*Hille* 1976a) is about 20 times higher than that of K-channels of frog skeletal muscle (*Stanfield* 1979a) while both affinities are about 10^6 times lower than that of the Na-channels of these cells for TTX. In addition, unlike TTX, which blocks only from the external solution, TEA^+ may block either from outside or from inside (*Koppenhöfer* and *Vogel* 1969, *Armstrong* and *Hille* 1972).

Table 2. Dissociation constants for the blocking reaction between *external* TEA^+ and its site of action, measured from block of K-permeability, in certain electrophysiological preparations

Preparation	Nature of K-channel	K_i (m*M*)	Reference
Squid giant axon	Potential-dependent	Not blocked	*Tasaki* and *Hagiwara* (1957), *Armstrong* and *Binstock* (1957)
Myxicola giant axon	Potential-dependent	24 m*M*	*Wong* and *Binstock* (1980)
Crayfish giant axon	Potential-dependent	Not blocked	*Shrager* et al. (1969)
Amphibian node of Ranvier			
Rana pipiens	Potential-dependent	0.4 m*M*	*Hille* (1967a)
Rana ribidunda	Potential-dependent	0.5 m*M*	*Mozhayeva* and *Naumov* (1972)
Xenopus laevis	Potential-dependent	0.6 m*M*	*Koppenhöfer* (1967)
Frog skeletal muscle	i. Potential-dependent	8 m*M*	*Stanfield* (1970a)
	ii. Inward rectification	20 m*M*	*Stanfield* (1970b), *Volle* et al. (1972)
Starfish egg	Inward rectification	Not blocked	*Hagiwara* et al. (1976)
Molluscan neuronal somata	Inward rectification	Not blocked	*Gola* (1972), *Eaton* and *Brodwick* (1976)
Bullfrog sympathetic ganglion cell	Inward rectification	Not blocked	*Koketsu* and *Nakamura* (1976)
Mammalian sympathetic ganglion cell	Inward rectification	Not blocked	*Christ* and *Nishi* (1973)
Molluscan neurones			
Helix pomatia	i. Transient outward current	20–80 m*M*	
	ii. Potential-dependent	5–12 m*M*	*Neher* and *Lux* (1972)
	iii. Ca-activated	0.7 m*M*	*Hermann* and *Hartung* (1981)
Helix aspersa	i. Potential-dependent	10 m*M*	
	ii. Ca-activated	10 m*M*	*Meech* and *Standen* (1975)
Tritonia	i. Transient outward current	100 m*M*	
	ii. Potential-dependent	8 m*M*	*Thompson* (1977)
	iii. Ca-activated	Insensitive	
Aplysia	i. Potential-dependent	6 m*M* at + 20 mV	
	ii. Ca-activated	0.5 m*M* at + 20 mV	*Hermann* and *Gorman* (1981)

11.1 External and Internal Block

The action of TEA^+ on K-conductance was first investigated in squid axon, where the ion has no blocking action from the outside (*Tasaki* and *Hagiwara* 1957). This finding cannot yield a general rule, however, and where block from inside and outside has been investigated, striking differences emerge in terms of structure-activity relationships and other qualities of the block. As has been discussed (Sects. 3.1 and 7.1.1) TEA^+ appears to be the most potent of the quaternary ammonium ions when it is applied outside (*Hille* 1967a,b, *Hermann* and *Gorman* 1981), but longer chain tetraalkylammonium ions are more effective than TEA^+ at blocking from inside (*Armstrong* 1969, 1971, *Armstrong* and *Hille* 1972, *French* and *Shoukimas* 1981, *Swenson* 1981). Further, internal quaternary ammonium ions are often (but not apparently universally, *Neher* and *Lux* 1972) more effective at blocking outward than inward currents, while external TEA^+ blocks inward and outward currents more or less equally, Nor is it a general rule that internal TEA^+ is more effective than external: in the case of Ca-activated K-conductance of *Aplysia,* internal TEA^+ has little blocking action, except at positive membrane potentials (*Hermann* and *Gorman* 1981). Except perhaps in heart, where external TEA^+ may have striking effects only in the long-term (see Sect. 5.1), the simplest assumption seems to be that TEA^+ applied externally acts externally, particularly if its action has a rapid onset and is readily reversed.

11.2 Specificity of Block of TEA^+

Externally applied TEA^+ is itself reasonably specific in its block of K-channels, though some reports argue for a small action on Na-channels in the node of Ranvier (e.g., *Koppenhöfer* 1967). Internally applied TEA^+ can block Na-channels if they have been modified by pronase treatment (*Rojas* and *Rudy* 1976; Fig. 3) and longer chain tetraalkylammonium ions ($TPrA^+$) block unmodified Na-channels of squid axon (*Armstrong* 1966b). Further, in mammalian nerve, longer chain quaternary ammonium ions [dodecyltriethylammonium (C12) particularly] have recently been promoted as local anaesthetics with an ultralong (days) action (*Scurlock* and *Curtis* 1981). This action appears to be due partly to a block of Na-channels and partly to K-channels, both actions probably following penetration of the membrane (*Curtis* and *Scurlock* 1981).

Nonetheless, in most experimental situations, it is a reasonable a priori assumption that TEA^+ block indicates a K-permeability, though the absence of block clearly does not indicate permeability to some other ion.

Certainly TEA^+ can be used to distinguish K^+ from Cl^- permeability (*Stanfield* 1970b, *Kenyon* and *Gibbons* 1979a). Further although such an action has sometimes been implied (*Ushiyama* and *Brooks* 1962, *Haeusler* et al. 1980, *Zucker* and *Smith* 1979), there seems little evidence for direct enhancement by TEA^+ of Ca-permeability (*Zucker* 1981).

11.3 Pharmacological Separation of K-Permeabilities

A number of workers have used TEA^+ as one agent to help distinguish different classes of K-permeability in a given cell. *Nakajima* (1966) appears to have been the first to do this (Sect. 7). Generally these pharmacological separations are most secure when the K-channels are also separable on the grounds of their physiological properties, as in skeletal muscle where inward rectification has a lower affinity for TEA^+ than does delayed rectification (Table 2; *Stanfield* 1970a,b) but where the two are separable on grounds of the form of their instantaneous current-voltage relations and of their dependence, in the first case, on voltage and $[K]_o$ and, in the second, on voltage alone (*Hestrin* 1981, *Leech* and *Stanfield* 1981, *Adrian* et al. 1970a). Similarly, in invertebrate neurones, the three K-permeabilities described in Sect. 7.1.1 are separable first on grounds of their kinetics and dependence either on membrane potential or on $[Ca^{2+}]_i$, and secondly of their affinities for TEA^+.

So far as neuronal K-permeability is concerned, although the results of *Thompson* (1977) show that Ca-activated K-conductance of *Tritonia* (the nudibranch mollusc) is little affected by TEA^+, just as in red cells (*Armando-Hardy* et al. 1975) and apparently in vertebrate neurones (Sect. 7.2), other molluscan neurones have Ca-activated conductances which are as sensitive as (*Helix aspersa, Meech* and *Standen* 1975), or much more sensitive (*Helix pomatia, Hermann* and *Hartung* 1981; *Aplysia, Hermann* and *Gorman* 1981) than the voltage-dependent K-conductances. Similarly, in skeletal muscle, Ca-activated K-conductance is blocked by TEA^+ (*Latorre* et al. 1982).

11.4 General Significance

The discussion of invertebrate skeletal muscle (Sect. 4.2) and of smooth muscle (Sect. 6), as examples, show that the use of TEA^+ has played a substantial role in the uncovering of Ca-permeability mechanisms of excitable cells. Further, the experiments with TEA^+ on squid axon discussed in Sect. 2.2 have had an influence in electrophysiology beyond the providing of a simple demonstration of K-channel block, but have stimulated developments in a number of areas.

First, the prolongation of the action potential, with its long plateau, found in squid axon after injection of TEA^+ (*Tasaki* and *Hagiwara* 1951) led to attempts to modify the equations of *Hodgkin* and *Huxley* (1952) in ways that would account for a plateau (*FitzHugh* 1960, *George* and *Johnson* 1961) such as occurs in the cardiac action potential also (*Noble* 1962, *McAllister* et al. 1975). Secondly, the inward rectification induced in squid axon by TEA^+ injection (*Armstrong* 1966a, 1969, 1971) has led to attempts to model the physiological inward rectification which occurs, for example, in skeletal muscle fibres (*Armstrong* 1975b, *Hille* and *Schwarz* 1978, *Standen* and *Stanfield* 1978, *Urban* and *Hladky* 1979, *Cleeman* and *Morad* 1979). Inward rectification might be produced by a TEA^+-like substance in skeletal muscle fibres, blocking outward movement of K^+ (*Armstrong* 1975b). Thirdly, the modelling of the requirement that K-channels be open before they can be blocked by TEA^+ has provided a theoretical framework for dealing with situations where activation of ionic channels and their subsequent inactivation may be linked. The models for block of K-channels by quaternary ammonium ions given in Sect. 2.2 from *Armstrong* (1975a) may be compared with that given for gating and inactivation of Na-permeability suggested by *Armstrong* (1981). Further, the differences between external and internal blocking effects in frog node (*Armstrong* and *Hille* 1972) have contributed to the development of models of the architecture of Na- and K-channels (*Armstrong* 1975a, 1981, *Hille* 1973, 1975).

Thus TEA^+ has been and remains a powerful weapon in the pharmacological and intellectual armoury of electrophysiologists.

Acknowledgements. I am indebted to my colleagues, Drs. Nick Standen and Tim Plant, for their comments on the manuscript and their discussions during the writing of this paper. I also thank the Medical Research Council for a grant for purchase of Cambridge Electronic Design computer system (PDP11–23 based) on which the calculations were made; the Computing Studies Unit of the University of Leicester for allowing me the use of a word processor in the preparation of the bibliography, and Mr. William King for help in this; and Mrs. J.A. Lewis, who prepared the final typescript, and Mrs. P. Harvey for help with the typing.

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Rev. Physiol. Biochem. Pharmacol., Vol. 97

Renal Magnesium Transport

GARY A. QUAMME and JOHN H. DIRKS *

Contents

1 Introduction

Magnesium handling by the mammalian kidney is normally characterized as a filtration-reabsorption system. Although magnesium secretion has been reported by a number of laboratories, further information from direct studies is required. Micropuncture studies of accessible tubular segments have indicated similar patterns of magnesium handling along the nephron in all species investigated to date. Experiments using direct micropuncture of surface glomeruli of the Munich-Wistar rat indicate that about 70%–80%

* Department of Medicine, Health Sciences Centre, Acute Care Unit, University of British Columbia, Vancouver, British Columbia/Canada

of the plasma magnesium is filtered at the glomerular membrane (*Brunette* and *Crochet* 1975; *LeGrimellec* et al. 1975). Free-flow micropuncture studies have demonstrated that the tubular fluid to ultrafilterable plasma concentration ratio (TF/UF) for magnesium increases along the proximal tubule as water is abstracted (*Morel* et al. 1969). This is in contrast to the concentrations of the other major cations, which remain close to that of plasma, and results in tubule fluid entering the loop of Henle with a magnesium concentration greater than the glomerular filtrate. Tubular fluid obtained from the early distal tubule contains significantly less magnesium than the glomerular filtrate, indicating that the loop of Henle is the major nephron segment reclaiming a significant portion of the filtered load. Comparison of the composition of the tubular fluid sampled at the bend of the descending limb of Henle's loop with that obtained from the early distal tubule indicates the importance of the thick ascending limb in reabsorption of the magnesium delivered distally (*De Rouffignac* et al. 1973; *Brunette* et al. 1974). The terminal segments of the nephrons, encompassing the distal convoluted tubule and collecting duct, reabsorb only a small portion of the filtered magnesium (Fig. 1). Thus, the overall reabsorption pattern for magnesium is characterized by functional heterogeneity along the various tubular segments. Moreover, although the transport of magnesium shares a number of characteristics with sodium and calcium reabsorption, a number of influences affect the transport of magnesium quite differently from that of these two cations.

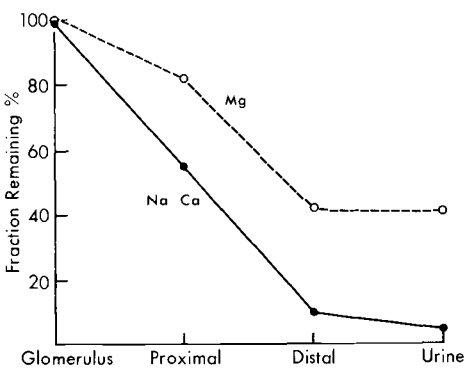

Fig. 1. Summary of the tubular handling of magnesium, calcium, and sodium in the rabbit. Free-flow micropuncture studies were performed on euvolemic rabbits maintained on normal rabbit chow. *Fraction remaining* indicates the percent of the filtered electrolyte which was not reabsorbed at the collection sites. Urinary magnesium excretion is markedly greater in the rabbit (45%) than in other species (5%–15%), presumably due to the higher dietary magnesium. As indicated, the loop of Henle reabsorbs the major fraction of the filtered magnesium. Tubular calcium handling is similar to that of sodium

2 Segmental Magnesium Transport

2.1 Glomerular Filtration

The ultrafilterable portion of the plasma magnesium is 70%–80% as determined by direct sampling of fluid from surface glomeruli of Munich-Wistar rats (*Brunette* and *Crochet* 1975; *LeGrimellec* et al. 1975) and by the use of artificial membranes (*Massry* et al. 1969; *Brunette* et al. 1974; *Carney* et al. 1980). In comparison the ultrafilterable plasma calcium is 60%–65% (*Massry* and *Coburn* 1973). Of the filterable magnesium, only 70%–80% is thought to be in the ionic form (Mg^{2+}), the remainder being largely complexed to anions, particularly phosphate, citrate, and oxalate (*Walser* 1973). The ultrafilterability of plasma magnesium, as determined by in vitro techniques, is not affected by magnesium deficiency, magnesium excess (*Carney* et al. 1980), or elevation of plasma calcium concentration (*Coburn* et al. 1970). Other factors that may directly affect in vivo glomerular ultrafilterability, such as alterations of plasma anion and protein constituents, remain to be evaluated.

2.2 Proximal Convoluted Tubule

Magnesium has a unique concentration profile along the length of the proximal convoluted tubule; the luminal concentration rises sharply with respect to the ultrafilterable magnesium (Fig. 2). *Morel* et al. (1969) were the first investigators to demonstrate this in the rat. The mean TF/UF magnesium ratio was 1.7 at a point in the nephron where 50%–55% of sodium

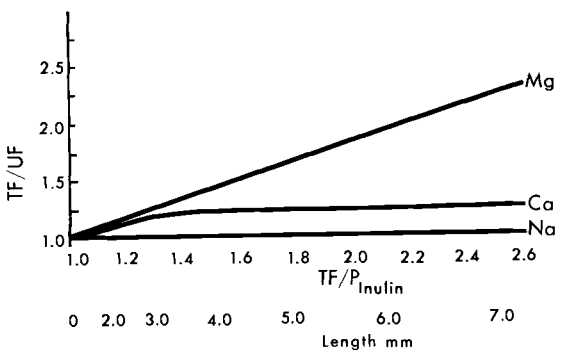

Fig. 2. Typical concentration profiles for magnesium, calcium, and sodium along the superficial proximal convoluted tubule of the dog. Other animal species studied to date, including rodents, monkeys, and rabbits, demonstrate similar proximal reabsorption patterns

Table 1. Segmental reabsorption of magnesium, calcium, and sodium under normal conditions

Nephron segment	Tubular fluid to plasma ratio			Fraction of filtered load remaining (%)		
	(TF/P)Na	(TF/UF)Ca	(TF/UF)Mg	Na^+	Ca^{2+}	Mg^{2+}
Proximal convoluted tubule	1.0	1.1	1.5	50	55	75
Descending limb of loop of Henle	2.5	2.5	4.0	45	45[a]	70–100[a]
Early distal tubule	0.4	0.4	0.6	10	10	12
Late distal tubule	0.25	0.25	0.8	5	5	10
Urine				<1	<1	10

[a] Controversial

Data are composite values from *Morel* et al. (1969), *de Rouffignac* et al. (1973), *Brunette* et al. (1974, 1975), *Quamme* et al. (1978), *Jamison* et al. (1979) and represent mean approximations at the end of the respective accessible nephron segments.

and water had been reabsorbed (*Morel* et al. 1969). This appears to be a common phenomenon in all species studied to date; it has been demonstrated in rats (*Morel* et al. 1969), desert rats (*Psammomys obesus*) (*De Rouffignac* et al. 1973), hamsters (*Harris* et al. 1979), *Perognathus penicillatus* (*Braun* et al. 1982), dogs (*Quamme* et al. 1978), monkeys (*Wong* et al. 1981b), and rabbits (*Whiting* et al. 1982). Thus, the overall fractional magnesium reabsorption in the proximal tubule is some 20%–30% of the filtered load, which is about 50%–70% the fractional reabsorption rate of sodium, potassium, and calcium (Table 1). The profile for the TF/UF magnesium ratio relative to the inulin concentration remains largely unchanged in a variety of experimental circumstances, including volume expansion, diuretic administration, metabolic acidosis and alkalosis, and changes in plasma levels of calcium and phosphate. Overall magnesium reabsorption in the proximal tubule closely follows changes in salt and water reabsorption, but at a lower fractional rate.

The observations that the tubule fluid magnesium may be 1.5-fold greater than the plasma concentration suggests that the proximal tubular epithelium possesses a low permeability for magnesium relative to sodium or calcium. Recently, a detailed analysis of magnesium transport in the proximal tubule was made using in vivo microperfusion techniques (*Quamme* and *Dirks* 1980). Tubules were perfused with Ringer solution containing various amounts of magnesium chloride, and magnesium was

measured by electron microprobe analysis of the samples collected downstream from the perfusion site. Absolute magnesium concentration increased along the perfused tubule in a linear manner with net water reabsorption, similar to that observed in free-flow micropuncture studies (Fig. 2). The collected to perfusate concentration ratio of magnesium rose whether luminal magnesium concentrations were markedly below or even very significantly above (tenfold) the ultrafilterable plasma magnesium level, indicating a relatively poor permeability of the superficial proximal tubule to magnesium. Further studies directed specifically at determining the level of backflux of magnesium from the peritubular compartment into the lumen revealed a very low level of magnesium backflux (*Quamme* 1980b). This latter observation was compatible with previous studies in which little isotopic magnesium was detected in the lumen following microinjections of ^{28}Mg into the superficial peritubular capillaries (*Brunette* and *Aras* 1971). A number of factors have been shown to increase permeability of the proximal tubule, including extracellular volume expansion. The movement of magnesium from the peritubular capillary into the lumen increases after volume expansion with saline infusions (*Quamme* 1980b). However, the backflux remains proportionately low relative to that of sodium and calcium (*Frick* et al. 1965). Bearing on this point are the observations in the proximal tubule of the dog subsequent to mannitol administration (*Wong* et al. 1979). Plasma osmolality was elevated from 295 to 395 mosmol/kg H_2O by infusion of hypertonic mannitol solutions. The late proximal tubule TF/P inulin ratio fell from 1.66 to 1.41 and TF/UF sodium decreased from 1.03 to 0.93; TF/UF calcium decreased from 1.06 to 0.97, and TF/UF magnesium fell from 1.43 to 1.04. Thus the fraction of sodium and calcium remaining at the proximal sampling site increased by 4%, and magnesium fell by 12%. This may be explained by a greater increase in plasma to lumen flux of sodium and calcium compared with magnesium. Alternatively, tubular permeability of magnesium may have increased, which would allow for a greater diffusional efflux of magnesium relative to sodium and calcium. Unidirectional magnesium flux and permeability have not been studied in proximal convoluted tubules, and an exact definition of active and passive transport mechanisms remains to be determined.

In the above in vivo perfusion studies (*Quamme* and *Dirks* 1980), it was demonstrated that magnesium reabsorption is dependent on intraluminal magnesium concentration. Perfusion of rat proximal tubules with solutions containing magnesium in excess of 5–7 m*M* failed to saturate the transport system. Further studies in dogs given magnesium chloride infusions support these observations (*Wong* et al. 1983). Graded elevations of plasma magnesium resulted in enhanced absolute magnesium reabsorption (Fig. 3). In summary, magnesium transport by the superficial proximal

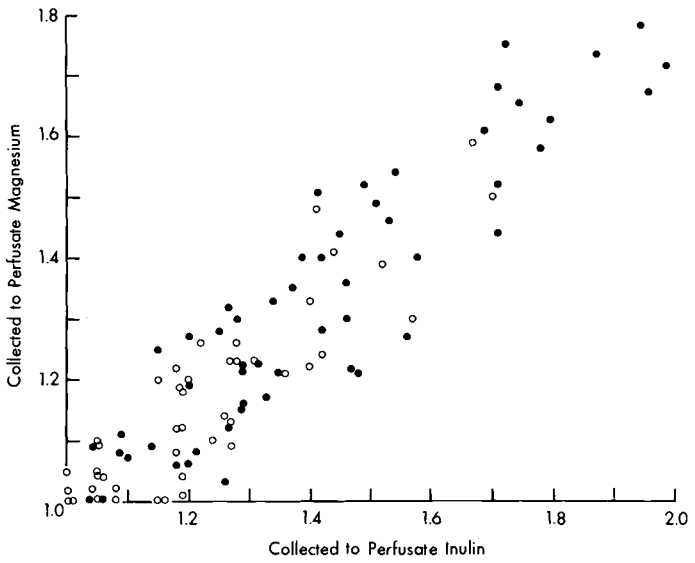

Fig. 3. Intraluminal magnesium concentration as a function of water reabsorption along the proximal tubule. Tubules were perfused in vivo with Ringer solutions containing 0.5 m*M* magnesium (○) or 5 m*M* magnesium (●). Magnesium concentration increased along the perfused tubule with net water abospriton whether the intraluminal concentration was lower or higher than the existing plasma magnesium concentration

tubule is mainly a unidirectional process and is dependent on the luminal or filtered magnesium concentration. Magnesium is reabsorbed at a relatively lower rate than sodium, although proportional to salt and water transport. Other factors which influence proximal sodium transport, such as inhibition of sodium-hydrogen ion exchange, have not been directly investigated.

It is relevant to speculate on some of the basic properties of magnesium in relation to calcium. The proximal tubule appears to be highly permeable to calcium, as it is to sodium (*Frick* et al. 1965; *Windhager* 1979). Evidence suggests that this is related to the nature of the paracellular pathway. *Williams* (1970) has summarized some of the distinguishing aspects of magnesium ions in aqueous solutions as compared with calcium, sodium, and potassium. The coordination number of magnesium is 6, whereas that of calcium is 8. Thus the Mg^{2+} ion is relatively larger and the free-solution mobility is only about two-thirds that of calcium and sodium. This may account for the lower convective movement of magnesium in comparison to calcium and sodium. Magnesium also differs in its intrinsically lower electrostatic energy, which may affect its permeation through a cationic selective pathway. *Sherry* (1969) predicted possible potency sequences of alkaline-earth cations by comparing differences in cationic electrostatic interaction energies with membrane negative sites. By varying site charge or spacing, *Sherry* predicted an interaction sequence of Ca^{2+} >

$Ba^{2+} > Sr^{2+} > Mg^{2+}$ on Na^+ movement. *Diamond* and *Wright* (1969) showed that the effects of these cations on most biological systems conform to this sequence and suggested that they may reflect the molecular structure of the site controlling permeation in the junctions of leaky epithelia and of the entry site in the apical cell membrane of tight epithelia. The permeability properties of magnesium and its relationship to sodium transport in the proximal tubule are not unique. For instance, the proximal epithelium is relatively impermeable to solutes such as glucose, amino acids, and electrolytes such as phosphate (*Deetjen* and *Boylan* 1968; *Loeschke* et al. 1969; *Dennis* et al. 1976). The reabsorption rates for these substances are all dependent on sodium and water transport in the proximal segment. The relationship appears to be highly dependent on the respective luminal concentration, which is thought to be the basis for the glomerulotubular balance observed for these solutes. This also appears to be the case for magnesium, i.e., an increase in filtration rate and the attendant sodium absorption leads to an increase in luminal magnesium concentration and magnesium transport. Transepithelial magnesium movement may be either transcellular or paracellular; the magnitude of both pathways is dependent on luminal concentration.

2.3 Proximal Straight Tubule and Thin Descending Limb of Henle's Loop

Present knowledge of magnesium transport by the proximal straight tubule is indirect and has come from evaluating the fluid collected by micropuncture from the bend of the loop of the surgically exposed papilla of young rats or desert rodents. Interpretation of comparisons of results derived from collections at the tip of the loop with collections made from the superficial proximal tubules is based on the assumption that the characteristics of the superficial nephrons are the same as the deep nephrons. These results principally describe events occurring in the deep nephrons. *De Rouffignac* et al. (1973) examined fluid collected from the late superficial proximal tubule and the tip of the long loops in *Psammomys* (*Jamison* et al. 1979). They determined that there was greater fractional magnesium concentration present at the end of the deep descending limb than at the end of the superficial proximal tubule. Since the rise in intraluminal magnesium correlated with osmolality of the tubule fluid rather than with water reabsorption, they suggested a net entry of magnesium in some segment prior to the hairpin turn of the loop. Presumably, the net entry of magnesium occurred either in the straight portion of the proximal tubule (pars recta) or the thin descending limb of the loop. Furthermore, the concentration of sodium, potassium, chloride, and to a lesser extent calcium

also rose towards the bend of the loop and correlated more strongly with the rise in osmolality than with water abstraction. It was proposed that the rise in electrolyte concentration, including that of magnesium, could be explained by entry into the pars recta or descending limb, with subsequent removal by the thick ascending limb and possibly the distal tubule, i.e., a process of medullary recycling (*De Rouffignac* et al. 1973). *Brunette* et al. (1974) observed little addition of magnesium up to the bend of papillary loops of normal rats. However, in rats given large infusions of $MgCl_2$, magnesium was present in amounts greater than could be accounted for by filtration and water reabsorption, which suggested net secretion of magnesium from the vasa recta or interstitium into the lumen of the straight proximal tubule or thin descending limb of the juxtamedullary nephrons (*Brunette* et al. 1975, 1978). This has not been supported by other studies on the superficial or cortical loop. Superficial loops were perfused, in vivo, from the late proximal convoluted tubule with magnesium-free solutions (*Quamme* and *Dirks* 1980). Little magnesium was detected in the fluid collected from the early distal tubule, suggesting that the epithelium of short cortical loops of the rat is relatively impermeable to magnesium. More recently, we have investigated magnesium transport in isolated straight proximal segments of the rabbit (*Quamme* 1982a). In vitro perfusion of superficial or juxtamedullary tubule segments with magnesium-free solutions resulted in little influx of magnesium from the bath to the lumen. Moreover, secretion was not detected when the bath magnesium concentration was elevated to 3.0 m*M*. The isolated straight proximal segments of the rabbit absorbed magnesium when they were perfused with artificial solutions similar to late proximal fluid, and the collected to perfusate magnesium concentrations rose with water abstraction, not unlike the rise observed for the proximal convoluted tubule. Thus, the reabsorption rate for magnesium is fractionally lower compared with the transport rates of water and sodium in both the cortical and juxtamedullary straight proximal tubules of the rabbit. In summary, the straight proximal tubule of the loop possesses a relatively high reflection coefficient for magnesium; reabsorption is small and is dependent on the delivery rate (luminal magnesium concentration) and the concurrent sodium transport rate.

Magnesium transport in the thin descending limb remains to be directly investigated with in vitro perfusion techniques; however, the solute permeabilities of the thin limbs of the rat and rabbit appear to be quite different, and species differences may explain some of the discrepancies mentioned above (*Jamison* 1981). Although no active transport has been found in the rat or rabbit thin descending limb, the rat descending tubule is much more permeable to sodium and urea than the rabbit descending limb. This could allow for diffusional flux of magnesium from the interstitium into

the lumen and account for the above observations in the rat and *Psammomys*. To test this idea, a precise evaluation of the diffusible magnesium concentration in the vasa recta and interstitium is required. The cortical to papillary concentration gradient for magnesium, unlike calcium and sodium, has not been well-delineated due to the rather large contribution of intracellular magnesium to total tissue magnesium (*Ullrich* and *Jarausch* 1956). *Brunette* et al. (1978) estimated the diffusible magnesium in the papillary vasa recta by determining the albumin concentration differences between vasa recta plasma obtained from rat papilla and that found in the circulating plasma. They found that the magnesium concentration was about twofold greater in the vasa recta than in the peripheral plasma, but lower than in the corresponding tubular fluid in the loop of Henle, implying net magnesium secretion. This secretion may be active, depending on the concurrent voltage. Confirmation of these observations, particularly with in vitro perfusions, would explain many reports suggesting that the deep long-loop nephrons contribute more magnesium to the final urine than the short, superficial loops (*LeGrimellec* et al. 1973b).

2.4 Ascending Limb of Henle's Loop

The early micropuncture studies of *Morel* and colleagues indicated that the loop of Henle was the major site of magnesium reabsorption (*Morel* et al. 1969; *LeGrimellec* et al. 1973a). Magnesium concentration in the early distal tubule fluid was distinctly lower than the ultrafiltrerable magnesium concentration, with a TF/UF ratio of 0.5–0.6. Hence some 50%–60% of the filtered magnesium was reabsorbed between the last accessible proximal tubule on the surface of the kidney and the early distal tubule. This represents a fractional reabsorption rate greater than that of sodium and calcium, based on the amount filtered at the glomerulus. More recent micropuncture studies by *Brunette* et al. (1974) in the rat and *De Rouffignac* et al. (1973) in *Psammomys* showed an increase in the mean TF/UF magnesium concentration at the bend of the descending limb of the loop, which indicated that the major site of the marked magnesium reabsorption must be located in the ascending limb of Henle's loop, most likely in the thick segment. Magnesium transport in the thin ascending limb has not been investigated. If one assumes that the thin ascending limb has relatively lower permeability to magnesium than the other tubular segments investigated, then it may be expected that magnesium delivery to the thick ascending segment would be greater than that of sodium and other solutes. This may be the basis for the differential transport of magnesium, relative to other cations in the loop, observed under a number of influences (*Quamme* 1981). A recent report by *Shareghi* and *Agus* (1979a), 1982b),

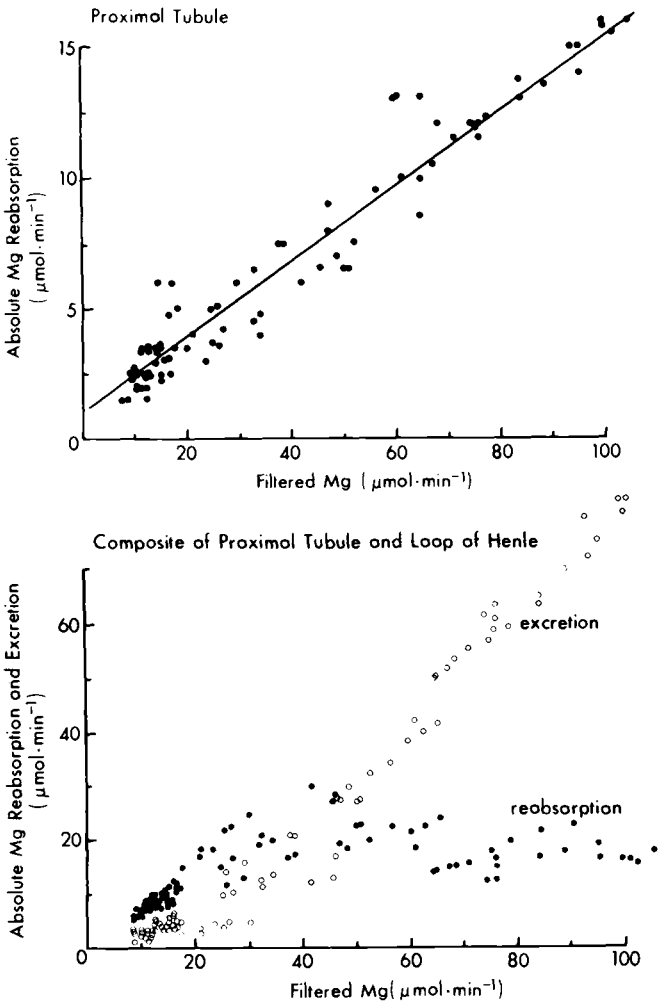

Fig. 4. Segmental magnesium reabsorption in the dog.
Thyparathyroidectomized dogs were given $MgCl_2$ infusions to determine the tubular transport capacity for magnesium. Magnesium reabsorption increased with filtration rate in the proximal convoluted tubule, with no evidence for saturation of the tubular reabsorption system. Magnesium transport initially increased in the loop of Henle with enhanced delivery from the proximal tubule, reached a maximum, and sharply fell with elevated plasma magnesium. The composition of proximal and loop reabsorption provides a maximal tubular reabsorption pattern (Tm). Thus the overall kidney demonstrates an apparent Tmax for magnesium reabsorption

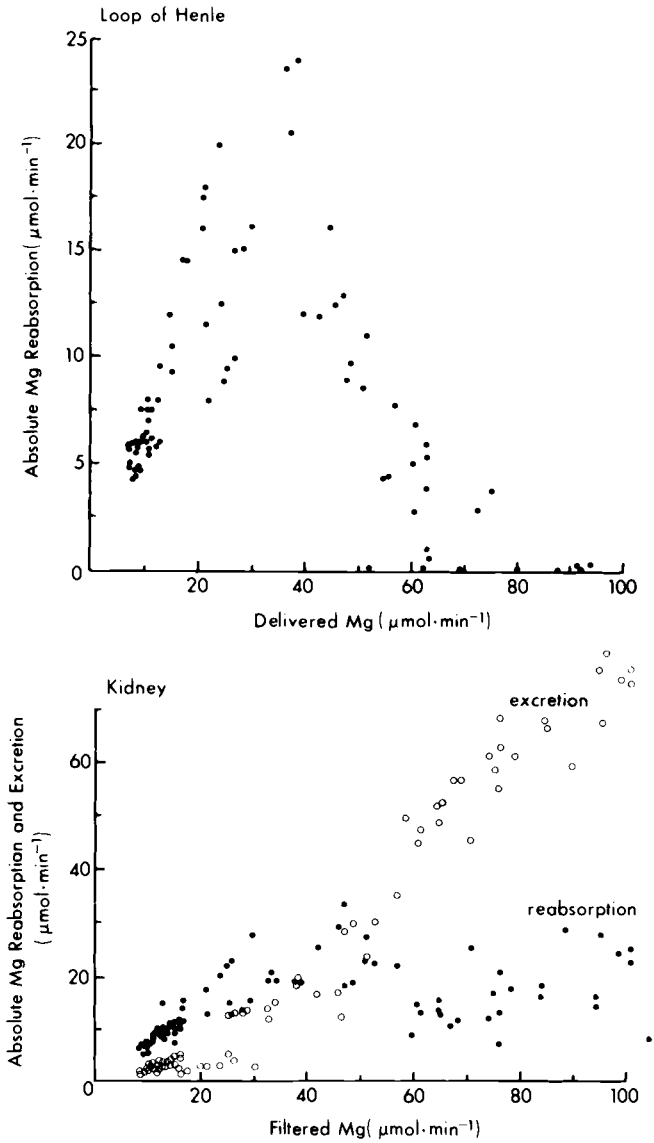

Fig. 4

using the isolated tubule perfusion technique, has confirmed the marked voltage-dependent magnesium transfer in the thick ascending limb.

We have recently examined, by in vivo perfusion techniques, magnesium reabsorption with the loop of Henle – which admittedly also included the straight portions of the proximal tubule and segments of the thin ascending limb, as well as the thick ascending limb (*Quamme* and *Dirks* 1980). It seems likely, however, that the major changes in transport that

were observed occurred within the thick ascending limb. Perfusion of the cortical short loops of rats, with magnesium-free solutions, led to little influx of magnesium under conditions of hydropenia and hypermagnesemia. Thus the loop of Henle was shown to be relatively impermeable to magnesium, not unlike the proximal tubule. Further experiments, in which the loop was perfused at constant flow rates with elevated magnesium concentrations and in the presence of normal plasma magnesium, provided evidence that the loop of Henle has a remarkable capacity to increase its absolute reabsorption with increased delivery. There was no indication of a saturation of magnesium transport (Tm) when the luminal magnesium concentrations were increased from 0 m*M* to 5 m*M*. In fact, there was a constancy of reabsorption approximating 80% of the delivered load at any level of luminal magnesium concentration; this was not unlike the fractional reabsorption rates of sodium and calcium. In contrast to changes in luminal magnesium concentration, elevation of plasma magnesium concentration (i.e., on the basolateral membrane) resulted in marked depression of magnesium absorption. As plasma magnesium concentration was progressively raised, absolute and fractional magnesium reabsorption fell sharply, suggesting that hypermagnesemia retards magnesium reabsorption in the loop of Henle by inhibition of magnesium transport from the basolateral membrane. These observations provide the basis for the Tm for magnesium which has previously been reported for magnesium reabsorption (Fig. 4). *Massry* et al. (1969) progressively elevated the plasma magnesium concentration in dogs and performed standard clearances. Overall kidney reabsorption of magnesium initially increased, reached an apparent saturation point, and at extreme levels appeared to fall. This phenomenon suggested a tubular maximum transport process for magnesium. However, free-flow micropuncture studies in dogs subjected to progressive hypermagnesemia indicated that the apparent Tm of magnesium transport may be a fortuitous composite of increasing proximal tubule magnesium reabsorption and, at the same time, progressively declining reabsorption in the loop with advancing hypermagnesemia; the latter observation reflects an action of magnesium from the basolateral side of the tubule. *Shareghi* and *Agus* (1982b) have recently supported these observations using the technique of in vitro perfusion of isolated rabbit tubules. They reported that elevation of bath magnesium significantly decreased magnesium transport in the cortical thick ascending limb segment, whereas elevation of luminal magnesium concentration increased magnesium absorption. These studies confirmed the earlier in vivo observations in the rat. It was of interest that hypermagnesemia also markedly inhibited calcium but affected sodium reabsorption only to a small extent. However, this was not observed in the isolated rabbit tubule (*Shareghi* and *Agus* 1982b). It has been amply demonstrated in the dog and rat that hyper-

calcemia inhibits magnesium reabsorption (*Coburn* et al. 1970; *Massry* and *Coburn* 1973; *Walser* 1973). With in vivo microperfusion studies in the rat it was observed that hypercalcemia inhibited both magnesium and calcium reabsorption in the loop of Henle (*Quamme* 1982b). Thus in the dog and rat there is significant suggestive evidence of a calcium-magnesium interaction from the basolateral side of the ascending limb cell that is not directly related to sodium chloride reabsorption. Generally, high plasma calcium and high plasma magnesium retard magnesium reabsorption more than that of calcium. Further studies are required to determine the difference in the rabbit in comparison with other species. Moreover, the definitive mechanism by which extracellular calcium and magnesium inhibit reabsorption is not known.

In addition to alterations in peritubular magnesium and calcium concentrations, magnesium reabsorption within the loop is influenced by the existing sodium chloride transport. Normally, changes in sodium chloride transport in the loop of Henle are reflected by proportional changes in magnesium reabsorption. Sodium chloride reabsorption in the loop of Henle, and presumably the thick ascending limb, may be altered by changes in the luminal flow rate and sodium chloride transport. Table 2 presents the results of altering each of these factors independently (Fig. 5). Superficial loops were perfused in vivo either at high perfusion rates (50 nl $\cdot$ min^{-1}) or with furosemide (10^{-6} *M* perfused at 25 nl $\cdot$ min^{-1}), and compared with control perfusions (25 nl $\cdot$ min^{-1}). Transport rates for sodium, calcium, and magnesium were determined by comparing deliveries to the early distal tubular collection site with those observed at the late proximal tubule. Elevation of perfusion rate and delivery resulted in an increase in absolute sodium, calcium, and magnesium absorption; however, fractional reabsorption fell for all three electrolytes (Figs. 5, 6). Moreover, fractional magnesium and calcium absorption was significantly less than that of

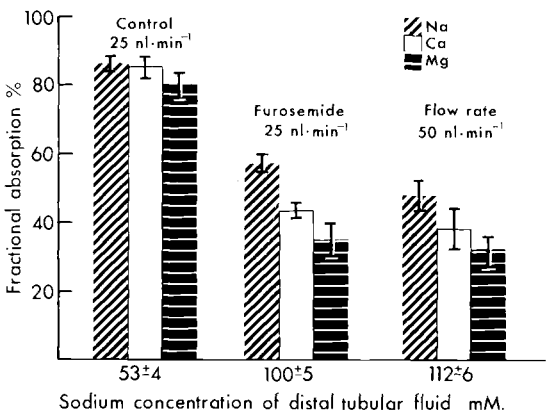

Fig. 5. Fractional sodium, calcium, and magnesium absorption as a function of luminal sodium chlroide concentration. Sodium concentration was elevated either through inhibition of active sodium chloride transport with furosemide (10^{-6} *M*) or by increasing the flow rate into the loop. Calcium and magnesium absorption were dependent on the sodium gradient regardless of the method of altering the salt gradient

Table 2. Effect of flow rate and voltage on the net absorption of sodium, calcium, and magnesium in Henle's loop

	Sodium				Calcium			
	Absolute delivery (p*M* · min^{-1})	Net absorption (p*M* · min^{-1})	Fractional absorption (%)	Distal [Na] (m*M*)	Absolute delivery (p*M* · min^{-1})	Net absorption (p*M* · min^{-1})	Fractional absorption (%)	Distal [Ca] (m*M*)
Control 25 nl · min^{-1}	2982 ± 154	2596 ± 167	86 ± 1	53	26.3 ± 2.0	22.4 ± 2.1	85 ± 3	0.42
(*n*)	(18)							
Control 50 nl · min^{-1}	6571 ± 2332	3215 ± 309	48 ± 4	112	53.7 ± 4.1	20.4 ± 3.2	38 ± 4*	1.18
(*n*)	(18)							
Furosemide 25 nl · min^{-1}	2946 ± 98	1643 ± 60	57 ± 2	98	23.9 ± 1.1	10.2 ± 0.8	43 ± 2*	0.87
(*n*)	(34)				(31)			

	Magnesium			
	Absolute delivery (p*M* · min^{-1})	Net absorption (p*M* · min^{-1})	Fractional absorption (%)	Distal [Mg] (m*M*)
Control 25nl · min^{-1}	16.0 ± 0.7	12.8 ± 0.8	80 ± 3	0.33
(*n*)				
Control 50 nl · min^{-1}	29.5 ± 2.1	9.4 ± 1.3	32 ± 4*	0.70
(*n*)				
Furosemide 25 nl · min^{-1}	25.6 ± 0.6	5.5 ± 0.8	35 ± 5*	0.95
(*n*)	(16)			

The perfusion rate was either 25 nl · min^{-1} or 50 nl · min^{-1} as indicated. Net absorption is the net absolute absorption and Fract. absorption indicates the fraction of the delivered load absorbed expressed as a percentage. * indicates significance ($P < 0.05$) from control

sodium (Fig. 5). Fractional magnesium and calcium absorption was also less than that of sodium following a half-maximal inhibitory concentration of furosemide. The sodium concentration was elevated similarly by either doubling the flow rate or inhibiting by half the sodium absorption. If the concentration of sodium in the distal tubular fluid reflects the transepithelial potential difference, then alteration of either the flow rate or active sodium chloride transport may lead to a decrease in magnesium and calcium absorption. These observations are readily explained if magnesium and calcium transport are passive and dependent on the sodium chloride-generated transepithelial potential. This may not be true, however, and separate active transport mechanisms must be considered (*Rocha* et al. 1977; *Suki* et al. 1980; *Imai* 1978). Moreover, magnesium and calcium are divalent cations, and presumably the transmembrane electrical potential difference would have a greater effect on these cations. This latter property may account for the greater effect on magnesium and calcium compared with sodium and, in turn, may reflect a lower limiting gradient for magnesium transport at a given transepithelial potential difference. This probably accounts in part for the magnesuria observed following maneuvers such as volume expansion. Any manipulation which alters sodium chloride transport within the ascending limb of Henle's loop affects magnesium transport more than sodium and calcium transport (*Massry* and *Coburn* 1973). For instance, furosemide inhibits sodium chloride transport but has a greater effect on magnesium absorption (*Quamme* 1981). This may be due to greater delivery of magnesium than sodium from the thin ascending limb or to a lower permeability of magnesium relative to calcium and sodium in the diluting segment. Thus at any given transmembrane voltage, magnesium transport would be proportionately less than sodium transport.

Since the thick ascending limb is the principle segment involved in control of magnesium transport, we will consider some of the cellular events that may occur in this segment even though further information is required to detail the transcellular movement precisely. For example, we do not know the magniutde, if any, of the bidirectional magnesium flux, nor do we know the intracellular ionic concentrations which would allow an analysis of transmembrane electrochemical gradients of magnesium. Nevertheless, the micropuncture and microperfusion data are suggestive of certain aspects of magnesium transport in the ascending limb. The transmembrane voltage has been demonstrated to be positive in the cortical (*Burg* and *Green* 1973) and medullary (*Rocha* and *Kokko* 1973) thick ascending limb. It is uncertain whether magnesium transport is voltage-dependent; however, a recent report suggests that in the isolated cortical ascending limb transport is altered by changes in transepithelial potential (*Shareghi* and *Agus* 1979a, 1982b). Relevant to this issue is

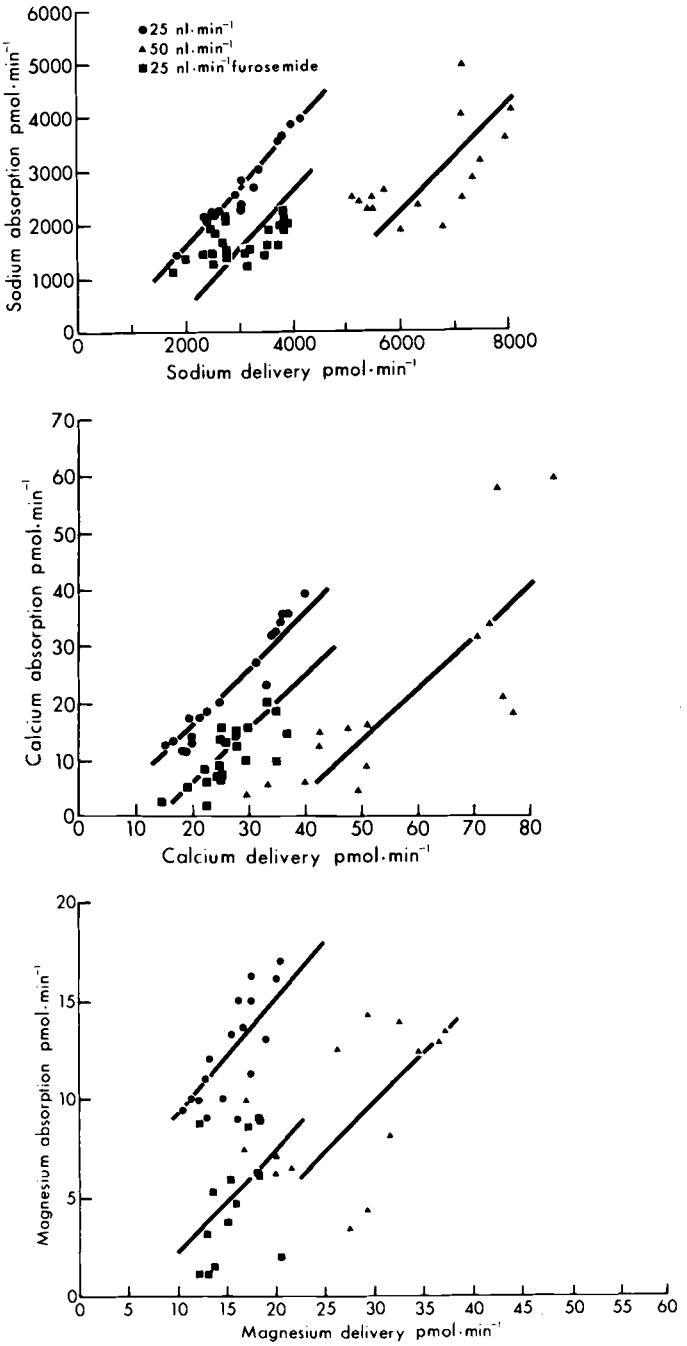

Fig. 6. Sodium, calcium, and magnesium absorption as a function of delivery rate. Loops of Henle were perfused in vivo with Ringer solutions and the luminal salt gradient, reflected by distal tubular fluid sodium concentration, was altered by addition of 10^{-6} *M* furosemide in the perfusate or by increasing the flow rate (see text for details of perfusion experiments)

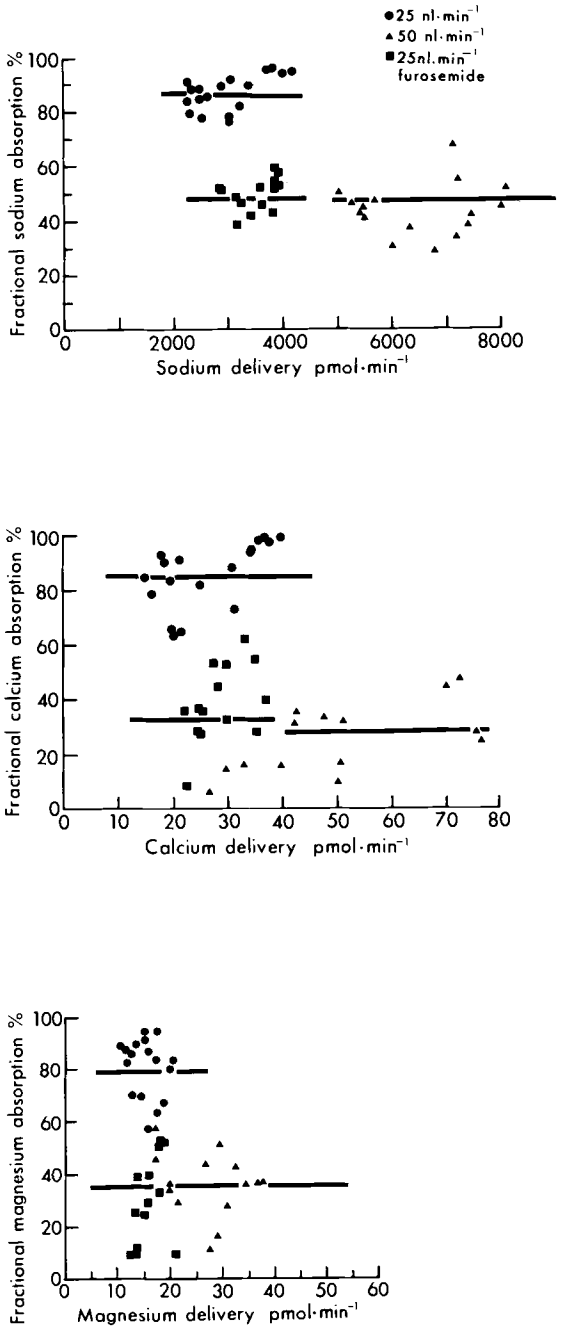
●25 nl·min⁻¹
▲50 nl·min⁻¹
■25nl.min⁻¹ furosemide
Fractional sodium absorption %
100
80
60
40
20
0
0
2000
4000
6000
8000
Sodium delivery pmol·min⁻¹
Fractional calcium absorption %
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
Calcium delivery pmol·min⁻¹
Fractional magnesium absorption %
100
80
60
40
20
0
0
10
20
30
40
50
60
Magnesium delivery pmol·min⁻¹

Fig. 6

whether calcium transport in this segment is active or passive. Calcium transport has been reported to be passive and voltage-dependent (*Shareghi* and *Stoner* 1978; *Bourdeau* and *Burg* 1979), or active (*Rocha* et al. 1977; *Imai* 1978). The major differences in these reports revolve principally around the issue of the presumed magnitude of single-file diffusion (*Bourdeau* and *Burg* 1980). More recently, *Suki* et al. (1980) have demonstrated active calcium transport in cortical thick ascending segments and passive voltage-dependent transport in the medullary segments, suggesting axial heterogeneity of calcium transport in the loop. The active calcium transport was clearly greater than any possible single-file diffusion and was not affected by furosemide, a potent inhibitor of sodium chloride transport. Clouding this issue further is the question of the mechanism involved in the generation of the intraluminal positive voltage. The initial in vitro perfusion experiments indicated that active chloride transport was the basis for the positive potential (*Burg* and *Green* 1973; *Rocha* and *Kokko* 1973). This conclusion was based on the existence of a positive potential difference in the presence of chloride and absence of sodium. More recent evidence, however, suggests that chloride transport is "secondary-active" and that the transepithelial potential is determined by an electrogenic, luminal sodium chloride transfer and a sodium diffusional potential (*Eveloff* et al. 1980; *Greger* 1981a, b; *Hebert* et al. 1981). The positive luminal voltage may be the basis for the paracellular movement of calcium and magnesium from the lumen to the basolateral compartment (*Bourdeau* and *Burg* 1979; *Shareghi* and *Agus* 1982b). However, magnesium absorption may be transcellular – either active or dependent on sodium chloride transport. The rate-limiting step in this transfer is unknown. Two interesting observations pertain to these speculations. First, the loop diuretic furosemide inhibits calcium and magnesium as well as sodium chloride in the loop of rats (*Duarte* 1968; *Quamme* 1981), dogs (*Edwards* et al. 1973), and rabbits (*Bourdeau* and *Burg* 1979; *Whiting* et al. 1982). This would suggest some dependence of calcium and magnesium transport on sodium chloride absorption. Furthermore, these cations, particularly magnesium, are inhibited to a greater degree than sodium chloride. This may suggest that the permeability coefficient for magnesium is lower than that of sodium at any given voltage setting for sodium chloride transport and that magnesium reabsorption is proportionately less than that of sodium. Second, increased flow rates into the thick ascending segment, or extracellular volume expansion, may alter the sodium diffusional gradient and the concurrent transmembrane voltage. Indeed, extracellular volume expansion leads to a greater fractional excretion of magnesium than sodium; for instance, a fractional sodium excretion increase from 1% to 2% is usually accompanied by a 10%–15% increase in magnesium excretion. As

mentioned above, this is probably due to a decrease in fractional sodium chloride transport and transmembrane voltage.

We can speculate further on the mechanisms involved in transcellular magnesium movement across the luminal and basolateral membranes (Fig. 7). Magnesium may cross the luminal membrane in a mediated manner, down its electrical gradient, which is established by the active Na-K ATPase system on the basolateral membrane. It is not known whether magnesium moves in the divalent form. Transfer of magnesium across the basolateral membrane is against its electrochemical gradient and must therefore be active or secondary-active transport. Both these situations are schematically illustrated in Fig. 7. Evidence from studies of other membranes, including the basolateral membrane of the proximal cell, has provided support for a sodium-calcium exchange mechanism (*Baker* 1976; *Blaustein* 1974; *Ullrich* et al. 1976; *Taylor* and *Windhager* 1979; *Gmaj* et al. 1979). This may also apply to magnesium transport in the loop, although at present there is no a priori evidence that this may occur. Factors which influence transcellular magnesium flux may involve (a) effects to the luminal membrane, as with furosemide; (b) effects on the electrochemical potential, such as the alteration of Na-K ATPase activity with ouabain or alteration of the intracellular magnesium pool; and (c) direct effects on the active or secondary-active transport across the basolateral membrane, such as hypermagnesemia and hypercalcemia.

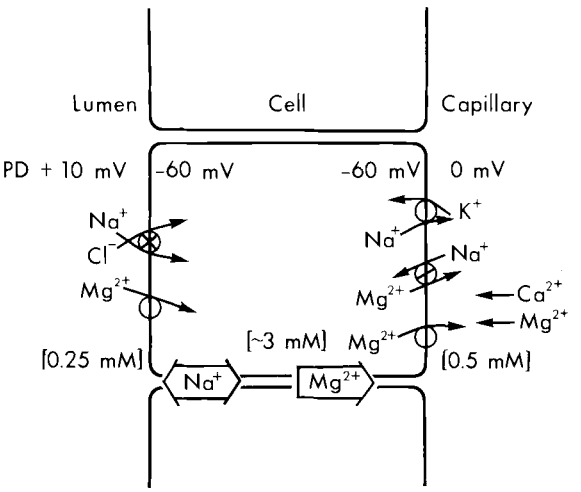

Fig. 7. Speculative model for magnesium transport by thick ascending limb of Henle's loop. *Arrows* indicate putative site of magnesium inhibition by extracellular calcium or magnesium. Interrelationships are discussed in the text

2.5 Distal Convoluted Tubule and Collecting Duct

Studies of the superficial distal convoluted tubule have used comparisons of the fractional magnesium deliveries to early and late collection sites, grouped according to TF/P inulin concentration ratios of randomly selected nephrons (*Carney* et al. 1980; *Harris* et al. 1979; *Wong* et al. 1981a), or comparisons of early and late puncture sites of the same nephron (*Brunette* et al. 1975; *Quamme* 1980a, 1981; *Quamme* and *Dirks* 1980). The TF/UF magnesium concentration ratio has been observed to rise along the accessible distal tubule, but usually less than that of inulin. Under normal conditions the distal convoluted tubule has the capability of reabsorbing a small but still significant portion of delivered magnesium, accounting for approximately 2%–5% of the filtered magnesium. However, fractional reabsorption is about one-third of either fractional sodium or calcium reabsorption in the distal tubule (*Quamme* 1980a; *Quamme* and *Dirks* 1980). We have recently shown that the transport of magnesium within the distal convoluted tubule is load-dependent (*Quamme* and *Dirks* 1980). As the delivery of magnesium was elevated, either by high magnesium concentration in the perfusate (*Quamme* 1980a; *Quamme* and *Dirks* 1980) or by inhibition of more proximal reabsorption by furosemide (*Quamme* 1981), the absolute reabsorption of magnesium increased. However, the fractional reabsorption rate fell significantly, inasmuch as fractional sodium and calcium reabsorption remained the same or increased (*Quamme* 1980a) with comparable increases in ion delivery. Distal magnesium reabsorption is normally close to capacity, whereas the rate of calcium and sodium transport is ordinarily unsaturated. Micropuncture and microperfusion studies of the distal tubule (*Costanzo* and *Windhager* 1978; *Shareghi* and *Stoner* 1978) have demonstrated that calcium reabsorption is independent of sodium transport and is not affected by the existing electrical potential difference, suggesting unique transport mechanisms for these two cations. Moreover, the luminal negative potential difference may be large in this segment (*Wright* 1971), implying active calcium transport. This may also apply to distal magnesium reabsorption. It would appear that the distal convoluted tubule is another site where magnesium may be dissociated from sodium and calcium, the details of which need to be elucidated.

The distal tubule, in this context, refers to the portion of the superficial nephron between the macula densa and the first confluence with another tubule. The above data demonstrating magnesium transport (*Quamme* 1980a; *Quamme* and *Dirks* 1980) were obtained from the accessible portion, which consists of about one-third to one-half (located at a distance between 20% and 60% from the macula densa) of the distal tubular length. The only accessible portion in the normal Wistar rat is the early part of the distal tubule. It is now well-recognized (*Morel* et al. 1976)

that the distal tubule is a heterogeneous structure made up of at least three morphologically different cell types (*Katz* et al. 1979; *Morel* et al. 1976); it remains to be shown whether this finding is relevant to distal magnesium transport.

All studies reported to date suggest that the collecting tubules and collecting ducts play a very limited role in renal magnesium transport (*Brunette* et al. 1974, 1978; *Carney* et al. 1980; *De Rouffignac* et al. 1973) and may account for the reabsorption of less than 1%–3% of the filtered magnesium. Most of our information concerning magnesium transport in the terminal segments is indirect and involves comparisons of the magnesium remaing in late distal tubule fluid with that appearing in the excreted urine. Inferences from differences between delivery rates at the distal tubule and final urine may lead to interpretative errors because of the cellular heterogeneity of tubule segments, and also because the collecting ducts receive distal tubules of both superficial and deep nephrons that may have very different functional properties. Only recently has the collecting tubule been investigated by direct means. *Brunette* et al. (1978) demonstrated little magnesium transport when they compared samples collected from early and late sites of the same collecting duct of exposed papillas of young rats. Although the samples may have represented a mixture of fluid from several collecting ducts, these observations support the conclusion that little magnesium is reabsorbed or secreted in the papillary collecting ducts. This was confirmed by more recent studies of *Bengele* et al. (1981), who determined magnesium transport along the inner medullary collecting duct of rats with the use of microcatheterization methods. They were unable to demonstrate magnesium transport in these segments. *Shareghi* and *Agus* (1982a), using in vitro perfusion techniques, were not able to detect net transport of either magnesium or calcium in the light segment of the rabbit cortical collecting tubule. These results are probably compatible with the above conclusions from in vivo studies, suggesting no reabsorption in either the cortical or medullary collecting tubules and ducts.

One controversial issue is the question of magnesium secretion in the terminal nephron segments subsequent to acute elevation of plasma magnesium. *Wen* et al. (1971) observed an addition of 10%–15% of the filtered magnesium load, comparing the micropuncture collections obtained from random distal tubules with the excreted urine, after infusion of $MgSO_4$ or $MgCl_2$ solutions into the volume-expanded dog, suggesting net magnesium secretion in the collecting tubule. *LeGrimellec* et al. (1973b) reported similar observations using magnesium-loaded rats. Urinary magnesium excretion was observed to exceed the delivery of magnesium to the distal tubule by 20%, expressed as a fraction of the filtered load. Thus, either magnesium was added to the tubule fluid beyond the surface distal tubule

by net secretion, or substantially less magnesium was reabsorbed by the deep long-loop nephrons, thereby permitting greater urinary magnesium excretion. These authors also suggested that a large medullary gradient of magnesium could have developed as a result of the magnesium loading and that this medullary pool could have provided a favorable diffusion gradient for magnesium movement into the collecting ducts. They further postulated that if such a pool exists, it could have augmented medullary recycling into the long loop and added magnesium to the descending limb of deep nephrons. One could speculate, if this were to happen, that the deep nephrons would contribute more magnesium to the collecting duct fluid than would be expected under normal circumstances. Unlike calcium or sodium, the cortical to papillary tissue concentration gradient for magnesium has not been well-delineated (*Ullrich* and *Jarausch* 1956) and remains to be determined in conditions of hypermagnesemia. In contrast to these studies, *Brunette* et al. (1975) and *Carney* et al. (1980) reported neither reabsorption nor secretion in the terminal segments of rats after elevation of ultrafilterable magnesium to about 2–3 m*M*, which was comparable to previous studies (*Wen* et al. 1971; *LeGrimellec* et al. 1973b). The reasons for these differences are not apparent. The possible significance of reabsorption or secretion, under the specialized circumstances mentioned, requires further investigation with more specific in vitro and direct in vivo experiments on the collecting tubule and collecting duct.

2.6 Summary of Segmental Magnesium Transport

Present evidence suggests that the renal handling of magnesium is normally a filtration-reabsorption process, since evidence for secretion remains inconclusive. Magnesium reabsorption has distinctive features compared with that of sodium and calcium. The proximal tubule concentration of magnesium rises to levels about 1.5 times greater than the glomerular filtrate, and only 20%–30% of the filtered magnesium is reabsorbed in this segment. Although the fractional reabsorption of magnesium is only half that of sodium, it changes in parallel with that of sodium in response to changes in extracellular fluid volume. These characteristics reflect a low permeability of the tubule to magnesium. The major portion of filtered magnesium (some 65%) is reabsorbed in the loop of Henle, especially in the thick ascending limb. Recent evidence suggests that magnesium reabsorption in the ascending limb may be voltage-dependent and secondary to active sodium chloride reabsorption. Present evidence also points to an important interaction between magnesium and calcium for transport from the contraluminal surface of the ascending limb cell. The loop of Henle appears to be the major nephron site where magnesium excretion is con-

trolled. About 10% of the filtered magnesium is delivered into the distal nephron. The distal tubule reabsorbs only a small fraction of the filtered magnesium and is readily saturated with increased magnesium delivery to the distal sites.

3 Factors Influencing Renal Magnesium Transport

Table 3 summarizes some of the factors that have been reported to influence magnesium excretion and indicates the various nephron segments which are affected. The most important factors relate to alterations in plasma magnesium or calcium concentration, changes in extracellular fluid volume, and the actions of parathyroid hormone and loop diuretics. We will limit our discussion to those factors which have been studied in some detail.

Table 3. Factors which may influence magnesium reabsorption by the nephron

Factors which increase reabsorption			Factors which decrease reabsorption	
	Factor	Example	Factor	Example
Proximal tubule	ECF volume contraction	1. Chronic thiazide administration	ECF volume expansion	1. Saline infusion 2. Aldosterone administration
			Proximal diuretics	1. Acetazolamide 2. Mannitol
Loop of Henle	Hormonal influences	1. Parathyroid hormone	Loop diuretics	1. Furosemide 2. Ethacrynic acid
			Osmotic diuretics	1. Mannitol 2. Urea 3. Glucose
	Hypocalcemia	1. Hypoparathyroidism 2. Calcitonin	Hypercalcemia	1. Vitamin D intoxication 2. Hyperparathyroidism 3. Malignancy
	Hypomagnesemia	1. Dietary Mg deficiency 2. Malabsorption	Hypermagnesemia	1. Excessive intake 2. Parenteral Mg administration
Distal tubule	Hormonal influences	1. Parathyroid hormone		

3.1 Glomerular Filtration Rate and Renal Plasma Flow

As is the case for sodium and calcium, there also appears to be glomerulotubular balance for magnesium. *Massry* et al. (1973) studied the acute effects of increased glomerular filtration rate (GFR) on magnesium excretion in the dog. They observed relatively little change in fractional magnesium excretion with alterations in filtration rate. Similar studies evaluating the effects of renal vasodilation with acetylcholine, bradykinin, and other agents have demonstrated only small increases in magnesium excretion, which were in precise proportion to that of sodium (*Massry* et al. 1973). The tubular sites of these changes have not been systematically studied, but they likely occur within the proximal tubule and the thick ascending limb. The basis for these observations within the proximal tubule and thick ascending limb has been given in detail above. Briefly, the inhibition of proximal tubular reabsorption increases distal delivery of salt and water, which may alter magnesium reabsorption in the loop.

Chronic renal failure, with its attendant reduction of GFR, results in a rise in fractional magnesium excretion, similar to that for calcium and sodium excretion (*Wong* et al. 1980a). Micropuncture studies performed in experimentally induced uremic dogs with reduced nephron populations and marked hyperfiltration per nephron demonstrated that fractional magnesium reabsorption was reduced within the loop of Henle. The reduction in fractional magnesium reabsorption was proportional to the reduction in sodium and calcium transport. Thus, factors which alter the single nephron GFR and the filtration fraction may change the peritubular capillary hydrostatic and osmotic forces and affect proximal reabsorption. In addition, enhanced distal salt delivery, and perhaps the putative natriuretic hormone, may affect magnesium transport in a way similar to sodium reabsorption in chronic renal failure.

3.2 Sodium Balance and Extracellular Fluid Volume Status

Extracellular volume expansion with saline or Ringer solution produces a brisk increase in magnesium excretion. This increase parallels that of sodium and calcium, but the fractional excretion rate is approximately twice that of these cations. The high fractional magnesium excretion rate due to volume expansion persists despite reduction in renal artery perfusion pressure (*Massry* et al. 1967a; *Massry* and *Coburn* 1973). The reduction in magnesium reabsorption occurs principally in the proximal tubule, so that fractional reabsorption falls from 30% to 15% when the extracellular volume is expanded to 5% body weight. The increased amount of magnesium delivered to the loop is largely excreted unchanged in the urine, due to the

concomitant increase in distal salt delivery from the proximal tubule. It would not be anticipated that acute volume expansion with colloid solutions, such as plasma, which does not alter proximal reabsorption, would increase urinary magnesium excretion.

3.3 Hypermagnesemia

The most striking changes in magnesium excretion occur after alterations in plasma magnesium concentration. With marked hypermagnesemia, due to either high intake or intravenous magnesium infusion, urinary magnesium excretion can approximate the filtered load of magnesium. Conversely, severe hypomagnesemia results in almost complete renal conservation of magnesium. Thus a major determinant of renal magnesium excretion appears to be the plasma magnesium concentration.

Large increases in magnesium excretion occur after hypermagnesemia; this is a result of the increased filtered load, a reduction in fractional reabsorption in the proximal tubule, and a more marked reduction in reabsorption in the ascending limb of Henle's loop. As mentioned earlier, with progressive elevation of luminal magnesium concentration in the proximal tubule, absolute magnesium reabsorption increases but the fractional reabsorption remains the same (*Quamme* and *Dirks* 1980). However, in the presence of hypermagnesemia the fractional sodium and water reabsorption is reduced in the proximal tubule, due to inhibition of unidirectional and net sodium efflux (*Dibona* 1971, 1974). This leads to proportional reduction in fractional magnesium reabsorption (*Brunette* et al. 1969; *Poujeol* et al. 1976; *Wong* et al. 1979). Thus, overall magnesium reabsorption is reduced in the proximal tubule during hypermagnesemia. *Brunette* et al. (1975) have demonstrated that beyond the proximal tubule the magnesium concentration of the tubule fluid increased more than could be accounted for by glomerular filtration and water absorption. These data suggest net addition of magnesium in the straight proximal tubule or in the descending limb during acute hypermagnesemia in the rat. More recently, *Brunette* et al. (1978) were unable to demonstrate a magnesium concentration gradient favoring magnesium transport from the vasa recta blood to the descending limb, suggesting active magnesium addition prior to the site of micropuncture in the papilla – either in the pars recta or in the thin descending limb. Studies involving in vivo and in vitro microperfusion of rat and rabbit tubules respectively were unable to provide evidence of significant entry of magnesium into the proximal tubule or loop of Henle during hypermagnesemia (*Quamme* and *Dirks* 1980; *Quamme* 1982a). This does not appear to be related to differences between superficial and deep nephrons, as similar results were obtained

with juxtamedullary tubules. More recent studies with perfused straight proximal tubules isolated from rabbits on a high-magnesium diet, failed to demonstrate significant magnesium entry despite large concentration differences between the bath and the lumen. The thin descending segment remains to be investigated by in vitro perfusion. Magnesium reabsorption in the loop of Henle is markedly reduced during hypermagnesemia due to the action of magnesium from the basolateral membrane of the thick ascending limb. At the level of the distal tubule, magnesium concentration rises sharply, exceeding the limited distal reabsorption capacity for magnesium, so that most of the magnesium rejected in the loop of Henle appears in the urine. As mentioned earlier, recent studies also serve as an explanation for the apparent Tm-limited magnesium transport which was observed in clearance studies (*Massry* et al. 1969). Hypermagnesemia also reduces calcium, but not sodium, reabsorption; conversely, hypercalcemia reduces both calcium and magnesium transport.

Some evidence for magnesium secretion, subsequent to marked hypermagnesemia, has been presented. *Averill* and *Heaton* (1966) reported magnesium secretion exceeding 150% of that filtered in rats which had been given magnesium salts chronically. *Wen* et al. (1971) demonstrated that the fraction of filtered magnesium remaining in the distal tubule was 15%–20% less than that excreted in the urine, suggesting magnesium addition beyond the distal tubule. However, others were unable to demonstrate secretion either by clearance techniques (*Alfredson* and *Walser* 1970) or by micropuncture studies of the distal tubule and collecting ducts (*Brunette* et al. 1975; *Carney* et al. 1980; *Quamme* and *Dirks* 1980). Although the aglomerular fish excretes magnesium in the urine (*Berglund* and *Forster* 1958), magnesium secretion could not be demonstrated in the chicken (*Robinson* and *Portwood* 1962). Present evidence would indicate that in higher animals magnesium is not secreted to any significant degree by the nephron. Thus, the basis of the massive magnesium excretion following hypermagnesemia rests with the remarkable ability of magnesium ions to reduce their own reabsorption in the loop of Henle (*Quamme* and *Dirks* 1980).

3.4 Hypomagnesemia and Magnesium Deficiency

In the presence of severe hypomagnesemia the kidney conserves almost all the filtered magnesium. The tubular sites of this magnesium absorption were recently studied in magnesium-deficient rats which had been depleted to about 30% of normal body magnesium (*Carney* et al. 1980). Plasma magnesium was depressed 50% and fractional magnesium excretion was only 3%, compared with an excretion of 15% in normal rats. Proximal

TF/UF magnesium increased in proportion to TF/P inulin along the length of the proximal tubule so that the normal fraction of magnesium was reabsorbed in the proximal tubule, but absolute magnesium reabsorption in the proximal tubule was reduced proportionally to the decrease in the amount that was filtered. The TF/UF magnesium in the early distal tubule was much lower than normal, indicating that more magnesium had been reabsorbed between the late proximal tubule and the distal tubule than normal, and this presumably occurred in the thick ascending limb. Fractional magnesium reabsorption in the loop was increased by 12% above normal, but the absolute magnesium reabsorption in the loop was below normal because of the much lower amount of magnesium delivered to this segment. Only 3% of the filtered load was delivered to the distal tubule and further magnesium reabsorption was not detected in the terminal nephron segments. Thus in magnesium deficiency the ascending limb is the major site at which magnesium reabsorption in enhanced. When an acute magnesium infusion was given to the magnesium-deficient rats in an amount sufficient to normalize plasma magnesium, the magnesium excretion increased to values approaching normal. Absolute magnesium reabsorption increased in the loop of Henle but remained somewhat below normal capacity, indicating some cellular impairment of magnesium reabsorption due to magnesium deficiency, which was not rapidly corrected by acute magnesium infusions. It should be mentioned that the rat is unique in its response to magnesium deficiency: hypercalcemia commonly occurs rather than hypocalcemia, which is usually observed in man and other animal species (*Gitelman* et al. 1968; *Wacker* and *Parisi* 1968). Since hypercalcemia can itself lower magnesium reabsorption, this may explain the initial refractoriness of the transport system to magnesium correction in the rat. Distal tubular reabsorption is very low even in magnesium deficiency and responds to increased delivery. Thus in magnesium deficiency the key nephron segment responsible for the marked magnesium conservation is the thick ascending limb of the loop of Henle.

3.5 Hypercalcemia and Hypocalcemia

Hypercalcemia is another important factor which markedly increases magnesium excretion. In fact after an acute calcium infusion, the fractional excretion of magnesium rises more markedly than the excretion of calcium. As mentioned above, hypermagnesemia results in a greater magnesuria than calciuria, and a common pathway for calcium-magnesium reabsorption has been suggested – presumably more sensitive to magnesium transport than to calcium transport. *Coburn* et al. (1970) demonstrated in a number of clearance studies in dogs that the elevation of plasma calcium

concentration progressively reduced the maximum ability of the kidney to reabsorb magnesium. This has been extensively reviewed by *Massry* and *Coburn* (1973). Subsequent micropuncture and microperfusion studies have provided additional understanding of magnesium transport during hypercalcemia. *LeGrimellec* et al. (1974) demonstrated that in acute hypercalcemia proximal TF/UF magnesium concentration remained unchanged, but overall magnesium reabsorption was reduced by 10% due to the reduction in isotonic reabsorption. Thus hypercalcemia increased magnesium delivery to the loop, secondary to the inhibition of sodium and water reabsorption. Early distal tubule fluid collections demonstrated elevated TF/UF magnesium concentrations, and magnesium delivery from the loop to the distal tubule was increased by 30%–40% indicating marked inhibition of magnesium reabsorption in the loop of Henle. Microperfusion studies have elucidated this further; hypercalcemia was shown to affect magnesium reabsorption to a greater degree than that of calcium, whereas sodium reabsorption remained near control values (*Quamme* 1982b). Absolute distal magnesium reabsorption was modestly increased because of the augmented delivery of magnesium resulting from loop inhibition, but distal reabsorption capacity was rapidly overwhelmed by the large magnesium load. These studies were performed on thyroparathyroidectomized rats in which there is presumably no circulating parathyroid hormone. These observations, in which increases in plasma calcium and magnesium affect the reabsorption of each other in the loop of Henle, strongly support the existence of a calcium-magnesium membrane interaction. As previously suggested, there is at present no knowledge of the nature of this process, but it may include competition for individual active transport steps for both calcium and magnesium or for some linked divalent cation transport system. This competition appears to occur from the basolateral membrane, as elevation of luminal calcium or magnesium has little effect on the respective absorption rates (*Quamme* 1982b; *Quamme* and *Dirks* 1980; *Shareghi* and *Agus* 1979a, 1982b). Hypomagnesemia may be secondary to the effect of elevated plasma calcium on the renal handling of magnesium. Conversely, hypocalcemia may lead to enhanced magnesium reabsorption (*Quamme* 1980a). It may be that hypocalcemia permits more complete calcium and magnesium reabsorption in the loop as a result of reduced competition for the transport system at the basolateral membrane.

3.6 Phosphate Depletion

Dietary phosphate restriction evokes appropriate conservation responses from the kidney, which increases its absorption of phosphate and develops a resistance to the phosphaturic action of parathyroid hormone. Interestingly, the syndrome of phosphate depletion also results in marked hypercalciuria and hypermagnesuria (*Coburn* and *Massry* 1970). In fact, the renal magnesium wasting may be sufficient to lead to overt hypomagnesemia (*Coburn* and *Massry* 1970; *Kreusser* et al. 1978). Micropuncture experiments in phosphate-depleted dogs indicated that this defect occurs in the thick ascending limb and distal tubule and may be corrected by the acute administration of parathyroid hormone or neutral phosphate infusions (*Wong* et al. 1980b). The cellular mechanisms responsible for the inability of the kidney to normally conserve magnesium in phosphate deficiency are unclear. *Kempson* et al. (1981) were able to restore the phosphaturic action of parathyroid hormone in phosphate-depleted animals by infusing large amounts of nicotinamide adenine dinucleotide (NAD), a naturally occurring cytosolic nucleotide involved in oxidation-reduction reactions. Surprisingly, this also corrected the renal calcium and magnesium leak (unpublished communications).

3.7 Parathyroid Hormone

No specific and unique hormonal control of magnesium absorption by the renal tubule has been defined to date. Parathyroid hormone most closely fulfils the criteria of a hormonal agent regulating magnesium transport. A number of other hormones, such as calcitonin, glucocorticoids, aldosterone, thyroid hormone, and growth hormone, have small effects on magnesium reabsorption, but these effects are probably indirect (*Sutton* et al. 1979).

The literature on the effects of parathyroid hormone on magnesium excretion has been confusing and controversial, largely because insufficient attention has been paid to the effects of the associated hypercalcemia which is normally observed following administration of parathyroid hormone. Early clinical investigations demonstrated that magnesium loss was associated with active hyperparathyroidism (*Wacker* and *Vallee* 1958; *Wacker* and *Parisi* 1968; *King* and *Stanbury* 1970). *Heaton* (1955) reported that parathyroid hormone increased magnesium excretion in the rat. However, the rats were hypercalcemic, and again the increase in magnesium excretion may have been secondary to the rise in plasma calcium concentration. *MacIntyre* (1967) showed that acute parathyroid hormone administration to rats resulted in a decrease in urinary magnesium excre-

tion, as well as that of calcium, provided there was no change in plasma calcium concentration. *Massry* et al. (1969) demonstrated in the dog that parathyroid hormone administration, superimposed on magnesium chloride infusion, resulted in a decrease in the fractional excretion of both magnesium and calcium. Thus it appears that parathyroid hormone increases magnesium reabsorption, but that the ensuing hypercalcemia in some cases may decrease reabsorption, leading to hypomagnesemia.

Micropuncture studies directed at assessing the site of action of parathyroid hormone on magnesium reabsorption have been performed in several animal species, which have displayed either no effect or small increases in reabsorption within the loop of Henle and distal tubule (*Kuntziger* et al. 1974; *Quamme* and *Dirks* 1980; *Quamme* 1981; *Wong* et al. 1980b). The most striking effects of parathyroid hormone were demonstrated in the golden hamster (*Harris* et al. 1979). Calcium and magnesium transport in the hamster is very sensitive to the action of parathyroid hormone, as parathyroidectomy results in the excretion of approximately 20% of the filtered magnesium and calcium. Subsequent administration of parathyroid hormone promptly reduces the fractional magnesium excretion to a normal level of less than 5%. Infusion of cyclic AMP or dibutyryl cyclic AMP has been shown to mimic this effect, suggesting that the effects of parathyroid hormone on magnesium, calcium, and phosphorus may be mediated through the production of cyclic AMP. The localization of the effects of parathyroid hormone and cyclic AMP on magnesium reabsorption in this study indicated that magnesium reabsorption was enhanced prior to the distal tubule, suggesting an action in the thick ascending limb of Henle's loop. Parathyroid hormone has also been reported to increase magnesium reabsorption in the loop in TPTX rats when magnesium reabsorption was initially reduced by either hypermagnesemia or furosemide (*Quamme* 1981; *Quamme* et al. 1980). This observation has been supported by a preliminary report of parathyroid-sensitive magnesium transport in the isolated rabbit thick ascending limb (*Shareghi* and *Agus* 1979b). A further increase in magnesium transport occurred beyond the distal tubule, which may reflect hormonal action within the deeper nephrons or reabsorption of magnesium within the collecting duct system of the hamster.

Since parathyroid hormone secretion appears to be modulated by changes in plasma magnesium concentration in the same manner as changes in calcium concentration (i.e., a fall in concentration of either ion stimulates parathyroid hormone release), and since parathyroid hormone promotes the renal retention of both ions, it is possible that the parathyroid glands could play a role in the maintenance of the normal plasma magnesium as well as calcium concentration. However, it is difficult to imagine how a single endocrine system could control the concentration of both

ions. It also appears that the sensitivity of the parathyroid glands to changes in plasma ionized magnesium concentration is lower than that to comparable changes in calcium concentration (*Carr* et al. 1965; *Habener* and *Potts* Jr. 1976). In summary, parathyroid hormone would appear to enhance magnesium reabsorption, and the resultant renal effects depend on the opposing forces of elevated serum calcium and magnesium concentrations.

3.8 Calcitonin

Several studies have indicated that calcitonin enhances renal magnesium reabsorption and calcium reabsorption. Acute administration of porcine or salmon calcitonin to thyroparathyroidectomized animals results in a decreases urinary calcium and magnesium excretion (*Alderd* et al. 1970; *Nielson* et al. 1971; *Quamme* 1980a; *Rasmussen* et al. 1967; *Sorensen* and *Hindber* 1972). However, these findings have not been consistent, particularly in the dog (*Clark* and *Kenny* 1967; *Littledike* and *Arnaud* 1971; *Pak* et al. 1970; *Puschett* et al. 1974; *Russell* and *Fleisch* 1968; *Wong* et al. 1977) and in man (*Ardaillou* et al. 1967; *Bijvoet* et al. 1971; *Cochran* et al. 1970; *Paillard* et al. 1972; *Singer* et al. 1969). Recently, *Chabardès* et al. (1976) demonstrated adenylate cyclase activity sensitive to calcitonin in isolated tubular fragments of medullary thick ascending limbs of Henle's loop obtained from the rabbit. Although there may be species differences (*Brunette* et al. 1979), the presence of receptors for calcitonin in the nephron suggests a functional role for this hormone. Support for such an action of calcitonin was not observed when the hormone was carefully studied in terms of its effect on renal tubular magnesium reabsorption. It was observed that calcitonin only increases magnesium reabsorption in the loop of Henle during hypocalcemia (*Quamme* 1980a). When the hypocalcemic effect of calcitonin in the rat was offset by continuous infusion of calcium, no change in magnesium reabsorption was observed in the loop of Henle. Thus the action of calcitonin on magnesium reabsorption would appear to be an indirect response to hypocalcemia. Although these observations are at variance with a recent report by *Poujeol* et al. (1980), they would explain the discrepant results reported in the literature. The majority of the negative responses to calcitonin have been obtained in large (presumably adult) dogs and adult human subjects who do not demonstrate large decreases in plasma calcium following calcitonin administration.

3.9 Adrenocortical Steroids

No report has yet indicated any consistent effect on magnesium excretion by glucocorticoids or mineralocorticoids. Chronic adminstration of glucocorticoids usually leads to an increase in urinary excretion of both magnesium and calcium in animals and humans (*Walser* 1973). This may be related to the catabolic effect on the bone, causing release of magnesium, or to the rise in glomerular filtration rate often observed in this situation. The acute administration of methylprednisolone to adrenalectomized dogs did not change the excretion of magnesium (*Massry* et al. 1967b). The acute administration to dogs of mineralocorticoids such as aldosterone decreased sodium excretion but did not influence calcium or magnesium excretion, indicating a dissociation of the transport of these three cations, probably at the level of the distal tubule. Chronic administration of aldosterone results in an increase in calcium and magnesium excretion (*Hanna* et al. 1957; *Horton* and *Biglieri* 1962; *Wacker* and *Vallee* 1958); this effect is reversed by spironolactone administration or adrenal insufficiency (*Walser* 1973). The modest increase in magnesium excretion after chronic mineralocorticoid administration probably reflects volume expansion due to mineralocorticoid action, with an increase in the delivery of sodium, calcium, and magnesium to the distal tubule, where mineralocorticoids may enhance sodium reabsorption without affecting calcium and magnesium transport.

3.10 Thyroid Hormone

Magnesium metabolism is altered in hyperthyroidism and hypothyroidism (*Bradley* 1978; *Jones* et al. 1966). Thyrotoxic patients not infrequently have a decreased serum magnesium concentration with increased urinary magnesium excretion. This is usually associated with an increase in plasma calcium concentration and hypercalciuria, which may provide an explanation for the above findings (*Bradley* 1978; *Epstein* et al. 1958; *Parfitt* and *Dent* 1970). Hypothyroidsm, on the other hand, is consistently associated with a mean increase in plasma magnesium concentration, possibly due to renal magnesium retention. Moreover, intracellular magnesium has been reported to be low in thyroid hormone deficiency (*Wacker* and *Parisi* 1968). In hypothyroid rats the reabsorption capacity for magnesium is elevated at all magnesium filtration rates (*Quamme* and *McCaffrey* 1980), despite the inability of the kidney to retain sodium and calcium (*Lebwohl* et al. 1976). The enhanced magnesium reabsorption in hypothyroidism was not dependent on changes in circulating parathyroid hormone. The cellular mechanisms responsible for these changes are unknown, but

thyroid hormones enhance Na-K ATPase activity, which is associated with parallel changes in sodium reabsorption. Increase in enzyme activity allows the cell to maintain a low intracellular sodium and calcium concentration and a relatively high intracellular magnesium concentration (*Ismail-Beigi* and *Edelman* 1973; *Smith* and *Edelman* 1979). These cellular changes may provide a common explanation for the alterations in renal handling of sodium, calcium, and magnesium in hypothyroidism. Overall, the changes observed in renal magnesium reabsorption in hypothyroidism and hyperthyroidism are small compared with the more marked extrarenal changes.

3.11 Other Hormones

Although the effects of the Vitamin D metabolites on parathyroid hormone and calcium metabolism have been extensively examined, few studies (*Hanna* 1961; *Coburn* et al. 1973) have been directed at their role on magnesium homeostasis. Studies are also required to determine the effect of alterations of intracellular magnesium on Vitamin D metabolism. Administration of growth hormone in humans results in an increase in urinary excretion of magnesium, as well as calcium (*Hanna* et al. 1961; *Henneman* et al. 1960). In addition, the urinary excretion of magnesium is increased in proportion to calcium and sodium excretion, following infusion of glucagon, angiotensin, or catecholamines (*Massry* and *Coburn* 1973). Estrogens and androgens have been reported to either increase or have little effect on magnesium excretion (*Wacker* and *Parisi* 1968). The mechanisms of these changes appear to be due to extrarenal events or hemodynamic alterations induced by the hormones rather than to direct tubular effects.

3.12 Diuretics

A number of drugs may decrease renal reabsorption of magnesium. The most important are the loop diuretics, such as furosemide, which can cause an impressive magnesuria along with a marked natriuresis and calciuria. Other diuretics, such as the carbonic anhydrase inhibitors, do not significantly alter magnesium excretion, although there may be a small decrease in proximal reabsorption, which is reclaimed in the loop of Henle. The acute use of thiazide diuretics may be accompanied by a mild magnesuria, which may persist with continued treatment. The effects on magnesium excretion of the distal potassium-sparing natriuretic agents, such as spironolactone and triamterene, are minor. Osmotic diuretics, such as mannitol and urea, greatly increase renal magnesium excretion.

The loop diuretics increase magnesium excretion more than that of calcium and sodium. Furosemide causes an abrupt increase in magnesium concentration in the early distal tubule after injection, which may approximate the ultrafilterable magnesium concentration. The effects of furosemide on magnesium transport are apparently the result of inhibition of secondary-active chloride transport and the associated decrease in reabsorption of magnesium. The somewhat greater effect on magnesium relative to sodium and calcium excretion may reflect the additional effects on a unique magnesium transport system. Ethacrynic acid and mercurial diuretics have effects on magnesium excretion similar to those of furosemide, but have not been directly studied by micropuncture. It is unlikely that there are any significant differences in their effects on segmental magnesium reabsorption.

The acute and chronic administration of thiazide diuretics may produce a mild magnesuria, even though this response is variable and is probably dependent on the magnitude of the proximal inhibition of salt and water. There is now clear evidence that thiazide diuretics reduce isotonic water reabsorption in the proximal tubule and hence magnesium transport, but the increased proximal delivery is normally reabsorbed in the loop of Henle. The natriuretic effect of thiazide diuretics depends on their action in the distal convoluted tubule. The evidence is now reasonably strong that at this site thiazides enhance calcium transport independent of the presence of parathyroid hormone (*Costanzo* and *Weiner* 1974, 1976; *Quamme* et al. 1975; *Costanzo* and *Windhager* 1978). Data obtained from micropuncture studies of the hamster demonstrate little or no change in magnesium reabsorption in the distal tubule after administration of thiazide diuretics (*Wong* et al. 1982). Modest amounts of magnesium escaping the loop of Henle may account for the variable magnesuric response that is often seen in some clinical studies.

The osmotic diuretics mannitol and urea impressively increase magnesium excretion in proportion to the osmotic load (*Wong* et al. 1979, 1981a). Magnesium excretion may rise to 40%–50% of that filtered when either mannitol or urea is administered to increase plasma osmolality by approximately 100 mosmol/kg. Recent micropuncture studies have demonstrated that the principle effect of mannitol infusions is directed to the loop of Henle (*Wong* et al. 1979). There was a marked reduction in magnesium reabsorption, so that the total increase in delivery of magnesium to the distal tubule approximated 30%–40% of the filtered load, which was largely excreted in the urine. The mechanism of the effect of mannitol on reducing electrolyte reabsorption in the loop of Henle appears to be related to the diminished water reabsorption in the descending limb of Henle's loop, as well as to a reduction in net electrolyte transport, and presumably the voltage, in the ascending limb. The pattern with progressive

infusions of urea is similar to that of mannitol (*Wong* et al. 1981). Urea markedly increases magnesium excretion by reducing magnesium transport in the loop of Henle. Hyperglycemia similarly causes marked increases in magnesium excretion, but these effects have not been specifically studied. In summary, osmotic diuretics appear to reduce reabsorption of magnesium principally within the loop of Henle.

3.13 Other Factors

A host of other factors have been examined for their effects of magnesium excretion. By and large, the results are often equivocal and usually of minor significance. The ingestion of a variety of metabolizable substrates such as glucose, galactose, fructose, and protein produce magnesuria without accompanying natriuresis (*Lindeman* et al. 1967; *Wacker* and *Parisi* 1968; *Walser* 1973). Best known for its effect on magnesium handling is alcohol, which, when administered acutely or chronically, increases fractional magnesium excretion by 5%–10% (*Wacker* and *Parisi* 1968). The mechanisms underlying these observations are unclear, but have been attributed to rapidly metabolized products which may have indirect effects on the tubule. Acute acidosis in experimental animals results in a magnesuria of similar proportion to the observed calciuria, and the effect occurs in the loop of Henle. However, *Lennon* and *Piering* (1970) were unable to demonstrate a magnesuria subsequent to chronic NH_4Cl-loading, which results in chronic hypercalciuria, in human patients. Presumably, this may be because the effects of acid-base disturbances on calcium transport occur in the distal convoluted tubule, a site where magnesium transport is low. A number of pharmacological agents, such as *cis*-platin (*Schilsky* and *Anderson* 1979; *Bar* et al. 1975), may selectively reduce magnesium reabsorption, and one would predict that this likely occurs in the loop of Henle. A primary inherited defect in renal reabsorption of magnesium has been reported in man (*Gitelman* et al. 1966; *Runeber* et al. 1975). Although these patients also have other defects (hypercalciuria and hypokalemia), they may provide a unique genetic model for investigations of magnesium reabsorption.

3.14 Summary of Factors Influencing Magnesium Transport

The present concept of renal tubular magnesium reabsorption suggests that it is essentially a unidirectional process throughout the whole nephron. Magnesium reabsorption proceeds at a slower rate in the proximal tubule than that observed for sodium and calcium. The primary site of

modifications of magnesium reabsorption is in the thick ascending limb of Henle's loop, and magnesium transport at this level is largely affected by plasma magnesium and calcium concentrations, as well as by the potent loop and osmotic diuretics, all of which focus their primary action on the ascending limb. No specific hormonal controls regulating magnesium transport have as yet been identified, though parathyroid hormone seems to have an enhancing effect on magnesium reabsorption in the loop of Henle and distal convoluted tubule.

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Rev. Physiol. Biochem. Pharmacol., Vol. 97

Gas Exchange in Fish Swim Bladder

RAGNAR FÄNGE *

Contents

* Department of Zoophysiology, University of Göteborg, Box 25059, S-400 31 Göteborg/Sweden

1 Introduction

Borelli (1681, cited in *Rauther* 1940) suggested that fishes fill the swim bladder with atmospheric air at the water surface and use the bladder to rise or sink in the water by muscular control of the gas volume. But the swim bladder hinders more than aids fishes in moving vertically. *Moreau* (1876) showed that many fishes live at an energy-saving constant depth, *"plan des moindres efforts"*, using physiological mechanisms to control the swim bladder volume.

The main function of the swim bladder is to compensate for downdrift caused by the high densities of the skeleton and the muscles. Freshwater fishes usually need a gas volume of 5.6%–8.3% of the body volume, seawater fishes 3.1%–5.6% (*Alexander* 1966). In lipid-rich fishes, such as the herring *(Clupea),* the swim bladder gas volume is correlated with the fat content of the body (*Srivastava* 1964).

The gas exchange in the swim bladder has been treated in several reviews: *Jaeger* (1903), *Koch* (1934), *Jones* and *Marshall* (1953), *Fänge* (1966, 1973, 1976), *Denton* (1961), *Steen* (1970), *Blaxter* and *Tytler* (1978), and *Blaxter* (1980). *Jones* and *Scholes* (1981) have discussed some ecophysiological aspects.

2 Structure

2.1 Morphological Diversity

Swim bladders and lungs are homologous. Lungs supposedly evolved in paleozoic freshwater vertebrates as an adaptation to hypoxic stress (*Romer* 1972; *Graham* et al. 1978). In the majority of fishes living now, the swim bladder is a hydrostatic organ with no respiratory function, but some primitive fishes and a few teleosts breath air by a lung or swim bladder. Fishes which in the adult have an open communication (pneumatic duct) between the gut and the swim bladder are termed *physostomes;* those with a closed swim bladder are called *physoclists.* Physoclists usually pass through a physostome stage during their larval development. A swim bladder is not found in all fishes (Table 1), but some teleosts which lack a swim bladder in the adult stage possess a swim bladder in the juvenile stage: pleuronectids (flounders), the angler *(Lophius),* etc. Among marine teleosts a swim bladder occurs in the majority of epi-, meso-, and benthopelagic species, but is lacking or regressed in bathypelagic ones. The benthopelagic fishes have a depth range of about 200–7000 m. They comprise relatively large fishes with a well-developed swim bladder:

Table 1. Main systematic divisions of fishes, approximate number of species within each division (*Nelson* 1976), and type of swim bladder (lung)

Division	Number of species	Swim bladder
Cyclostomata (hagfish, lamprey)	63	Absent
Chondrichthyes (sharks, rays, chimaeras)	600	Absent
Crossopterygii (coelacanth)	1	Regressed
Dipnoi (lungfish)	6	Respiratory
Polypteriformes (bichir)	11	Respiratory
Chondrostei (sturgeons, paddlefish)	25	Nonrespiratory
Holostei (bony ganoids: gars, bowfin)	8	Respiratory
Teleostei (modern bony fish)	18,000	Respiratory (very few) Nonrespiratory or absent

macrourids (rat-tails), morids (deep-sea cods), brotulids, halosaurs, notacanths, and deep-sea eels (*Marshall* 1970, 1972, 1979).

The comparative anatomy of the swim bladder was treated by *Rauther* (1940), but although thousands of fishes have a swim bladder, this organ has been examined more closely only in relatively few species.

2.1.1 Lungs and Respiratory Swim Bladders

Extinct lobe-finned fishes are assumed to have possessed a primitive lung, but the only representative living now, the coelacanth *(Latimeria chalumnae),* has a regressed swim bladder replaced by a mass of wax esters (*Nevenzel* et al. 1966).

Lungfishes (Dipnoans). The African *(Protopterus)* and the South American *(Lepidosiren)* lungfishes have paired lungs connected with the gut via a glottis. The Australian lungfish *(Neoceratodus)* has a single lung.

Polypterids (Bichirs). Polypterus and *Calamoichtys* are African freshwater fishes with paired lung-like swim bladders. They are facultative air-breathers practicing swim bladder respiration during the night, or at low aquatic oxygen pressure (*Magid* 1966; *Pfeiffer* 1968; *Marquet* et al. 1974).

Holosteans (Bony Ganoids). The gars *(Lepisosteus)* and the bowfin *(Amia calva)* are related to extinct ancestors of teleost. They possess a highly vascularized swim bladder resembling a primitive lung. In the gar the swim bladder has a volume of 8%–10% of the body volume. The highly folded internal surface of the bladder of a gar of 1.73 kg body weight was about 1200 cm^2 (*Rahn* et al. 1971). The gars use the swim bladder

regularly for respiration, but the bowfin *(Amia)* breathes air only at high water temperature (*Johansen* et al. 1970).

Teleosts (Modern Bony Fish). Compared with the great number of species, extremely few teleosts have a swim bladder adapted for oxygen uptake from the air (Table 2). Teleostean respiratory swim bladders have an alveolated of spongy richly vascularized inner lining and a short, wide pneumatic duct. *Pantodon* has air-filled processes from the swim bladder which penetrate into the vertebrae, forming avian-like "pneumatic bones" (*Poll* and *Nysten* 1962).

Table 2. Teleosts with respiratory swim bladder. All except *Megalops* are freshwater inhabitants (*Johansen* 1970; *Fänge* 1976; *Graham* et al. 1978)

Family	Genus
Notopteridae	*Notopterus, Xenomystus*
Osteoglossidae	*Arapaima* [a], *Heterotis*
Pantodontidae	*Pantodon*
Gymnarchidae	*Gymnarchus*
Megalopidae	*Megalops*
Umbridae	*Umbra*
Phractolaemidae	*Phractolaemus*
Erythrinidae	*Erythrinus, Hoploerythrinus*
Lebiasinidae	*Piabucina*

a Obligate air breather

2.1.2 Nonrespiratory Swim Bladders

Physostomes

Chondrosteans (Ganoids). Acipenser, Huso, Scaphirhynchus (sturgeons) and *Polyodon* (paddlefish) are primitive physostomes with poorly vascularized swim bladders.

Teleosts (Modern Bony Fish). Herring (clupeids), salmon and whitefish (salmonids), pikes (esocids), catfishes (siluroids), carps (cyprinids), and eels (anguillids) are teleostean physostomes. The eels (anguillids: *Anguilla, Conger*) are provided with a physostomatous swim bladder structurally and functionally closely resembling the euphysoclist type (Fig. 1D).

Physoclists

A great number of teleosts are physoclists. In those all gas exchange takes place between the swim bladder and the circulating blood through specialized parts of the swim bladder wall. The pneumatic duct is absent.

The more highly organized physoclists are called *euphysoclists.* Their swim bladder, or the inner membrane of the swim bladder, is divided into two parts, one *secretory* and one *resorbant* (*Rauther* 1940; *Fänge* 1953). A great number of fishes are euphysoclists, for instance members of the families Gadidae, Macrouridae, Stromateidae, Triglidae, Cichlidae, Sciaenidae, Gerridae, Labridae, and Percidae.

Structural diversity among teleostean swim bladders is very large. Many physoclists (physoclists comprise only teleosts) possess a closed swim bladder structurally unlike that of euphysoclists: the marlin (*Makaria: Lamonte* 1958, Scombresocidae and Cyprinodontidae (*Rauther* 1940), the Scabbard fish or "Espada" (*Aphanopus: Bone* 1971), deep-sea eels (*Polycanthodus, Synaphobranchus: Wittenberg* et al. 1980), *Argentina* (*Fänge* 1958; *Fahlén* 1965), and so on.

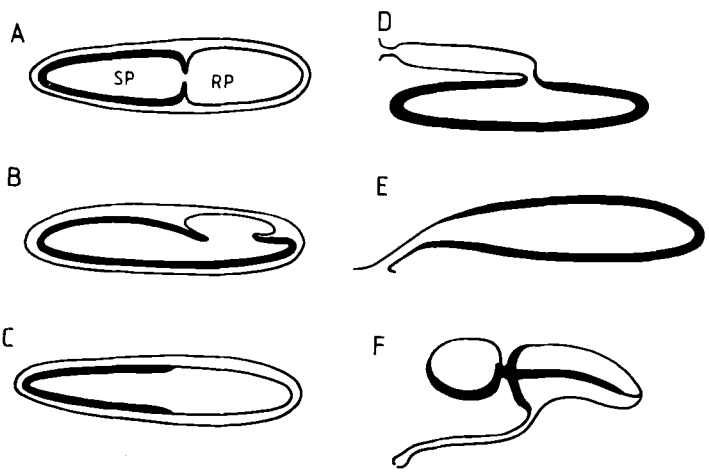

Fig. 1. Diagram of a few types of teleostean swim bladders. *A, B, C,* euphysoclists; *D,* eel *(Anguilla); E,* salmonid; *F,* cyprinid (carp); *RP,* resorbent part; *SP,* secretory part. *Thick lines* indicate smooth muscle layers with α-adrenergic responses to catecholamines. These muscles act in gas exchange reflexes (p. 131, 132)

2.2 Vascularization

2.2.1 Lungs and Respiratory Swim Bladders

Müller (1845) defined lungs as oxygen-absorbing structures which receive venous blood via arteries and give off arterial blood via veins. A circulation of this type is found in lungfishes (dipnoans), in which the lungs are supplied by pulmonary arteries, and oxygenated blood returns to the heart

via pulmonary veins. The primitive pulmonary circulation of lungfishes is kept partly separate from the systemic circulation by incomplete septa in the heart (*Johansen* 1970).

Tendencies toward a pulmonary type of circulation are met within several fishes with respiratory swim bladders. Genuine pulmonary arteries are found in bichirs *(Polypterus).* In most fishes with a respiratory swim bladder, the oxygenated blood when it returns to the heart avoids the hepatic circulation and passes via the posterior cardinal vein, hepatic veins, or a special "pulmonary vein" directly to the heart (*Carter* 1957).

Fishes have a simple circulation, and oxygenated blood from a respiratory swim bladder becomes mixed with venous blood from different parts of the body when passing the heart (except in dipnoans; see above).

2.2.2 Nonrespiratory Swim Bladders

Physostomes

The swim bladders of physostomes are supplied with blood from the coeliacomesenteric artery, and from the dorsal aorta. In the sturgeon *(Acipenser stellatus)* and herring *(Clupea harengus)* the arterial branches are accompanied by veins joining the hepatic portal system (*Jasiński* 1965; *Fahlén* 1967b). These paired vessels might constitute low efficiency countercurrent systems. In salmonids (*Fahlén* 1959), the pike (*Esox lucius: Jasiński* 1965), and carps (Cyprinids: *Rauther* 1940; *Fänge* and *Mattisson* 1956) branches of small arteries and veins form very distinct countercurrent bundles or retia mirabilia. In salmonids these have the character of small two-dimensional "microretia". In most physostomes countercurrent vascular bundles in the swim bladder are relatively weakly developed, but retia mirabilia of considerable size are found in the pike *(Esox)* and in eels *(Anguilla, Conger).*

Physoclists

A double circulation is found in the swim bladder of the euphysoclists (and the eels; *Anguilla, Conger*). The *secretory part* of the swim bladder, usually supplied from the coeliacomesenteric artery, has well-developed countercurrent vascular bundles (retia mirabilia). All the blood entering and leaving the gas gland area and the secretory mucosa passes the countercurrent vessels. The veins leaving the countercurrent bundles join the hepatic portal system. The resorbent part of the swim bladder is supplied from the dorsal aorta, or from a branch of the coeliacomesenteric artery that bypasses the countercurrent systems (Fig. 2). The arteries of the resorbent part divide into a rich plexus of capillaries, which form a continuous layer ("Kapillaris": *Saupe* 1940; *Ross* 1979a) beneath the inner

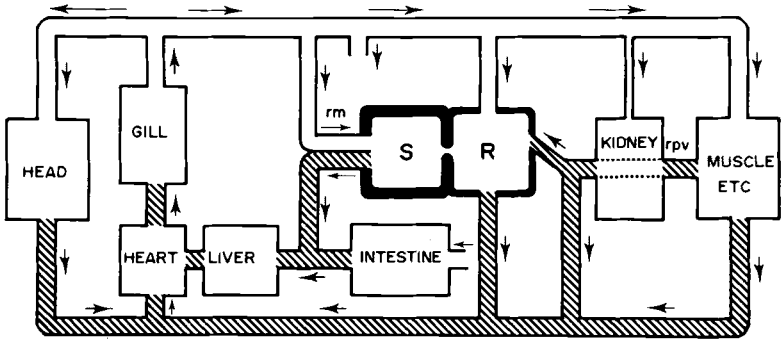

Fig. 2. Blood circulation in the euphysoclist type of swim bladder in relation to the body circulation. *R*, resorbent part of the swim bladder; *S*, secretory part of the swim bladder; *rm*, rete mirabile; *rpv*, renal portal vessels. *Shaded area*, venous blood

epithelium. The veins from the resorbent capillary plexus join the posterior cardinal vein system. This plexus probably also receives a blood supply from the renal portal vein system. In the perch (*Perca fluaviatilis*, 18–20 cm body length) the total length of the capillaries of the resorbent area (the oval) was about 45 m (*Saupe* 1940). *Ross* (1979a) calculated a total length of 31.3 m of capillaries in the resorbent area (the "oval") of a saithe *(Pollachius virens)*. The resorbent capillary plexus covers 40% of the internal surface of the swim bladder.

In eels *(Anguilla, Conger)* two well-developed rete mirabile portions receive blood from the swim bladder artery and supply the secretory part of the swim bladder. The rete vessels deliver blood to the hepatic portal vein. The pneumatic duct of the eels, which serves as resorbent area, is vascularized by a pulmonary-like capillary network. The structure and the mode of vascularization of the eel pneumatic duct are reminiscent of conditions in an amphibian lung. From the resorbent capillary plexus of the pneumatic duct a vein, much like a "pulmonary vein", carries blood to the ductus Cuvieri, or directly to the atrium of the heart (*Mott* 1950).

2.2.3 Structure of the Rete Mirabile

Anatomically *rete mirabile* means a blood vessel dividing into branches which rejoin to form a larger vessel. The type of rete mirabile found in the fish swim bladder is a double one. It consists of one arterial and one venous part which form a countercurrent system of closely packed parallel capillaries.

Woodland (1911a) distinguished two types of swim bladder rete mirabile: (1) unipolar and (2) bipolar (Fig. 3). *Krogh* (1929) found that the bipolar rete mirabile (composed of two separate lobes) of a moderate-sized

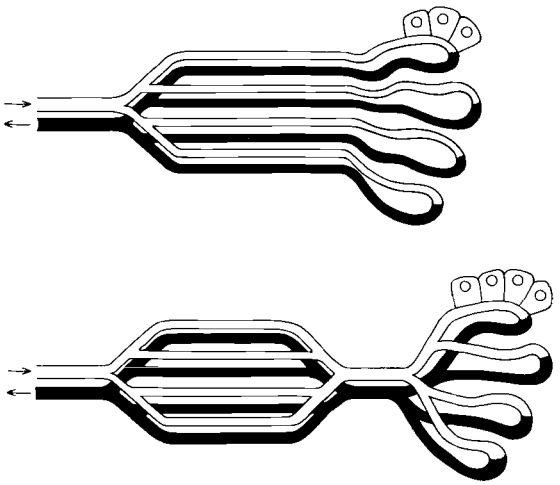

Fig. 3. Two main types of swim bladder countercurrent vascular bundles as distinguished by *Woodland* (1911). *Upper*, unipolar rete mirabile (the most common type). *Lower*, bipolar rete mirabile (mainly in eels: *Anguilla, Conger*). The number of parallel capillaries in a single bundle (rete) varies from extremely few (in so-called microretia) to hundreds of thousands

eel *(Anguilla)* consists of 88 000 venous and 116 000 arterial capillaries. This well-developed rate mirabile consists of arterial (afferent) and venous (efferent) capillaries separated only by a 100- to 150-μm-thick basement membrane (*Stray-Pedersen* and *Nicolaysen* 1975). In the arterial capillaries the endothelium is relatively thick with no fenestrae. The cytoplasm is penetrated by great numbers of intracellular channels with constrictions indicating peristaltic or pinocytotic activity (*Bendayan* et al. 1974; *Rasio* et al. 1977). The venous capillaries have a thin endothelium with many pores or fenestrae and structurally resemble capillaries of endocrine glands and the kidney (*Jasiński* and *Kilarski* 1971).

2.3 Histology and Ultrastructure

2.3.1 Swim Bladder Wall

Nonteleosts. Lungs and respiratory swim bladders of nonteleosts are structurally adapted for gas exchange by a richly folded alveolar or spongy inner lining with extensive capillary blood supply. No countercurrent systems occur. The inner epithelium of dipnoan (lungfish) lungs contains intracellular osmiophilic bodies similar to those of the lungs of higher vertebrates (*Hughes* 1973). The ultrastructure of the lung-like swim bladder of a bichir *(Polypterus)* was examined by *Marquet* et al. (1974). Layers of smooth or striated muscles are found in the swim bladders of lungs of several nonteleostean fishes (*Rauther* 1940), but few detailed studies have been made.

Teleosts. Fahlén (1967a,b, 1968, 1971) investigated the histology and ultrastructure of the swim bladder of certain physostomes: salmonids (*Salmo, Coregonus,* etc.) and the herring *(Clupea harengus).* The swim bladder of rainbow trout *(Salmo gairdneri)* was studied by *Brooks* (1970), who described osmiophilic lamellar bodies within the inner epithelial cells.

Histological studies on the swim bladder of physostomes show a considerable diversity in the structure of the wall (cyprinids: *Evans* 1925; *Fänge* and *Mattisson* 1956; *Umbra: Rauther* 1940; *Dallia: Crawford* 1974; *Anguilla: Dorn* 1961). Layers of smooth muscles are found in most physostome swim bladders. These muscles either form a thin continuous layer in the whole swim bladder (clupeids, salmonids, esocids) or form distinct bands and sphincters in special parts of the swim bladder (characids and cyprinids: *Müller* 1845; *Rauther* 1940; *Fänge* and *Mattisson* 1956; *Evans* 1925, 1930) (Fig. 1F).

The euphysoclist swim bladder wall consists of three main layers: tunica externa, submucosa, and mucosa. The two innermost layers are sometimes called tunica interna. The tunica externa is a dense connective tissue capsule around the two inner layers. It is composed of collagen fibrils (*Morris* and *Albright* 1975) but may contain some smooth or striated muscles. The striated muscles are sound producing (*Eichelberg* 1977; *Trewavas* 1977). The submucosa, which separates the tunica externa from the mucosa, consists of a loose connective tissue, often jelly-like in consistency. It contains nerves and vessels (such as retia mirabilia). The mucosa, or the inner lining of the bladder, consists of an epithelium which is associated with blood capillaries, a delicate connective tissue membrane (lamina propria), and a smooth muscle membrane or muscularis mucosae. The smooth muscle bundles constituting the muscularis mucosae form a basket-like net-like layer as described by *Saupe* (1940) in the perch *(Perca fluaviatilis)* swim bladder.

The division of the euphysoclist swim bladder into a "secretory" and a "resorbent" part usually concerns only the two inner layers, the submucosa and the mucosa (*Fänge* 1953; *Lappenas* and *Schmidt-Nielsen* 1977). The boundary between the two regions is often formed by a mucosal fold called the "diaphragm" or "oval". Circular and radial smooth muscles are arranged in a sphincter-like way in this fold (*Woodland* 1913; *Nilsson* 1971; *Ross* 1979a). The secretory mucosa-submucosa is much thicker than the resorbent mucosa-submucosa. The muscularis mucosae of the secretory part of the swim bladder is strongly developed, but it is very thin in the resorbent part. The submucosa of the secretory part is impregnated with guanine crystals (*Lappenas* and *Schmidt-Nielsen* 1977), lipid membranes (*Wittenberg* et al. 1980), or platelets of unknown nature (*Brown* and *Copeland* 1977, 1978). These materials are thought to make the secretory submucosa impermeable to gases (*Denton* et al. 1970, 1972).

Removal of the guanine-containing "silvery layer" from the swim bladder of the conger eel increases the gas permeability about 100 times (*Denton* et al. 1972). The encrustations with guanine, etc., make the secretory submucosa difficult to study histologically, because fixatives, embedding substances, and stains do not penetrate the tissue. Under the electron microscope it is seen to consist of great numbers of folded thin membranes (*Morris* and *Albright* 1975).

The resorbent submucosa-mucosa is extremely thin and closely associated with the resorbent capillary network.

2.3.2 Gas Gland

The gas gland consists of gland-like cells supplied with blood from the countercurrent vascular bundles. The gas gland cells typically derive from the inner epithelium, but in certain physostomes supposed gas gland cells look like modified connective tissue cells, and their epithelial nature is uncertain (cyprinids: *Fänge* and *Mattisson* 1956; *Umbra: Rauther* 1940; *Dallia: Crawford* 1974; *Argentina* – a deep water physoclist: *Fänge* 1958).

In physoclists the gas glands are generally more highly developed than in physostomes. *Woodland* (1911a) classified gas glands into two groups: (1) gas glands in which the epithelium is single layered, unfolded or folded, (2) gas glands consisting of a many-layered epithelium. Transitional forms exist between the two types. Many-layered gas glands are penetrated by clefts, ducts, or anastomosing canals communicating with the swim bladder lumen.

The gas gland cells, cylindrical, cubical, or polyhedric, are arranged around blood capillaries (*Jasiński* and *Kilarski* 1972). In histological sections the cytoplasm appears finely granulated or vacuolated, and slightly eosinophilic. The cells have a polarized structure with the apex directed toward the lumen or a duct communicating with this, and the cell base is in contact with a blood capillary. Giant cells measuring 100 μm or more occur in the gas glands of deep-sea fishes (*Marshall* 1960) and in certain shallow-water forms such as the perch, *Perca fluviatilis* (*Woodland* 1911a; *Fänge* 1953; *Horn* 1975).

When observed in the living condition under the microscope, gas gland cells appear filled with refringent granules and large yellowish spheres. The latter may be released at the cell apex, forming amorphous masses at the surface of the gas gland. The cells are stained intensely brown by OsO_4, indicating the presence of lipids (*Woodland* 1911a,b). *Jacobs* (1930) distinguished three types of cells representing different stages of secretory activity in the gas gland of the perch *(Perca fluviatilis).* The cells seemed to secrete a fluid into the blood capillaries. According to *Woodland* (1911a) intracellular gas vacuoles do not appear, but gas bubbles

occur in a thin liquid layer covering the gas gland (*Fänge* 1953). Histochemical studies show that the cytoplasm is rich in glycogen particles (*Copeland* 1951; *Fänge* 1953; *Jasiński* and *Kilarski* 1972; *Schwartz* 1971). *Dorn* (1961) demonstrated the presence of carbonic anhydrase in gas gland cells of the eel *(Anguilla).*

Electron microscopic investigations show that the gas gland cells contain rough and smooth endoplasmic reticulum, glycogen particles, a Golgi complex, and lipid-like spherical formations (multilamellar or dark bodies) showing very strong reduction of OsO_4. The osmiophilic bodies resemble multilamellar bodies in vertebrate lungs (*Morris* and *Albright* 1975) and are identical to the "gas"-forming bodies of *Copeland* (1969). Mitochondria are small and filamentous with few tubular cristae. They are relatively numerous in some species and sparse in others (*Copeland* 1969; *Jasiński* and *Kilarski* 1972; *Morris* and *Albright* 1975).

The basal paravascular cell base of the gas gland cells is folded in a complex way. The basal infoldings are unique in that they lack mitochondria. The gas gland capillaries have very thin fenestrated endothelial cells with large mitochondria. In the presence of fenestrae they resemble the venous capillaries of the rete mirabile (*Copeland* 1969; *Morris* and *Albright* 1975).

2.4 Innervation

Nerve cells and vagal nerve fibers have been found in lungs and swim bladders of nonteleostean fishes (*Rauther* 1940). Fine varicose adrenergic fibers, mainly distributed around blood vessels, occur in the wall of the swim bladder of the gar *(Lepisosteus)* (*Nilsson* 1981), but no adrenergic nerve elements were detected in the lungs of lungfish *(Protopterus)* (*Abrahamsson* et al. 1979).

The teleostean swim bladder is well provided with nerve fibers and nerve cells of vagal and sympathetic origin. The main nervous supply is from the ramus intestinalis of the vagus. This ramus is in reality a vago-sympathetic trunk containing strong sympathetic components which join the vagus close to the brain (*Nilsson* 1976, 1982). Sympathetic fibers, partly anastomosing with the vagus, also reach the swim bladder from the celiac ganglion. Some vagal fibers may be sensory (*Quotob* 1962), but most are efferent fibers supplying smooth muscles, blood vessels, and probably the gas gland cells. *Evans* (1925) described a ganglionic layer in the isthmus that connects the two chambers of the carp (Cyprinid) swim bladder. *Dorn* (1961) observed large nerve cells in the pneumatic duct of the eel *(Anguilla anguilla).* In euphysoclists such as the cod *(Gadus morhua),* and the perch *(Perca fluviatilis),* a "gas gland ganglion" consisting

of thousands of neurons is associated with the rete mirabile, and outside the swim bladder in the bundles of nerves entering the gas gland region a "swim bladder nerve ganglion" is found (*Fänge* 1953; *McLean* and *Nilsson* 1981). The gas gland ganglion consists of relatively small (20 μm) nerve cells showing strong acetylcholine esterase activity but no catecholamine fluorescence. High activity of the acetylcholine synthesizing enzyme choline acetyltransferase is found in the gas gland and the secretory mucosa (*Fänge* and *Holmgren* 1982). Fibers with strong acetylcholine esterase activity, probably originating in the "gas gland ganglion", are distributed at the base of the gas gland cells. The "swim bladder nerve ganglion" comprises relatively large (40 μm) cells, which are positive for catecholamines and acetylcholine esterase. These cells may constitute adrenergic sympathetic postganglionic neurons (*McLean* and *Nilsson* 1981).

The smooth muscle layers of the swim bladder of physostomes (*Fahlén* 1965; *Fahlén* et al. 1965; *Fänge* et al. 1976; *McLean* and *Nilsson* 1981) contain rich plexuses of catecholamine-fluorescent nerve fibers. The fibers are also distributed within the rete mirabile and the gas gland.

Chemical estimations show that the muscularis mucosae of the secretory part of the swim bladder of the wrasse *(Ctenolabrus)* and the cod *(Gadus)* contain considerable amounts of noradrenaline and small quantities of adrenaline (*Abrahamsson* and *Nilsson* 1976; *Fänge* et al. 1976).

2.5 Depth Adaptations of Swim Bladder Structures

Anatomical modifications of the skeleton supposed to increase the strength of the swim bladder have been found in certain deep-water living cod fishes (gadids: *Hagman* 1921).

The amount of guanine in the swim bladder wall increases with depth (*Blaxter* and *Tytler* 1978; *Ross* and *Gordon* 1978). The swim bladder of the conger eel *(Conger)*, which lives deeper than the common eel *(Anguilla)*, contains more guanine and is about ten times less permeable to O_2 and CO_2 than the swim bladder of the latter (*Denton* et al. 1972; *Kutchai* and *Steen* 1971). However, when the common eel metamorphoses to the silver eel stage before migrating to spawning depths, the guanine content of the swim bladder increases drastically (*Kleckner* 1980b).

A direct relationship exists between the degree of development of the gas gland-rete mirabile complex and the depth range. In deep-living fishes the numbers and sizes of the retia and the surface of the gas gland increase with depth (*Denton* 1961; *Marshall* 1979). Whereas the retia of surface-living fishes are less than 1 mm long, those of deep-sea fishes measure 25–65 mm in length (*Marshall* 1979; *Wittenberg* et al. 1980). In the common eel *(Anguilla)* the two retia increase considerably in size during the spawning migration (*Kleckner* 1980a).

The large size of the countercurrent vascular system (rete) in deep-sea fishes is important both for the deposition of gases, and by serving as a lock against diffusion loss of gases (*Scholander* 1954). The rediffusion of oxygen across the rete capillaries permits deep-sea fishes to keep an oxygen tension in the swim bladder blood vessels, which is perhaps 1000–3000 times larger than that in the systemic blood vessels (*Rasio* et al. 1977).

3 The Swim Bladder Gases

The swim bladder contains the same gases that occur in the air, the ambient water, and the body fluids. In the living fish the swim bladder volume is normally kept constant, but gases are continuously entering and leaving the swim bladder at the same rate (steady state).

3.1 Steady State Composition

3.1.1 Oxygen

In shallow-living fishes the oxygen concentration in the swim bladder is usually near that of the air (*Scholander* and *Van Dam* 1953; *Green* 1971), but in the eel *(Anguilla)* and the toadfish *(Opsanus)* high percentages of oxygen may occur (*Wittenberg* 1961). The percentage of oxygen in the swim bladder increases with depth (Table 3), as shown originally by *Biot* (1807). The values in Table 3 are from newly caught fishes. Because it takes a considerable time to haul up deep-water fishes, the composition of their swim bladder gases may have changed from normal. But the errors are probably small, because diffusion of gases through the swim bladder wall is slow. Nevertheless *Sundnes* et al. (1977) report that sampling of swim bladder gases in an underwater laboratory gives more constant and thus more reliable values than conventional methods. In extreme deep-sea fishes, such as *Bassogiagas profundissimus,* living at 7000 m depth (*Nielsen* and *Munk* 1964) the oxygen partial pressure, calculated with an average oxygen percentage of 90% in deep-sea fish swim bladders, may exceed 600 atmospheres. The buoyancy effect of the swim bladder of deep-sea fishes must be rather low because of the increased densities of the compressed gases. The values of the swim bladder as an oxygen store for respiration ought to be higher in these fishes than in shallow-living ones, but it is not known whether this is of biological importance.

Table 3. Oxygen in the swim bladder of fishes caught from various depths in seawater (SW) of fresh water (FW) (the list of examples could easily be made longer)

Fish	Depth (m)	% O_2	Approximate pO_2 (atm)	Authors
Eucinostomus gula (SW)	0	21	0.2	*Green* (1971)
Perca fluviatilis (FW)	0	20	0.2	*Jacobs* (1930)
Anguilla anguilla (SW, FW)	0	44.7	0.4	*Jacobs* (1898)
Opsanus tau (SW)	0	50	0.5	*Fänge* and *Wittenberg* (1958)
Salvelinus namaycush (FW)	0–6	0.3	0.02	*Saunders* (1953)
Perca flavescens (FW)	0–6	31.9	0.4	*Saunders* (1953)
Perca flavescens (FW)	20	48.4	1.4	*Saunders* (1953)
Catostomus commersoni (FW)	6–11	4–42	0.07–0.8	*Saunders* (1953)
Lota lota (FW)	6–11	34.4	0.7	*Saunders* (1953)
Lota lota (FW)	120	87.8	11	*Saunders* (1953)
Argentina silus (SW)	80	77.4	7	*Fänge* (1958)
Leucichthys artedi (SW)	90	68	6.8	*Saunders* (1953)
Bathypelagic sp. (SW)	> 100	54–93	6–10	*Kanwisher* and *Ebeling* (1957)
Aphanopus carbo (SW)	1000	82.1	83	*Bone* (1971)
Conger conger (SW)	1000	87.7	89	*Jacobs* (1898)

3.1.2 Carbon Dioxide

The concentration of carbon dioxide in the swim bladder is normally low. *Scholander* and *Van Dam* (1954) measured 0.17–1.02% CO_2 in marine fishes. *Tait* (1956) and *Saunders* (1953) reported the range 0.2–2.1% in swim bladders of freshwater fishes.

3.1.3 Nitrogen

About 99% nitrogen is found in the swim bladders of certain species of physostomes living at 60–70 m depth (resembling 7–9 atm hydrostatic pressure) (*Hüfner* 1892; *Tait* 1956; *Saunders* 1953; *Scholander* et al. 1956; *Sundnes* 1959; *Sundnes* et al. 1969; *Abernethy* 1972). In deep-sea fishes the percentage of nitrogen is low, but at the high hydrostatic pressures of nitrogen of 5–15 atm in the swim bladder of fishes caught from considerable. Thus *Scholander* and *van Dam* (1953) reported partial pressures of nitrogen of 5–15 atm in the swim blader of fishes caught from 900 m depth. Obviously both in physostomes and physoclists nitrogen may occur normally in the swim bladder at partial pressures above 0.8 atm, the approximate pN_2 of the atmosphere and the water.

3.1.4 Other Gases

Argon, like nitrogen, is enriched in the swim bladder of deep-water fishes. The Ar-N_2 ratio in the swim bladder is usually about the same or slightly

higher than in the air, where it is 1.8 (*Schloesing* and *Richard* 1896; *Scholander* 1954; *Tait* 1956; *Wittenberg* 1958; *Abernethy* 1972). Because argon is inert, it does not pass into the swim bladder as a result of a chemical reaction, but some "physical mechanism" must exist for the transport of argon and other inert gases (*Piiper* et al. 1962).

Probably all gases dissolved in the surrounding water are deposited into the swim bladder. *Traube-Mengarini* (1889) showed the presence of hydrogen in the swim bladder of physostomes *(Cyprinus)* and a physoclist *(Mugil)* kept in seawater containing dissolved hydrogen. *Wittenberg* and *Wittenberg* (1961) obtained high concentrations of carbon monoxide (CO) in the swim bladder of toadfish *(Opsanus tau)* kept in seawater saturated with mixtures of oxygen and carbon monoxide. The toadfish, like certain other teleosts, survives for long periods of hypoxia. The deposition of helium, neon, xenon, and krypton in the swim bladder of the toadfish has been studied by *D. Wittenberg* et al. (1981).

3.2 Newly Secreted Gas

The gas mixture introduced into the swim bladder by secretion differs in composition from the steady state composition. Oxygen is the main gas deposited, but *Jacobs* (1932) showed that in the perch *(Perca fluviatilis)* carbon dioxide reaches high values. Concentrations of CO_2 of up to 24.6% were measured during gas secretion. Similar values were obtained by *Meesters* and *Nagel* (1935). *Wittenberg* (1958) measured up to 25% CO_2 (average 10%–12%) in newly secreted gas in the scup *(Stenotomus)*, and *Wittenberg* et al. (1964) found 17%–37% CO_2 (61.2%–79.2% O_2) in the swim bladder of another marine species, the bluefish *(Pomatomus saltatrix)* during gas secretion. They concluded that the newly secreted gas contains 65%–86% O_2, the rest being mainly CO_2.

The highest values of CO_2 occur at the peak of gas secretion, when resorption of gases is minimized due to expansion of the gas-impermeable secretory mucosa-submocosa (= oval closure). In the cod *(Gadus morhua)* the maximal gas secretion occurs 3–6 h after initiation of secretion by puncture and emptying of the swim bladder. During this period the values of CO_2 in the swim bladder gases increase to 12%–13% and the oxygen values may rise to 80% (*Fänge* 1953). *Scholander* (1956) found in the cod that gas bubbles collected under a plastic film at the surface of the gas gland contain 5.0%–15.8% CO_2 and 30.1%–81.1% O_2, which reflects the composition of the newly secreted gas. In the barracuda *(Sphyraena barracuda)* gas bubbles collected by the same method contained 3.6%–5.3% CO_2. In the wrasse *(Ctenolabrus)* the percentage of CO_2 never exceeded about 3% during any phase of gas secretion

(*Fänge* 1953). *Green* (1971), studying gas secretion in a tropical fish *(Eucinostomus)*, reported 1.4%–11.0% CO_2 and 89.5%–98.6% O_2 in newly secreted gas.

Concentrations of CO_2 and O_2 deposited by the slowly functioning gas secretory systems of physostomes are of similar magnitudes to those in physoclists. *Jacobs* (1934) found up to 11.2% CO_2 and 63.6% O_2 in the swim bladder of minnow *(Phoxinus)* and 15.8% CO_2 and 68.3% O_2 in the pike *(Esox)* during gas secretion induced by evacuation of the swim bladder. The values cited are the maximal ones reported. The average values are lower. Salmonids deposit a gas mixture containing 33.4%–69.3% O_2 during seasonal migration to deep water *(Coregonus lavaretus: Sundnes* 1963; *Sundnes* et al. 1969).

4 Gas Transport Properties of Fish Blood

4.1 Oxygen

Oxygen is transported in fish blood bound chemically as oxyhemoglobin and physically dissolved in the plasma. The number of hemoglobin-containing cells measured as hematocrit varies from about 50% in active fishes *(Scomber, Trachurus, Clupea, Pollachius)* to 17% in sluggish fishes such as the angler *(Lophius piscatorius)* (*Larsson* et al. 1976; *Everaarts* 1978). Antarctic icefish of the family Chaenichthyidae almost completely lack hemoglobin. In this fish the oxygen is transported solely in physical solution. The capacity of the plasma of icefish to dissolve oxygen may be favored by low temperature (*Ruud* 1954, 1965). The icefish has no swim bladder.

In hemoglobin-rich fishes with high hematocrits the oxygen capacity of the blood varies within the range of 5%–15%. For the marine bluefish *(Pomatomus saltatrix)* 14 vol.% is reported (*Wittenberg* et al. 1964), and *Scholander* (1956) measured an oxygen capacity of 10–13 vol.% in the barracuda *(Sphyraena barracuda)*. The oxygen capacity of mammalian blood is about 20 vol.% (human arterial blood). Thus fish blood generally has a lower oxygen capacity than mammalian blood.

The amount of oxygen carried by fish blood depends not only on the concentration of hemoglobin, but also on the molecular properties of the hemoglobin, which are influenced by the temperature, the pH (or CO_2 content), and by the presence of organic cofactors such as ATP (*Powers* et al. 1979). High temperature, like the Root effect, reduces the oxygen capacity of fish blood. This may restrict certain antarctic fishes *(Trematomus)* to cold water with a temperature close to zero (*Grigg* 1967).

The oxygen dissociation curves of fish blood are usually hyperbolic, or of complex shape due to multiple hemoglobins (*Scholander* 1957; *W. Wittenberg* et al. 1981).

If the blood pH is lowered by lactic acid or carbon dioxide, oxygen is usually liberated from the oxyhemoglobin, a process known as the Bohr or Root effect. The Bohr effect concerns mainly the oxygen affinity, while the Root effect, considered as an extension of the Bohr effect, also concerns the oxygen capacity (*Baines* 1975; *Root* 1931). In some deep-sea fishes the Root effect persists at oxygen pressures as high as 140 atmospheres, but in others it disappears at high oxygen pressures (*Scholander* and *van Dam* 1954; *Scholander* 1957). The sensitivity of fish blood to acidification varies greatly between species (*Willmer* 1934; *Ledebur* 1937; *Black* 1940).

The reactions of CO_2 taking place in the red cells during the Bohr effect are rate limiting for this effect, which has physiological significance. Thus the movement of O_2 out of human red cells as a consequence of reduced ambient pO_2 has a half-time of 0.012 s, but the release of O_2 under the influence of CO_2 (Bohr effect) requires about 0.12 s (*Craw* et al. 1963). Similar rate relationships have been found in fish blood (*Forster* and *Steen* 1969).

4.2 Carbon Dioxide

In fish blood CO_2 is transported as bicarbonate and to a small extent as physically dissolved CO_2. The binding of CO_2 as bicarbonate, and the release of CO_2 from bicarbonate, require the interaction of hemoglobin and carbonic anhydrase in the erythrocytes. Carbonic anhydrase catalyzes the "chloride shift" – the exchange of HCO_3^- for Cl^- across the cell membrane – in fish erythrocytes (*Cameron* 1978). Fish red cells, as red cells of other vertebrates, are rich in this enzyme (*Maren* 1967).

The carbon dioxide content of fish blood is much lower than that of mammalian blood, partly because in fishes CO_2 rapidly disappears by diffusion into the water, when the blood is passing through the gills. Most fishes carry 5–10 vol.% (2–3 mm Hg) CO_2 in the blood (*Rahn* 1966; *D'Aoust* 1970), whereas mammalian blood holds about 50 vol.% CO_2 (human venous blood). Fishes living in water high in CO_2 and low in O_2 carry higher concentrations of CO_2 in the blood than species from CO_2-poor and O_2-rich waters, and their blood has a higher buffering capacity. Examples of such fishes are the obligatory air-breathing African lungfish *(Protopterus),* and the gar *(Lepisosteus)* (*Grigg* 1974; *Lenfant* and *Johansen* 1968). In air-breathing fishes the CO_2 tension of the blood may reach 90 mm Hg (*Rahn* et al. 1971). Even a few water-breathing

fishes living in oxygen-rich and CO_2-poor waters (for instance the mackerel, *Scomber scombrus*) have a high blood buffering capacity, perhaps as an adaptation to high production of lactic acid and CO_2 by the swimming muscles (*Root* 1931).

5 Gas Exchange Through the Pneumatic Duct

5.1 Lungs and Respiratory Swim Bladders (Table 1)

5.1.1 Nonteleosts

The African *(Protopterus)* and South American *(Lepidosiren)* lungfishes are obligate air-breathers with degenerated gills. The South American lungfish consumes 42 ml O_2/kg body weight per hour, more than 90% of which comes from inhaled air (*Sawaya* 1946). The Australian lungfish *(Neoceratodus)*, a facultative air breather, uses its lung during nocturnal active periods as an accessory structure for oxygen uptake (*Grigg* 1965; *Pfeiffer* 1968). In lungfishes (dipnoans) inspiration is brought about by a buccal pumping mechanism. A high intrapulmonary pressure enhances O_2 uptake (*Wood* and *Lenfant* 1976). The buccal pump muscles are phylogenetically derived from gill ventilatory muscles (*McMahon* 1969). Nerve-induced opening and closing of a glottis are involved in the chain of events of pulmonary ventilation. Expiration is largely passive, but smooth muscles of the lung wall may have some importance. These muscles are innervated by the vagus nerve. The motor nerve fibers may be cholinergic, since nerve-induced contractions are potentiated by eserine (10^{-5} *M*), and strips of muscles are contracted by acetylcholine or carbacholine, the effects being antagonized by atropine (10^{-8}–10^{-6} *M*). Catecholamines contract or relax the muscles, but the effects are weak (*Abrahamsson* et al. 1979).

Buccal pump mechanisms are also responsible for inspiration in bichirs *(Polypterus, Calamoichtys)*, gars *(Lepisosteus)*, and the bowfin *(Amia calva)*. The ventilation of the lung-like swim bladder in gars was investigated by *Rahn* et al. (1971). Gars *(Lepisosteus)* inhale through the mouth but exhale through the opercular openings. At low water temperature the gills suffice for the O_2 uptake, but at warm temperature (20–25°C) the gars obtain 70%–80% of their O_2 requirements from the swim bladder. Elimination of CO_2 proceeds entirely through the gills at low temperature, but at warm temperature up to 8% of the respiratory CO_2 is excreted through the swim bladder. The gar swim bladder is ventilated several times per hour. Expiration is thought to be passive, brought about by the hydrostatic pressure. However, the physiological importance of

the striated and smooth muscles in the wall of the swim bladder of gars (*Potter* 1927; *Nilsson* 1981), bowfin (*Amia*), and bichirs (*Polypterus, Chalamoichtys*) has not been investigated.

The blood streaming to the lung/respiratory swim bladder comes from the efferent gill arteries. If, as may often happen with air-breathing fishes, the ambient water is poor in oxygen, oxygen may diffuse into the water when the blood passes the gills. In the Australian lungfish (*Neoceratodus*) oxygen loss from the gills is reduced by a high oxygen affinity of the blood. In other dipnoans reduction of gill function protects against diffusion loss of O_2 when the fishes are surrounded by stagnant water (*Wood* and *Lenfant* 1976). In gars (*Lepisosteus*) O_2 loss from the gills is probably minimized by shunts allowing some blood to bypass the gills when the fish is breathing air (*Rahn* et al. 1971). Such shunting of part of the ventral aortic blood past the gas exchange surface of the gills is supposed to take place in several air-breathing fishes (*Satchell* 1976). However, *Randall* et al. (1981) found no shunts in the bowfin (*Amia*).

The neuromuscular control of the ventilatory movements in air-breathing nonteleostean fishes is imperfectly known. The brain center, which produces rhythmical signals to the respiratory muscles, seems to be more sensitive to lack of O_2 than to excess of CO_2.

5.1.2 Teleosts

Quite a number of teleosts of different families possess anatomical structures used for the uptake of atmospheric O_2. Organs occasionally adapted for aerial respiration include the skin, gills, mouth and buccal cavity and special structures within these, intestine, and swim bladder (*Carter* 1957; *Johansen* 1970). Only a handful of species use the swim bladder for O_2 uptake (Table 2).

Most air-breathing teleosts live in stagnant tropical fresh waters. As a rule they are facultative air-breathers, using the swim bladder as an accessory respiratory organ when the O_2 content of the water is low. A minority are obligate air-breathers: *Arapaima, Pantodon, Gymnarchus* (Table 2).

The "pirarucu", *Arapaima gigas*, is the largest fish of the Amazonas and may sometimes grow to a body length of 5 m. It has reduced gills and depends on the lung-like swim bladder for its normal respiration. The adult pirarucu comes to the surface every 10–15 min to breath air (*Lüling* 1971), but young fishes ventilate their swim bladder every 1–2 min (*Stevens* and *Holeton* 1978). The mode of ventilation is unusual in that the pirarucu inspires by aspiration, probably using the diaphragm-like ventral wall of the swim bladder to produce suction (*Farrel* and *Randall* 1978). The swim bladder gets its blood supply from the dorsal aorta. Because of gill reduction this carries blood with an unusually low pO_2 (*Farrel* and *Randall* 1978).

Among the characins, which are carp-like freshwater fishes, the families Erythrinidae and Lebiasinidae use the swim bladder for oxygen uptake. As in carp (cyprinids), the swim bladder consists of chambers connected by a narrow canal or isthmus. The highly vascularized posterior chamber is used for O_2 uptake. The characin *Hoploerythrinus* ("jeju") comes to the surface every 3 min to inhale air by a buccal force mechanism. *Kramer* (1978) carried out cinematographic analyses of the air exchange. Each time the fish comes to the surface, it ventilates the swim bladder two to three times in rapid succession. The gas pressure in the swim bladder exceeds the ambient hydrostatic pressure by abut 12 cm H_2O (*Farrel* 1978; *Farrel* and *Randall* 1978). In *Hoploerythrinus* the swim bladder contains about 7% O_2 and 0.7% CO_2 during aquatic respiration, but when the fish is breathing air the swim bladder contains 1.8% O_2 and 1.5% CO_2 (*Carter* and *Beadle* 1931).

Piabucina, another characin, can maintain normal O_2 consumption by aquatic respiration in water with O_2 pressures down to about 70 mm Hg, but if exposed to water containing less O_2, it switches over to swim bladder respiration (*Graham* et al. 1977). *Piabucina* normally keeps an O_2 concentration of about 8% in the swim bladder, but if hindered from gulping air through the pneumatic duct the O_2 drops to 3% (*Carter* and *Beadle* 1931).

Facultative air-breathing teleosts with a respiratory swim bladder use this almost exclusively for O_2 uptake and depend on gills and the body surface for the excretion of CO_2. The gill epithelium has a high activity of the enzyme carbonic anhydrase which is supposed to catalyze the formation of molecular CO_2 from blood bicarbonate (*Maren* 1967). In contrast to mammals, teleosts cannot use the carbonic anhydrase of the erythrocytes for the release of CO_2 in the air-breathing organ. *Burggren* and *Haswell* (1979) found that in *Hoploerythrinus* (and in the bichir, *Calamoichtys*), the respiratory swim bladder lacks carbonic anhydrase, which makes elimination of CO_2 through the swim bladder impossible, or difficult. *Randall* et al. (1978) found that increasing artificially the CO_2/HCO_3^- reaction velocity in the blood of *Hoploerythrinus* by infusion of bovine carbonic anhydrase significantly raises the CO_2 exchange ratio of the swim bladder and reduces hypercapnic acidosis caused by air exposure.

The obligatory air-breathing pirarucu *(Arapaima)*, unlike other teleosts, has a relatively high excretion of CO_2 via the swim bladder (*Farrel* and *Randall* 1978). Due to reduced gill function this fish excretes insufficient amounts of CO_2 via the gills. It is not known whether carbonic anhydrase, in the swim bladder or the blood, plays a role in the excretion of CO_2 via the swim bladder in this species.

The ventilation of teleostean respiratory swim bladders is based on nervous reflexes similar to those controlling gas exchange through the

pneumatic duct of other physostomes. The characins *Hoploerythrinus* and *Erythrinus* increase the frequency of swim bladder ventilation if the pO_2 of the air decreases (*Stevens* and *Holeton* 1978), which indicates that a nervous respiratory center for the breathing movements is sensitive to low pO_2 in the blood. When air-breathing teleosts reach the water surface they first inspire, then immediately expire. The initial inspiration may serve to extend the swim bladder wall, triggering a reflex similar to the "gas-spitting reflex" (*Franz* 1937). Nervous control of the pneumatic sphincter and smooth muscles in the wall of the swim bladder are probably important in the ventilatory activities.

5.2 Nonrespiratory Physostome Swim Bladders

Physostomes change their swim bladder volume both by gas secretion and resorption, and by intake and extrusion of gases via the pneumatic duct.

5.2.1 Air Intake Through the Pneumatic Duct

If the swim bladder is emptied by pressure decrease or puncture, most physostomes are able to fill it again by gulping air at the surface. The process is completed within minutes (*Overfield* and *Kylstra* 1971). The air is pumped into the swim bladder by buccal muscles (*Jacobs* 1934). Intake of air through a pneumatic duct is essential for the initial inflation of the embryonic swim bladder in certain physoclists (*Jacobs* 1938; *Kitajima* et al. 1981).

5.2.2 Expulsion of Gases via the Pneumatic Duct

The term *"gasspuckreflex"*, or gas-spitting reflex means a release of gases via the pneumatic duct, which is initiated by decompression. A release of gases may also be initiated by various disturbing factors (vibrations, sudden illumination, etc.; *Verheijen* 1962). *Harvey* et al. (1968) distinguish between a passive *gasspuckreflex,* in which the pneumatic sphincter serves as a safety valve, and an active release of gases initiated by frightening. The *gasspuckreflex* in the sense of *Franz* (1937) involves opening of the pneumatic sphincter, contraction of smooth muscles of the swim bladder wall, and expulsion of gas bubbles from the mouth. A reflex center may be situated within the diencephalon of the brain. Excess internal pressure in the swim bladder (60–80 mm Hg in cyprinids: *Jones* and *Marshall* 1953), contraction of the body wall muscles, and the external hydrostatic pressure contribute to the expulsion of gases through the pneumatic duct. When frightened fishes (salmonids) dive or "sound" into deeper water, as much as 34% of the swim bladder gases is ejected, probably due to active contraction of the swim bladder muscles (*Harvey* et al. 1968).

5.3 Nervous Control of Gas Expulsion Through the Pneumatic Duct

After bilateral vagotomy, and during narcosis, a larger pressure decrease than usual is needed to initiate the *gasspuckreflex* (Table 4). Vagotomy also means that air cannot be swallowed through the pneumatic duct into the swim bladder (*Plattner* 1941). *Kuiper* (1916) and *Plattner* (1941) assumed that the pneumatic sphincter is innervated by vagal inhibitory and sympathetic tonus-increasing fibers. *Fänge* (1953) pointed out a principal similarity between the *gasspuckreflex* of physostomes, and the deflatory reflex of physoclists. Both responses involve opening (relaxation) of a sphincter area, and simultaneous contraction of smooth muscles of the swim bladder wall. Pharmacological results show that the sphincter or the whole pneumatic duct of physostomes contracts by acetylcholine and relaxes by catecholamines, the latter response being mediated by beta-adrenergic receptors. The smooth muscles of the physostome swim bladder wall contract by catecholamines, probably an alpha-adrenergic effect (*Fänge* 1953; *Nilsson* and *Fänge* 1967). Adrenergic influences provoke or facilitate gas release from the swim bladder in the sockeye salmon (*Oncorhynchus: Harvey* et al. 1968) and the herring (*Clupea: Srivastava* 1964).

Table 4. Pressure decrease (mm Hg) needed to evoke release of gas bubbles through the pneumatic duct ("duct release pressure") after different treatment in various physostome species

Fish	Treatment	Control	Experiment	Reference
Carassius	Narcosis	179	282	*Kuiper* (1915) *Plattner* (1941)
Tinca	Narcosis	50–85	81–350	*Plattner* (1941)
Tinca	Vagotomy	10–100	300–400	*Plattner* (1941)
Leuciscus	Vagotomy	60		*Evans* and *Damant* (1928)
Phoxinus	Vagotomy	26–31	69–96	*Franz* (1937)
Anguilla	–	250	–	*Haempel* (1909)
Oncorhynchus	Atropine	28.1	0.1	*Harvey* et al. (1968)

Information is scattered and incomplete, but indicates that adrenergic influences decrease swim bladder volume in physostomes by opening the pneumatic sphincter and contracting the swim bladder muscles. Cholinergic stimuli may close the sphincter more firmly (Table 4; *Oncorhynchus*). However, great species differences probably exist.

6 Gas Secretion

The deposition of gases from the blood into the swim bladder is conventionally termed gas secretion, although what is "secreted" by the gas gland cells may be lactate, hydrogen ions, and CO_2, and not gases. For practical reasons, the term is retained. Gas secretion seems to be unique to the teleost swim bladder, but a related O_2-concentrating mechanism is found in the eye of teleosts and one holostean fish, *Amia calva* (bowfin: *Wittenberg* and *Wittenberg* 1974). Of historical interest is the hypothesis that under extraordinary conditions oxygen secretion occurs in the mammalian lung (*Bohr* 1891; *Haldane* 1935). In deep-water fishes gas secretion takes place against very steep pressure gradients. Energy for the process supposedly comes from the metabolism of the gas gland cells, and from the mechanical pumping of the blood through a counter current capillary system (rete mirabile) by the heart. The amount of energy needed may not be extremely large (*Parr* 1937). *Scholander* (1954) calculated that the work of compressing one mole of oxygen 1000 times at 8°C = $1.99 \times T \times 2.3 \log (P_1/P_2)$ kcals = 3.5 kcals. Somewhat more than 3% of this quantity of O_2 would be enough to generate metabolic energy for the pumping process.

6.1 Rates of Gas Secretion

Most teleostean physostomes are able to fill the swim bladder by gas secretion. In clupeids (herring) the situation is unclear. The herring lacks obvious gas depository structures (rete mirabile, gas gland), but has relatively high O_2 values in the swim bladder (up to 21.5%: *Fahlén* 1967b), and observations of release of gas bubbles from vertically migrating herrings (*Sundnes* and *Bratland* 1972) indicate that some gas secretion may occur. Gas secretion is known from the swim bladder of salmonids (perhaps not all forms), esocids (pike), umbrids (*Umbra,* mudminnow), cyprinids (carp), siluriforms (catfish), and eels *(Anguilla, Conger),* etc. In the goldfish, *Carassius auratus,* gas secretion is linear in time and independent of swim bladder volume. It is inhibited by increasing the ambient pressure above 1.5 atm (*Overfield* and *Kylstra* 1971). *Krohn* and *Piiper* (1962) noticed that gas secretion in the tench *(Tinca tinca)* is abolished in water quilibrated with O_2 at 1 atm pressure. Possibly gas gland cells of the tench in contrary to those of physoclists are sensitive to high pO_2 (*D'Aoust* 1969). Many physostomes require a considerable time to complete gas secretion, but in the common eel *(Anguilla)* gas secretion is as rapid as in physoclists (Table 5).

In physoclists gas secretion is generally fast (Table 6). In the bluefish, *Pomatomus saltatrix,* the time needed for filling the swim bladder after puncture is less than 4 h (*Wittenberg* et al. 1964).

Table 5. Time for filling the swim bladder by gas secretion in different physostomes (families and species)

Fish	Time	Authors
Esocidae		
Esox lucius (pike)	5–17 days	*Jacobs* (1934)
Salmonidae		
Salmo alpinus (char)	2–9 days	*Sundnes* and *Bratland* (1972)
Salmo gairdneri (rainbow trout)	13 days	*Wittenberg* (1958)
Cyprinidae		
Pimephales promelas (fathead minnow)	2 days	*Gee* (1968)
Rhinichtys cataractoe (longnose dace)	3–4 days	*Gee* (1968)
Carrassius auratus (goldfish)	5–17 days	*Overfield* and *Kylstra* (1971)
Phoxinus laevis (minnow)	6–15 days	*Jacobs* (1934)
Tinca tinca (tench)	Several weeks	*Jacobs* (1934)
Anguillidae		
Anguilla rostrata (common american eel)	12–24 h	*Wittenberg* (1958)

Table 6. Rates of gas secretion in physoclists

Fish species	Secretory rate ml/kg/h	Authors
Pollachius virens (saithe)	1.67–2.50	*Tytler* and *Blaxter* (1973)
Gadus morhua (cod)	4.8	*Jones* and *Scholes* (1981)
Pomatomus saltatrix (bluefish)	3.0–14.7 (O_2)	*Wittenberg* et al. (1964)
Lepomis macrochirus (bluegill sunfish)	1.36–3	*McNabb* and *Mecham* (1971)

6.2 Biochemistry of Gas Secretion

6.2.1 Acid Secretion by the Gas Gland

It has been known for many years that the gas gland cells produce an acid (*Hall* 1924; *Akita* 1936; *Ledebur* 1937). *Hogben* (1958) showed that hydrogen ions are extruded from the serosal surface of the isolated gas gland. Lactic acid is formed by the gas gland (*Ball* et al. 1955; *Kuhn* et al. 1962); *Steen* 1963; *Enns* et al. 1967). This is a relatively strong

acid (pK = 3.70 at 25°C). Taking advantage of the bipolar structure of the rete of the eel *(Anguilla) Steen* (1963) managed to analyze blood from different parts of a functioning gas secretory system. Blood leaving the gas gland epithelium was about 1 pH unit more acid than blood arriving via the swim bladder artery; lactic acid increased to concentrations of up to 188 mg%. Other authors obtained analogous but less complete results (Table 7). The lactic acid concentration in fish blood circulation is highly variable and influenced by stress (*Larsson* et al. 1976). But because the countercurrent effect causes rediffusion of lactic acid within the rete, lactic acid production in the swim bladder does not influence the general circulation. Lactic acid is only moderately increased in the blood leaving the swim bladder (Table 7: postrete vein). Excess of lactic acid is carried by the portal vein to the liver and metabolized.

Table 7. pH value and lactic acid concentration (mg%) in blood from different gas gland vessels during gas secretion (means and ranges)

Fish	Prerete artery	Prerete vein	Postrete vein	References
Monacanthus	pH 7.7	–	pH 7.5	*Akita* (1936)
Anguilla	39 (23–49)	–	57 (28–136)	*Kuhn* et al. (1962)
Anguilla	pH 7.5 (7.3–7.7)	pH 6.7 (6.5–6.9)	pH 7.4 (7.2–7.6)	*Steen* (1963)
	45 (14–88)	119 (45–188)	69 (14–118)	
Sphyraena	40 (25–75)	–	50–100	*Enns* et al. (1967)
Opsanus	–	pH 6.5 (calculated)	–	*W. Wittenberg* et al. (1981)

The source of lactic acid is glucose, which is absorbed from the blood and partly stored within the gas gland cells as glycogen (*Copeland* 1951, 1952; *Fänge* 1953; *Jasiński* and *Kilarski* 1964; *D'Aoust* 1970; *Schwartz* 1971; *Morris* and *Albright* 1975). *Ball* et al. (1955) examined the metabolism of the isolated gas gland of the scup, *Stenotomus chrysops*, a species with a very fast rate of gas secretion (*Wittenberg* et al. 1964). The gas gland of the scup rapidly converts glucose into lactic acid under both aerobic and anaerobic conditions. Lactate formation accounts for 70%–90% of the glucose that disappears from the medium. According to *Deck* (1970) the isolated gas gland of the bluegill sunfish *(Lepomis macrichirus)* continues to produce lactic acid for 20–22 h. Glucagon increases the lactic acid formation 33%–66%. *Rasio* (1973) showed that in the eel *(Anguilla)* even the rete mirabile has a considerable glycolytic activity.

Glycogen disappears when lactic acid is formed during gas secretion (*Fänge* 1953), but most lactate emanates from direct metabolism of glucose absorbed from the blood or the medium (*D'Aoust* 1970).

6.2.2 Metabolic Enzyme Pattern of the Gas Gland

In accordance with its capacity for lactic acid production gas gland tissue shows high activities of glycolytic enzymes such as lactate dehydrogenase (LDH) and aldolase (*Fänge* 1953), but activity of cytochromoxidase (COX) is very low (*Gesser* and *Fänge* 1971). In contrast to other glycolytically active cells the gas gland cells show no Pasteur effect, i.e., the cells produce lactic acid at a high rate in the presence of high partial pressures of oxygen (*Ball* et al. 1955; *D'Aoust* 1970). Nevertheless the lactate dehydrogenase of the gas gland is predominantly of the anaerobic type found in skeletal muscles (*Gesser* and *Fänge* 1971).

Following an approach by *Pette* and *Bücher* (1963) and *Pette* (1971) in studies of skeletal muscle metabolism, *Boström* et al. (1972) examined metabolic enzyme activity patterns in gas gland tissue of the cod, *Gadus morhua.* The results were compared with those obtained on skeletal muscle (Table 8). The enzymes hexokinase, glucose-6-phosphate dehydrogenase (G6PDH), 6-phosphogluconate dehydrogenase (6-PGDH), lactate dehydrogenase (LDH), and malate dehydrogenase (MDH) showed considerably higher activities in the gas gland than in skeletal muscle, but the opposite was the case with four other enzymes: phosphofructokinase (PFK), glycerophosphate dehydrogenase (GPDH), fumarase (FUM), and cytochromeoxidase (COX). Pyruvate kinase (PK) showed the same activity in both tissues (Table 8). Closely similar results were obtained with gas gland tissue from ten other species of fishes (*Boström, Johansson,* and *Fänge* 1982, unpublished).

Table 8. Ratios of activities of metabolic enzymes in the gas gland to those of the same enzymes in white skeletal muscle in the cod *(Gadus morhua).* (After *Boström* et al. 1972)

Enzyme		Ratio activity gas gland/muscle
Hexokinase	(EC 2.7.1.2)	14.5
G6PDH	(EC 1.1.1.49)	19.9
6-PGDH	(EC 1.1.1.44)	10.2
PFK	(EC 2.7.1.11)	0.2
GPDH	(EC 1.1.99.5)	0.4
PK	(EC 2.7.1.49)	1.0
LDH	(EC 1.1.1.27)	4.3
MDH	(EC 1.3.99.1)	1.8
FUM	(EC 4.2.1.2)	0.6
COX	(EC 1.9.3.1)	0.2

The absence of the Pasteur effect in the gas gland depends on the dominating role of the hexosemonophosphate pathway, which is not inhibited by products of the citric acid cycle (ATP, etc.), and on the weak representation of this cycle. The unique metabolic enzyme pattern of the gas gland tissue explains its efficient production of lactic acid at the high oxygen pressures found within the swim bladder. The uptake of glucose from the blood is favored by the high activity of hexokinase. The hexose monophosphate shunt enzymes G6PDH and 6-PGDH are 10–20 times more active in the gas gland than in skeletal muscle, while the activity of the glycolytic key enzyme PFK is low. This leads to glucose being mainly metabolized by the hexose monophosphate shunt and only to a minor degree by the ordinary glycolytic pathway. Due to low activities of cytochrome oxidase and other mitochondrial enzymes the pyruvate formed by the breakdown of glucose is mainly transformed into lactic acid by the very active lactate dehydrogenase.

6.2.3 Insensitivity of Swim Bladder Tissue to Hyperbaric Oxygen

The cells of the swim bladder of deep-sea fishes are exposed to oxygen at pressures highly toxic to most animal tissues. *D'Aoust* (1969) showed that the Pacific rockfish *(Sebastodes miniatus)* dies if exposed to oxygen partial pressures equal to those of its own swim bladder. However, toadfish *(Opsanus tau)* subjected to seawater saturated with pure oxygen survived 48 h if the change from air to O_2 was made slowly (*Wittenberg* et al. 1981a). The metabolism of the gas gland, with dominance of the hexosemonophosphate pathway and little importance of the citric acid cycle, resembles biochemical adaptations in mammalian cells subjected to hyperbaric O_2 (*Gorman* et al. 1971). The enzyme superoxide dismutase, thought to protect tissues against toxic effects of O_2, has been demonstrated in the swim bladder of the toadfish *(Opsanus tau)* (*Morris* and *Albright* 1981), but its specific importance in the swim bladder is uncertain.

A high resistance against oxygen toxicity is observed in the retina of teleosts (*Ubels* and *Hoffert* 1981). The fish retina, like the gas gland of the swim bladder, is adapted to a high pO_2.

6.2.4 Lipids in the Swim Bladder

The swim bladder has a high capacity for lipid biosynthesis, perhaps as a consequence of the predominant hexosemonophosphate shunt pathway in the gas gland cells (*Boström* et al. 1972). The shunt activity produces NADPH, which participates in lipid synthesis. A similarity between swim bladder lipids and surfactants of the vertebrate lung has been suggested (*Phleger* and *Saunders* 1978). The low surface tension of phospho-

lipids may enhance the release of free gas (*Phleger* 1971), and the great solubility of oxygen in lipids may facilitate secretion of oxygen at high pressures (*Phleger* and *Benson* 1971). *Copeland* (1969) attributes great significance to intracellular lipid droplets of the gas gland, which he designates "gas-forming bodies". On the other hand, *Wittenberg* et al. (1980) suggest that lipid-rich layers of the swim bladder wall constitute a barrier against loss of gases.

The swim bladder of deep-sea fishes contains a "swim bladder foam" formed by expansion of gases within the swim bladder wall when fishes are hauled to the surface from deep water. The foam as seen in the electron microscope consists of bilayered membranes (*Phleger* et al. 1978). The chemical composition resembles that of cell membranes: neutral fat, phospholipids, cholesterol, and lipoproteins. The principal lipids are cholesterol and phospholipids at a ratio of about 1:1. Sphingomyelin is the major phospholipid in *Parabassogigas,* phosphatidylcholine in *Coryphaenoides* (*Phleger* and *Holtz* 1973). The fatty acids are highly unsaturated in spite of the high oxygen pressure in the swim bladder. Certain steps in the biosynthesis of cholesterol may be enhanced by the high concentrations of oxygen (about 90%) and by the high total pressure (100 atm or more) in the swim bladder of deep-sea fishes. However, even in shallow-living fishes the gas gland synthesizes more cholesterol and phospholipids than the liver (*Phleger* and *Benson* 1971; *Phleger* et al. 1978).

6.3 Countercurrent Concentration of Swim Bladder Gases

6.3.1 Oxygen

The theory that gas secretion in the swim bladder is brought about by a countercurrent-concentrating mechanism was presented by *Jacobs* (1930) and *Koch* (1934) and further developed by *Kuhn* and *Kuhn* (1961), *Kuhn* et al. (1962, 1963), *Kuhn* and *Marti* (1966), *Lesslauer* et al. (1966), *Sund* (1977), and others. According to *Kuhn* and *Kuhn* (1961) lactic acid produced by the gas gland lowers the pH of the blood, causing a Bohr or Root effect that forces oxyhemoglobin to release oxygen. In addition an increased plasma ionic concentration may increase tensions of physically dissolved gases ("salting out effect": *Koch* 1934). By these combined effects, the pO_2 in the efferent capillaries of the rete reaches higher levels than in the afferent capillaries. This "single concentrating effect" (*Kuhn* et al. 1963) causes a continuous diffusion of oxygen from the efferent (venous) into the afferent (arterial) capillaries, as circulation goes on in the rete. The Bohr and Root effects surpass the salting out effect in importance for oxygen accumulation (*Gerth* and *Hemmingsen* 1982).

For the proper functioning of the countercurrent-concentrating mechanism the time relationships of the Root (Bohr) shift are very important (*Röskenbleck* and *Nisel* 1962). As in mammalian blood (*Craw* et al. 1963) the reactions of CO_2 in the red cells in fish blood are rate limiting during the Root (Bohr) shift. *Forster* and *Steen* (1969) measured the reaction velocities of the Root effects in eel blood: O_2 leaves the oxyhemoglobin (Root "off-shift") in less than 0.1 s but recombines with hemoglobin as pH increases (Root "on-shift") in 10–20 s (half-time). The circumstance that the pO_2 of the efferent capillaries decreases very slowly when the pH increases along the rete (due to the slow Root "on-shift") is of crucial importance for the countercurrent multiplication of oxygen in the rete.

Berg and *Steen* (1968) describe what happens in the rete during gas secretion:

1. Arterial blood with normal pH and pO_2 enters the rete. When flowing through the afferent capillaries it receives O_2, CO_2, and lactic acid by diffusion from the efferent capillaries. As the blood enters the gas gland, it is enriched in O_2 and has a low pH.

2. During its passage through the gas gland the blood receives more lactic acid, which increases the pO_2 further by Root and "salting out" effects. Oxygen diffuses across the cells into the lumen of the swim bladder.

3. When the blood leaves the gas gland and passes into the efferent capillaries it has a lower pH, lower O_2 capacity of the plasma, higher pO_2 (but slightly lower O_2 content), and higher pCO_2 than when it entered the gas gland. As the blood flows within the efferent capillaries, O_2 diffuses according to the pO_2 difference from the efferent into the afferent capillaries. Lactic acid and CO_2 diffuse in the same direction. Although the pH increases along the rete, the gradient of pO_2 persists because the Root "on-shift" is slow. Probably the rate of blood flow is such that the maximal release of oxygen (Root "off-shift") takes place in the efferent rete capillaries rather than in the gas gland capillaries.

For the system to function it is necessary that the rete capillaries are permeable to lactic acid (*Berg* and *Steen* 1968; *Sund* 1977). This was demonstrated experimentally by *Stray-Pedersen* (1975).

6.3.2 Carbon Dioxide

The CO_2 deposited in the swim bladder during gas secretion is of double origin: (1) from bicarbonate of the blood and (2) from the metabolism of the gas gland cells (and the rete cells: *Rasio* 1973).

Wittenberg et al. (1964) calculated that only about 5% of the swim bladder CO_2 derives from the cell metabolism. According to *D'Aoust* (1970) the production of lactate exceeds that of carbon dioxide in the

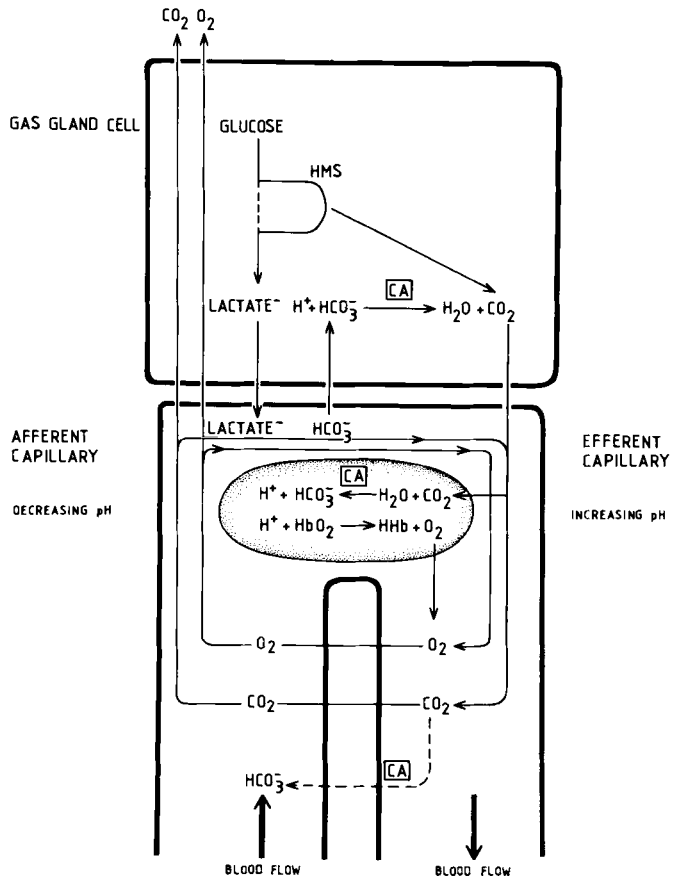

Fig. 4. Possible mechanisms responsible for deposition of O_2 and CO_2 in the swim bladder and role of carbonic anhydrase in gas secretion. In the gas gland cells carbonic anhydrase *(CA)* causes dehydration of HCO_3^-, whereas in the red cells and in the rete cells the same enzyme may serve to transform CO_2 into HCO_3^-. Most of the CO_2 released during gas secretion comes from bicarbonate of the blood plasma, but some is produced by the hexosemonophosphate shunt activity *(HMS)* in the gas gland cells (based on *Fänge* 1953, 1973; *Maetz* 1956; *Fairbanks* et al. 1974)

gas gland of a marine fish *(Sebastodes)* about 100 times. The major part of the CO_2 derives from the bicarbonate of the blood. However, the CO_2-releasing effect of acidification of the blood during one single passage through the gas gland is not enough to explain the high CO_2 values noted in the swim bladder of some fishes during gas secretion. *J.B. Wittenberg* et al. (1964) and *W. Wittenberg* et al. (1981) concluded that CO_2 must be further concentrated by countercurrent multiplication. Figure 4 illustrates the processes which possibly take place in the gas gland-rete complex during O_2 and CO_2 secretion.

6.3.3 Inert Gases

The high percentage of nitrogen, in some cases 99%, in the swim bladder of freshwater physostomes living at depths with pressures of up to 15 atm has interested investigators since *Hüfner* (1892). The slow-functioning gas secretory system of these fishes transports an oxygen-rich mixture of gases into the swim bladder. The final high percentage of nitrogen may result from preferential absorption of carbon dioxide and oxygen (*Piiper* et al. 1962; *Sundnes* et al. 1969; *Abernathy* 1972). However, this mechanism is not enough to explain the deposition of nitrogen into the swim bladder at great depths (*Scholander* and *van Dam* 1953). Countercurrent multiplication mechanisms must also be involved. *D. Wittenberg* et al. (1981) maintained toadfish *(Opsanus tau)* in seawater equilibrated with mixtures of oxygen with nitrogen, helium, neon, argon, krypton, or xenon. The more soluble inert gases were enriched in the swim bladder in relation to the less soluble ones. The authors concluded that partial pressures of inert gases in the swim bladder are elevated by countercurrent multiplication of a salting-out effect. A previous theory based on transport of inert gases by microbubbles (*Wittenberg* 1958) was not confirmed.

Gerth and *Hemmingsen* (1982) measured the effect of lactic acid on N_2 and Ar solubilities in water. They showed that the salting-out effect with lactic acid is very small and they therefore concluded that for a theoretical explanation of deposition of inert swim bladder gases in deep-sea fishes one must pay great attention to factors such as the unequal number and size of the arterial and venous capillaries of the rete, and to dissolved gas backdiffusion along the rete.

6.3.4 Countercurrent Concentration of Oxygen in the Fish Eye

The retina of teleost fish is dependent on high pO_2 for its function (*Hoffert* and *Fromm* 1972), and a countercurrent multiplier for O_2 termed the choroidal rete mirabile is found in the eye of most teleost and one holostean *(Amia calva)* (*Wittenberg* and *Wittenberg* 1962, 1974; *Wittenberg* and *Haedrich* 1974). Oxygen tensions above 1000 mm Hg have been measured in the eyes of fishes (*Wittenberg* and *Wittenberg* 1974).

The retina has a high glycolytic rate (*Krebs* 1927), and the fish retina must produce lactic acid needed for the O_2-concentrating mechanism (*Fairbanks* et al. 1974). Some fishes when transferred from cold O_2-rich water into warm water suffer from development of gas bubbles in the eyes. The gas exophthalmia is prevented by inhibition of carbonic anhydrase, indicating that carbonic anhydrase of the O_2-concentrating system of the eye is involved in the origin of the gas bubble disease (*Dehadrai* 1966).

6.4 Carbonic Anhydrase

Carbonic anhydrase, which catalyzes the hydration of CO_2 and the dehydration of bicarbonate, has a high activity in the swim bladder (*Leiner* 1937, 1940; *Fänge* 1953; *Maetz* 1956), both in the gas gland epithelium and in the rete (*Dorn* 1961; *D'Aoust* 1970). Inhibition of carbonic anhydrase blocks deposition of gases in the swim bladder (*Fänge* 1950, 1953; *Skinazi* 1953; *Maetz* 1956) and O_2 concentration in the fish eye (*Fairbanks* et al. 1969, 1974). Several theories attempt to explain the role of carbonic anhydrase.

The hypothesis by *Copeland* (1951) that carbonic anhydrase from the pseudobranch is carried by the blood to the swim bladder is contradicted by the fact that fish blood plasma lacks carbonic anhydrase activity and contains inhibitors of the enzyme (*Maetz* 1956; *Haswell* and *Randall* 1976).

Maetz (1956) suggested that the gas gland cells resorb bicarbonate from the blood in exchange for lactate ions. Carbonic anhydrase within the cells dehydrates the bicarbonate:

$$HCO_3^- + H^+ \overset{CA}{\rightleftharpoons} CO_2 + H_2O$$

Fairbanks et al. (1969, 1974), following another suggestion by *Maetz* (1956), assume that carbonic anhydrase in the endothelium of the venous capillaries, by hydration of CO_2 to bicarbonate ions, prevents short-circuiting of CO_2 into the arterial capillaries and too early dissociation of oxyhemoglobin. The process may be combined with active transport of HCO_3^- into the arterial capillaries.

Berg and *Steen* (1968) found that inhibition of erythrocytic carbonic anhydrase of fish blood lengthens the time for the Root "off-shift" from about 0.05 s to 30 s, which displaces the site of release of O_2 from oxyhemoglobin in the rete, putting the countercurrent multiplicatory process out of order. This explanation seems plausible but it does not exclude that carbonic anhydrase also functions at sites other than in the erythrocytes (Fig. 4).

Carbonic anhydrase occurs at a high concentration in the fish eye, where it is needed for the O_2 concentration mechanism (*Hoffert* 1966; *Fairbanks* et al. 1969). It may also function in the formation of vitreous fluid (*Maetz* 1956; *Fairbanks* et al. 1974).

6.5 Nervous Control of Gas Secretion

The "inflatory reflex" (*Fänge* 1953, 1966) results in increased gas secretion and decreased resorption. The nerve effects are exerted upon gas gland cells, blood vessels, and smooth muscles (muscularis mucosae).

The existence of special fibers which stimulate gas gland cells is indicated by experimental cutting of the vagus nerve (*Bohr* 1894; *Kuiper* 1915; *Franz* 1937; *Fänge* 1953, 1973; *Fänge* and *Nilsson* 1972). The fibers may be cholinergic, because gas secretion is blocked by cholinergic-blocking agents (atropine, mecamylamine: *Fänge* 1953; *Fänge* et al. 1976). Other facts supporting the theory of a cholinergic secretory innervation are high activities of acetylcholine esterase, choline acetyltransferase, and the presence of a gas gland ganglion (*Augustinsson* and *Fänge* 1951; *McLean* and *Nilsson* 1981; *Fänge* and *Holmgren* 1982). *Dreser* (1892) reported that pilocarpine increases the O_2 content of the swim bladder of the pike *(Esox lucius),* but this cholinergic drug effect has so far not been confirmed (*Fänge* 1953, 1973).

The function of the gas gland-rete mirabile system is greatly influenced by the blood flow. In carp (cyprinids), section of the sympathetic nerves to the swim bladder produces a slight increase of the O_2 and gas content in the swim bladder (*Moreau* 1876; *Franz* 1937), possibly due to removal of nerve-induced vasoconstrictor tonus.

In the cod *(Gadus morhua)* electrical stimulation of splanchnic and vagal nerves to the swim bladder, or perfusion with small amounts of catecholamines, constrict gas gland arterioles. The nerve effects are blocked by alpha-adrenergic blocking agents and reduced by atropine, indicating that vasoconstriction may be caused by both adrenergic and cholinergic influences. The adrenergic vasoconstrictor effects seem to dominate (*Nilsson* 1972; *Wahlqvist* 1982).

A marked vasodilation occurs in the active gas gland (*Hall* 1924; *Fänge* 1953). This may be due to a reflex inhibition of an adrenergic vasoconstrictor tonus, but metabolites from the gas gland (CO_2, lactic acid) could contribute to the effect. *Jacobs* (1930) assumed that the gas gland cells secrete a vasodilatory substance into the blood. Vasodilator nerves have never been found in the swim bladder, but it may be of some interest that nerve fibers containing vasoactive intestinal polypeptide (VIP) or a related peptide are present in the wall of the swim bladder artery (*Lundin* and *Holmgren* 1982, recent unpublished finding). In mammalian salivary glands release of VIP may be responsible for vasodilation caused by stimulation of secretomotor fibers (*Lundberg* 1981).

Yohimbine, an alpha-adrenergic blocking agent, considerably increases CO_2 and O_2 in the swim bladder of the cod *(Gadus morhua).* This effect may be due to blocking of resorption (oval closure) and dilation of the gas gland arterioles (*Fänge* 1953; *Fänge* and *Nilsson* 1972). The effect is prevented by vagotomy, indicating that certain vagal fibers (secretory?) are essential for gas gland activity.

In some species gas secretion may be continuous, the rate of gas filling of the swim bladder being controlled mainly by changes of the rate of

resorption. *Ledebur* (1929) blocked resorption in the marine fish *Serranus cabrilla,* using a ligature to close the opening in the diaphragm of the swim bladder. The swim bladders of the operated aminals were overinflated with a gas mixture containing up to 85%–94% O_2 . *Stray-Pedersen* (1970) suggests that increased deposition of gases is induced by (central) nervous inhibition of adrenergic impulses to the swim bladder.

6.6 Invertebrate Gas Depository Mechanisms

The protozoan *Arcella* forms cytoplasmic gas vacuoles when anoxic. The gas may be oxygen produced by an oxidase (*Cicak* et al. 1963). Bubbles of oxygen, possibly produced by peroxidases, are also observed in water-living insect larvae *(Chironomus)* when transferred to an anaerobic environment (*Harnisch* 1958). Other insect larvae *(Sciara* and *Corethra* or *Chaeborus)* possess rigid-walled gas-filled spaces formed by the tracheal system. Gas-filled rigid chambers are also found in cephalopods: the pearly nautilus and the cuttlefish. The gas is mainly nitrogen, released by a pressure gradient caused by osmotic resorption of ions and water (*Denton* 1961).

Siphonophores are colony-forming jelly-like marine animals, some of which possess a gas-filled float or "pneumatophore". In *Physalia* (Portuguese man-of-war) the pneumatophore contains up to 1000 ml gas, 0.5%– 13% of which is carbon monoxide (*Wittenberg* 1960). In *Nanomia,* a deep-sea form, the pneumatophore lumen of 1 mm^3 contains up to 87.4% CO (*Pickwell* 1967). The CO is produced by a pink "gas gland" containing folic acid. The amino acid serine is a substrate for a CO-producing enzymic reaction in siphonophores. The siphonophore gas gland is able to secrete gases against pressures of 50–120 atm.

It may be concluded that although processes superficially resembling gas secretion are found in certain invertebrates, they are based on principles different from those of gas secretion in fishes.

7 Gas Resorption

7.1 Rates of Resorption

In air-breathing physostomes (Table 2) the swim bladder functions like a primitive lung. *Umbra limi,* a teleost with a respiratory swim bladder, resorbs O_2 from the swim bladder at a rate of 28 ml/kg per hour (*Gee* 1981). But in the majority of physostomes, which do not use the swim

bladder for respiratory uptake of O_2, the absorption of O_2 is slow (*Black* 1942), because poor vascularization of the swim bladder wall prevents rapid diffusion. Some species of physostomes have an efficient gas secretory mechanism but no extensive resorption (*Crawford* 1974).

The physoclist swim bladder represents a mixture of gases enclosed within tissues (*Piiper* et al. 1962). Due to the continuous O_2 consumption by the tissues, the total pressure of gases within the swim bladder slightly exceeds that of the tissues. This pressure gradient forces gases to disappear by diffusion. The individual gases differ in their diffusion rates through the tissues. *Krogh* (1919) found the following relative diffusion rates through biological membranes for the atmospheric gases:

N_2	0.4–0.7
O_2	1
CO_2	35.7

Carbon dioxide diffuses so easily that the concentration within the swim bladder is usually close to zero. Oxygen is the main gas which is exchanged during volume control of the swim bladder, while N_2 is least movable. The rate at which O_2 is resorbed depends upon how fast it can be carried away by the blood. Important factors are

1. The size of the gas exchange area (controlled by smooth muscles)
2. The rate of blood flow in the resorbent area (controlled by vasomotor activity)
3. The O_2 capacity of the blood
4. The O_2 pressure gradient between the swim bladder and the blood

According to *Jones* and *Scholes* (1981) a 1-kg cod *(Gadus morhua)* must remove from or add to the swim bladder 5 ml of gases (at STP) for each meter it moves vertically. Whereas the maximal secretory rate, independent of depth, never exceeds about 4.8 ml/kg per hour, the rate of resorption increases with depth, as more O_2 dissolves in the plasma. At the surface (1 atm gas pressure) the rate of resorption is 3.6 ml O_2/kg per hour, but at 190 m depth (20 atm gas pressure) about 190 ml/kg per hour. Thus in deep water resorption is much faster than secretion (although the rates of both processes are influenced by nerve reflexes).

Some species of fish probably maintain neutral buoyancy only when actively swimming. During resting periods they may stay at the bottom with a half-filled swim bladder (*Jones* and *Scholes* 1981).

Ross (1979a) calculated that the maximal O_2 resorption rate in the saithe *(Pollachius virens)* permits a maximal ascent rate of only 3.2 m/h. This calculation is founded on the assumption that the resorbent capillaries contain blood which is about 85% saturated with O_2. However,

since the resorbent capillary plexus may be connected to the renal portal veins, the degree of blood oxygenation could actually be lower than 85%, which would increase the O_2 resorption rate.

Ross (1979b) determined the rate of blood flow in the oval using radio-labeled microspheres. The flow rate was considerably increased if a "deflatory reflex" was initiated.

7.2 Nervous Control of Resorption

The structures of importance for gas resorption, the resorbent capillary plexus and the muscularis mucosae, are controlled by autonomic nerve reflexes, which have been investigated mainly in euphysoclists (*Fänge* 1966).

During the *deflatory reflex* (increased resorption) the inner gas impermeable membrane (= secretory mucosa-submucosa) contracts toward the gas gland, leaving the resorbent mucosa relaxed and thin. Simultaneously the resorbent blood vessels dilate. The contraction of the smooth muscles (muscularis mucosae) of the secretory mucosa-submucosa is produced by adrenergic impulses from the vagus nerve, but cholinergic innervation may also exist. Isolated strips of the secretory mucosa-submucosa are contracted by α-adrenergic agonists and by acetylcholine, the responses being antagonized by α-adrenergic antagonists (phentolamine, yohimbine) and cholinergic-blocking agents (atropine, mecamylamine), respectively (*Fänge* et al. 1976).

Relaxation of the resorbent mucosa and dilatation of the resorbent capillary blood vessels may be largely a β-adrenergic effect. The very thin resorbent mucosa, when isolated, contracts by acetylcholine and relaxes by β-adrenergic agonists (*Ctenolabrus: Fänge* et al. 1976; *Anguilla: Nilsson* and *Fänge* 1967). The circular muscles of the oval, which react like the resorbent mucosa, may have cholinergic innervation (*Ross* 1978).

During the *inflatory reflex* (increased gas secretion) the secretory mucosa relaxes and expands over the inside of the swim bladder, covering the resorbent area to a varying degree and blocking its gas exchange. At the same time the gas gland arterioles dilate, the gas gland cells increase their metabolism, and the resorbent capillaries contract. Relaxation of the secretory mucosa results from inhibition of adrenergic influences, but release of a peptide may have additional importance. VIP occurs along the blood vessels of the swim bladder wall and has a relaxing influence on the secretory mucosa (*Lundin* and *Holmgren* 1982, personal communication).

The very thin resorbent mucosa, when isolated, contracts by acetylcholine and relaxes by β-adrenoceptor agonists (*Ctenolabrus: Fänge* et al.

1976; *Anguilla: Nilsson* and *Fänge* 1967). The circular muscles of the oval, which react like the resorbent muscularis mucosae, may possess cholinergic innervation (*Ross* 1978). However, the mode of nerve control of the resorbent mucosa is unclear. Possibly it relaxes by adrenergic nerve impulses and contracts by cholinergic impulses, but tissue elasticity may be a major cause for its contraction (*Nilsson* 1971; *Fänge* et al. 1976).

Adrenergic influence on the swim bladder, which increases resorption ("inflatory reflex") may be exerted via nerve fibers and via circulating catecholamines. Circulating catecholamines emanate from chromaffin cells situated along veins of the head kidney ("pronephros"). These cells are controlled by sympathetic nerves. The release of catecholamines from chromaffin cells may be provoked by asphyxia and other stress conditions (*Nilsson* et al. 1976; *Abrahamsson* and *Nilsson* 1976).

The swim bladder reflexes are initiated by signals from sense organs. Stretch receptors of the swim bladder wall (*Quotob* 1962) and the balance organ may be involved. But little is known about the reflex pathways and the location of the reflex centers in the brain.

8 Discussion

When physoclistous fish is neutrally buoyant, gases are deposited and resorbed at the same rate. The constancy of the swim bladder volume is the result of a continuous flux of gases through the membranes of the swim bladder wall. The passage of gases is finely adjusted by the autonomic nervous system.

Mathematical models have successfully been used to explain details of the gas exchange in the swim bladder. *Scholander* (1954) treated the barrier function of the rete mirabile. *Kuhn* and co-workers (1961, 1962, 1963, 1966) and *Sund* (1977) examined the countercurrent multiplication of gases. *Piiper* et al. (1962) studied theoretically and practically gas resorption from isolated pockets. So far formulae which describe the overall gas exchange in the swim bladder have not been worked out. Such formulae would have to be complicated, because many parameters must be considered: changing dimensions of diffusion areas, nervous control of blood flow, metabolism of the gas gland cells, diffusion of individual gases with different rates across the biological membranes, large species variation, etc. However, the physiological principles governing swim bladder gas exchange are the same that are valid for gas exchange in vertebrate tissues generally. The special functions noted in the swim bladder, such as gas secretion, can probably be explained by adaptations or specializations of the circulatory system, the structure of the swim

bladder wall, the biochemistry of the inner epithelium, and the physicochemical properties of fish blood, such as a large Bohr (Root) effect. It is remarkable that as far as is known the principle of countercurrent concentration of O_2 has evolved only in fishes, not in other vertebrates. Possibly the greater availability of O_2 for air-breathing vertebrates made the O_2 concentration mechanism unnecessary during evolution.

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Author Index

Page numbers in *italics* refer to the bibliography

Subject Index