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REVIEW

THE COMPOSITION OF ANIMAL CELLS: SOLUTES CONTRIBUTING TO OSMOTIC PRESSURE AND CHARGE BALANCE

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Abstract—1. The cytoplasmic solutes of vertebrates and invertebrates, other than Na, K and Cl, are surveyed in relation to their influence on ionic regulation through osmolality and charge balance.

2. The most abundant include MgATP, phosphagens, amino acids, various other nitrogen and phosphorus compounds and sometimes anaerobic end products and antifreeze agents.

3. Differences in muscle osmolality, e.g. between marine and non-marine animals, affect mainly nitrogenous solutes of no net charge, such as certain amino acids, taurine, betaine, trimethylamine oxide and urea.

4. The high osmolality of axoplasm in marine invertebrates is due more to anions such as aspartate, glutamate and isethionate.

INTRODUCTION

The composition of cytoplasm is constrained by the requirement of electroneutrality and by the total contribution of the various solutes to osmotic pressure. More specifically, these are constraints on intracellular Na, K and Cl and, in an earlier review (Burton, in press), the total contributions to osmolality and charge balance of substances other than Na, K and Cl were quantified for a variety of tissues and animals. Here the actual solutes are surveyed. The interest here is thus primarily in ionic and osmotic regulation, though Na, K and Cl concentrations are not themselves discussed and the information is of wider interest. A relevant general picture of the organic constitution of cytoplasm can prove elusive in the biochemical literature since this often places little emphasis on molecular ionization and *in vivo* concentrations. However, some balance sheets have been published that show the contributions of individual substances to osmotic pressure and compare total charges on known anions and cations. Tissues so treated include muscle of frog (Conway, 1945), rat (Conway, 1945, 1950), *Nephrops* (Robertson, 1961), cephalopods (Robertson, 1965), *Squalus* (Robertson, 1975), *Myxine*, *Chimaera* (Robertson, 1976) and echinoderms (Robertson, 1980) and also nerve (axoplasm) of *Carcinus* (Lewis, 1952), cephalopods (Koechlin, 1955; Deffner, 1961) and *Myxicola* (Gilbert, 1975).

In the present context, it is helpful to think of animals as falling roughly into three categories: (I) those with internal osmolalities like that of their marine environment; (II) those, like most vertebrates and many non-marine arthropods, with body fluids of about 200–500 mOsm/kg water; (III) those, e.g. many freshwater invertebrates, with more dilute body fluids. Data for muscle cells (Burton, in press) show that the concentration of osmotically active solutes other than Na, K and Cl (designated α/V) is typically 600–900 mmol/kg water in category I, 60–450 mmol/kg water in category II and as low as 16 mmol/kg water in category III (i.e. in *Anodonta*, Potts, 1958). The net anionic charge on substances other than Na, K and Cl, calculated as ([Na] + [K] − [Cl]) and designated $z\alpha$/V, is about 110–250 m-equiv/kg water in both categories I and II, but lower in category III. Muscle cells of category I thus differ from those of category II mainly in containing more solute of no net charge. Such solutes include urea, amino acids, taurine, betaine and trimethylamine oxide, the importance of each depending on the species. Appropriately, they are those most important in intracellular isosmotic regulation in muscles of euryhaline animals. This has been reviewed elsewhere (e.g. Florkin and Schoffeniels, 1965; Gilles, 1975), so it is not treated here; data given for marine species relate to animals in full-strength sea water. Neurons of marine invertebrates differ from muscle in that the high osmotic pressure is due much more to organic anions. Some substances are noted below as occurring in marine invertebrates even though contributing little to charge balance or osmolality. However, the same concentrations could be very significant in animals of categories II and III. Some minor solutes are included simply to establish the fact of their insignificance.

Cell composition varies with both tissue and species and the relevant information, though abundant, is scattered and uneven so that the account given here is necessarily incomplete. Only a minority of references can be cited and data have, therefore, been chosen to indicate either maximum known concentrations or, because of their wide interest, concentrations in mammalian tissues. The information is presented mainly by substance rather than by tissue or species.

The principal ionization states of substances, at pH 7, are indicated by positive and negative signs in parentheses. Many solutes are zwitterions (+ −) with no net charge. Concentrations are given in terms of

mmol, recalculated as necessary. Where possible, they relate to cell water, but often, of necessity, to total tissue water or to total tissue wet wt. In muscle fibre water, the concentrations of substances absent from extracellular fluid are usually about 50% higher (40–70%) than in the total wet tissue, as indicated by inulin spaces. Substances described by other authors as occurring 'in large amounts' are not always significant in the present context.

HCO_3, SO_4, NH_4, Ca *and* Mg

Cells are typically more acid than extracellular fluid and HCO_3 (−) concentrations are correspondingly lower inside than outside, perhaps by a factor of a half or a quarter. Most water-breathing animals have low extracellular HCO_3 levels, so that HCO_3 can contribute little to internal charge balance or osmolality. In mammalian muscle fibres the HCO_3 concentration is about 10 mmol/kg water (e.g. Khuri *et al.*, 1976) and in neurons of *Helix*, where the total solute concentration is lower, it is about 15 mmol/kg water (Thomas, 1976) and so is very significant.

Inorganic SO_4 (− −) can exceed 25 mM in extracellular fluid of some marine invertebrates, but is present at only about 1 mmol/kg cell water in muscle of *Nephrops* (Robertson, 1961) and *Squalus* (Robertson, 1975). It is more concentrated in squid axoplasm (7.5 mmol/kg water, Deffner, 1961) and in fast adductor of *Mytilus* (8.8 mmol/kg cell water, Potts, 1958), but is still not very significant in the present context.

Ammonium ions (+) might be expected to occur at higher levels inside cells than outside both because of the membrane potential and because of the lower pH inside. Robertson (1961, 1965, 1970, 1975, 1976) and Boyd *et al.* (1977) found mean values of 1.3–6.6 mmol/kg cell water in muscles of marine animals, all much higher than in the extracellular fluids, but nevertheless low in the present context. Analyses could sometimes be affected by autolytic production and diffusive loss.

Cell Ca (+ +) is mostly bound. Precipitated or otherwise out of solution it makes no contribution to osmolality, unlike dissolved complexes. One of the latter is Ca citrate (−), which can reach 22 mmol/kg wet tissue in parts of the oviducts of laying hens (Hertelendy and Taylor, 1964). Mg too is largely bound, with levels of the free ion (+ +) being about 0.6–6 mmol/kg cell water (references in Burton, 1980). Especially in muscle, much of the Mg is complexed with adenosine triphosphate (ATP), as MgATP (− −), and total concentrations of Mg and of ATP are, therefore, correlated (Burton, 1980). This binding of Mg to ATP does not affect charge balance, but it does reduce their joint contribution to osmolality.

Phosphates and phosphoryl compounds

Phosphorus compounds are often important both as osmolytes and as anions, but their contributions are sometimes hard to assess because the phosphagens and ATP may release inorganic phosphate during tissue processing. Both inorganic and hexose phosphates may become artifactually raised from their normal low levels in frog muscle (Dawson *et al.*, 1977). The ionization of some phosphates (e.g., inorganic phosphate, glycerophosphate) is pH-dependent in the physiological range (so that they act as buffers), but their total contribution to charge balance is often little affected inasmuch as those most pH-sensitive tend to be present at low levels. ATP, mostly present as MgATP, and also phosphorylcreatine and phosphorylarginine, are little affected by pH in the normal intracellular range.

Total acid-soluble phosphorus is about 39–66 mg atoms/kg wet tissue in mammalian muscle (i.e. about 60–100 mg atoms/kg cell water) (Long, 1961) and about 75–170 mg atoms/kg cell water in muscle of various marine animals (Robertson, 1961, 1965, 1970, 1975, 1976, 1980). In the axoplasm of *Loligo* Caldwell (1960) found only 14 mg atoms/kg, contributed mostly, and about equally, by orthophosphate, phosphorylarginine and ATP. Intracellular precipitates of Ca and Mg phosphates are common in invertebrates; these are only relevant here inasmuch as the osmotic inactivity of their components must be recognized.

Phosphorylcreatine (+ − − −) is the phosphagen of vertebrates. That and phosphorylarginine (+ + − − −) are the most widespread phosphagens of invertebrates, though five others have been found in the Annelida, namely the *N*-phosphoryl derivatives of taurocyamine, hypotaurocyamine, glycocyamine, opheline (each + − − −) and lombricine (+ + − − − −) (Robin, 1964). The literature on phosphagens has been reviewed by Ennor and Morrison (1958) and by Thoai and Roche (1961). Muscle typically contains more phosphagen than do other tissues (e.g. Long, 1961; Beis and Newsholme, 1975). Thus, resting rat and frog muscle usually contains about 20–26 mmol/kg wet tissue of phosphorylcreatine (Conway, 1945; Beis and Newsholme, 1975; Dawson *et al.*, 1977) as compared with 2.4 mmol/kg wet tissue in mouse brain (Beis and Newsholme, 1975). The highest sarcoplasmic concentrations of phosphagens seem to occur in some marine invertebrates. In the upper range for phosphorylarginine are the averages of 52 mmol/kg wet muscle in *Pecten* (Beis and Newsholme, 1975) and 81 mmol/kg muscle fibre water in *Nephrops* (Robertson, 1961).

ATP, mostly as MgATP, also tends to occur at higher concentrations in muscle (e.g. 2–8 mmol/kg wet tissue) than in most other tissues (e.g. Beis and Newsholme, 1975; Burton, 1980). There is no obvious tendency for muscle to contain more in marine invertebrates than in mammals. Concentrations of adenosine diphosphate and monophosphate seem generally to be low enough to be ignored here (Beis and Newsholme, 1975). In any case, adenosine diphosphate in muscle may be largely bound to actin (Seraydarian *et al.*, 1962).

In exercising muscle, inorganic phosphate may accumulate as it is released from phosphagen and ATP (e.g. Dawson *et al.*, 1977). In muscle of *Periplaneta*, the phosphate released from phosphorylarginine and ATP accumulates as glycerophosphate (more − − than −) (Kubišta, 1957).

Muscles of *Bufo* contain phosphoethanolamine (mostly + − −, some + −) and glycerophosphorylethanolamine (+ −), each at a few mmol/kg wet tissue (Gordon, 1965). L-serine ethanolamine phosphate (+ + − −) and L-threonine ethanolamine phosphate (+ + − −) occur in brain, heart and gut of lower vertebrates at concentrations up to about 8 mmol/kg wet tissue (Porcellati *et al.*, 1965). Little of either is found in skeletal muscle.

In vertebrate erythrocytes, various phosphates regulate the oxygen affinity of haemoglobin, with binding to haemoglobin presumably reducing their osmotic activity. Myoinositol pentaphosphate (probably 6–7 negative charges per molecule) has this role in birds, at up to 6 mmol/l of cells (Rapoport and Guest, 1941; Bartlett and Borgese, 1976). Many mammals utilize 2,3-diphosphoglycerate (3–4 negative charges per molecule). This occurs in mammalian and some other vertebrate erythrocytes at up to about 9 mmol/l (Rapoport and Guest, 1941; Bartlett, 1976; Bartlett and Borgese, 1976). Concentrations of ATP can be high in erythrocytes, reaching 15 mmol/l of cells in the 24-day embryo of the emu (Bartlett, 1982a). The concentration is 6.5 mmol/l of erythrocytes in newborn *Mustelus*; there is less in the adult, but then the erythrocytes contain guanosine triphosphate at about 4 mmol/l (Bartlett, 1982b). Comparable concentrations of both substances have been found in other fish erythrocytes (Bartlett, 1978).

Guanidine compounds related to phosphagens

The phosphagens are formed by phosphorylation of the respective guanidine compounds, notably creatine (+ −) and arginine (+ + −), and these too can occur in substantial concentrations. This is especially so in exercising muscle as a result of phosphagen breakdown, but the concentrations given below are intended as resting or normal values. Artifactual dephosphorylation after tissue sampling may account for some high concentrations that have been reported. Beis and Newsholme (1975) tabulate average concentrations of creatine and arginine for vertebrates and invertebrates. With one exception, they are between 1.6 and 18.4 mmol/kg wet tissue. Skeletal muscle samples had been freeze-clamped. The exception—white muscle of *Scylliorhinus*—contained 36.6 mmol creatine/kg; Beis and Newsholme thought it unlikely that this high value was a result of degradation and proposed a role for creatine in osmoregulation in this elasmobranch. Little free arginine occurs in the muscle of many, at least, of the animals utilizing phosphorylcreatine, but in muscle samples of marine crustaceans and molluscs utilizing phosphorylarginine, arginine levels are commonly 20–70 mmol/kg wet tissue (Florkin and Schoffeniels, 1965; Gilles, 1975). Arginine is one of the protein-forming amino acids discussed below. Hypotaurocyamine (+ −) has been found in *Arenicola* muscle at 160 mmol/kg wet tissue (Jacobsen and Smith, 1968).

Creatinine (uncharged) has been found in skeletal muscle of *Raja* at 20 mmol/kg wet tissue and in brain of *Dasyatis* at 9 mmol/kg wet tissue (Boyd *et al.*, 1977), but there is much less in muscle of *Squalus* (Vyncke, 1970; Robertson, 1975). Creatinine may sometimes be overestimated since it forms from creatinine in acidified samples that are kept too long before analysis.

Other anaerobic end-products

During vigorous exercise or anoxia, various metabolic end-products may accumulate to concentrations of several tens of mmol/kg. Lactate (−) is the major product of anaerobic glycolysis in vertebrates, crustaceans, insects (Grieshaber, 1982) and some molluscs (Livingstone, 1982). Normal levels in mammalian tissues are low, provided that anoxia is avoided. Then there are 1–5 mmol/kg wet tissue in rat skeletal muscle and less than 3 mmol/kg wet tissue in rat liver, while concentrations of pyruvate and Krebs cycle intermediates are much lower (Williamson and Brosnan, 1974). Many of the higher levels reported for lactate in mammalian tissues are probably not valid resting values, though normal in exercising muscle.

Major products of anaerobic metabolism also include succinate (− −) and alanine (+ −), e.g. in some diving vertebrates (seal, sea lion, porpoise, turtle) and in molluscs and *Ascaris*, propionate (−) in annelids, nematodes, trematodes and cestodes and proline (+ −) in trematodes. Some annelids, nematodes and cestodes also produce isobutyrate (−), isovalerate (−) and methylbutyrate (−) (Pandian, 1975).

Octopine (+ − −) is an important anaerobic end-product in some molluscs, *Sipunculus* and probably other invertebrates (Grieshaber, 1982; Livingstone, 1982). It forms by condensation of pyruvate with arginine, so that, in muscle of *Loligo* and *Pecten*, arginine does not accumulate in exercise despite the breakdown of phosphorylarginine (Grieshaber and Gäde, 1976, 1977). In some molluscs and annelids, pyruvate condenses with alanine or glycine to form alanopine (− −) or strombine (− −) (Grieshaber, 1982; Livingstone, 1982).

Free amino acids and other nitrogenous solutes

The term 'amino acid' has been used in the literature variously to denote just the usual amino acids of proteins and, more generally, to include other ninhydrin positive substances. We consider first the former. Most are dipolar (+ −), but aspartate and glutamate each have a second carboxyl group (+ − −) and lysine and arginine each have a second positively charged amino group (+ + −). Histidine is a buffer in the physiological pH range and so has a variable net positive charge. However, cells seem rarely to contain much free histidine, though Abe (1981) found 0.4–92.5 mmol/kg wet tissue in muscle of various fish.

Considering first mammals, levels of free amino acids vary from tissue to tissue in any one species (e.g. rat; Friedberg and Greenberg, 1947, Awapara *et al.*, 1950; rat and also caiman: Herbert *et al.*, 1966; rabbit: Dubreuil and Timiras, 1953), but rat muscle may be taken as representative. Concentrations there have consistently been found to be about 15 mmol/kg wet tissue both by the gasometric ninhydrin method (Friedberg and Greenberg, 1947; Flock and Bollman, 1962) and by summation of individually determined amino acids (Sassenrath *et al.*, 1954; Herbert *et al.*, 1966). About half is contributed by alanine, glutamine, glutamate and particularly glycine. The net charge is close to zero. The photometric ninhydrin method yields values near 35 mmol/kg wet tissue (Awapara *et al.*, 1950; Herbert *et al.*, 1966), but this method determines also taurine, sarcosine and peptides such as carnosine, anserine and glutathione. Lysine, with its net positive charge, can replace potassium in muscle and kidney in potassium deficiency (Eckel *et al.*, 1958).

Gilles (1975) tabulated data on fifteen amino acids in muscle of a terrapin and three teleost fish. Totals, excluding taurine, were 16.5–23.1 mmol/kg wet tissue in sea water and 4.6–10.0 mmol/kg wet tissue in fresh water, glycine predominating.

Concentrations of free amino acids substantially lower than in rat tissues occur in tissues of invertebrates with more dilute body fluids. These include *Anodonta* and *Hirudo* (Potts, 1958; Florkin and Schoffeniels, 1965) and also *Otala* and *Helix* (Campbell and Speeg, 1968).

In elasmobranchs, free amino acids contribute substantially to the higher osmotic pressure, for example 80–305 mmol/kg cell water in erythrocytes and muscle of *Raja* and *Dasyatis* (Forster and Goldstein, 1976). High concentrations also occur in muscle of *Squalus* (Vyncke, 1970; Robertson, 1975).

Many marine invertebrates also contain much free (protein-forming) amino acid. The proportions of each vary even within phyla, but certain amino acids are repeatedly found to predominate in muscle, notably glycine, proline and alanine (also in the marine ciliate, *Miamiensis*—Kaneshiro *et al.*, 1969). The following points are based on data compiled by Florkin and Schoffeniels (1965) and by Gilles (1975). Glycine abounds in muscle of annelids, crustaceans and some molluscs. It is particularly abundant in muscle of *Arenicola* (288 mmol/kg tissue water) and *Callinectes* (362 mmol/kg cell water). Proline is low in molluscan muscle, but high in crustaceans and annelids, where the highest value tabulated is 104 mmol/kg wet tissue in *Homarus* muscle. Alanine (see above) can be as high as 159 mmol/kg wet tissue, in muscle of *Mya arenaria*, but the variability of amino acid patterns is illustrated by the very low level, 2 mmol/kg wet tissue, in another mollusc, *Acanthochitona*. Other amino acids sometimes occur at quite low levels that would, nevertheless, be significant in animals with more dilute body fluids. These include aspartate, glutamate, threonine and serine. Asparagine occurs at about 240 mmol/kg dry weight in body wall of *Glycera*, with lesser amounts of alanine, serine and threonine (Costa *et al.*, 1980). Glutamate analyses may include glutamine and figures for arginine, often high, may include phosphorylarginine.

It is in axoplasm of squids, crustaceans and *Myxicola* that aspartate and, to a lesser extent, glutamate, contribute most to osmotic and charge balance (Lewis, 1952; Koechlin, 1955; Deffner, 1961; Gilbert, 1975). In the circumoesophageal connectives of *Homarus*, about half of the potassium is balanced by aspartate and the concentrations of potassium and aspartate are strongly correlated (Hanig and Freeman, 1980). Glutamate occurs in substantial amounts in vertebrate brains (e.g. 4 20 mmol/kg wet tissue), together with small amounts (several mmol/kg) of glutamine (+ −), γ-amino butyrate (+ −), aspartate and acetylaspartate (− −) (Tallan, 1962). The brain of the stingray *Dasyatis* contains glutamate at 15 mmol/kg wet tissue, with lesser, but still significant, amounts of glutamine, γ-amino butyrate and aspartate, and also glycine and β-alanine (+ −) (Boyd *et al.*, 1977).

We turn now to amino acids other than those generally present in proteins. β-alanine and γ-amino butyrate (see above) are occasionally found free in high concentrations, as in the electric lobe of *Torpedo*, where Davies and Dowe (1977) found β-alanine at 39 mmol/kg wet tissue. In *Raja* Boyd *et al.* (1977) found 41 mmol/kg of wing muscle and 51 mmol/kg of erythrocytes. Lesser amounts of β-alanine occur in skeletal muscle of *Rana cancrivora* (Gordon and Tucker, 1968). γ-Amino butyrate averages 15 mmol/kg water in erythrocytes of *Platichthys* living in sea water; with taurine it is involved in intracellular isosmotic regulation (Fugelli and Zachariassen, 1976). It has this role, with proline and alanine, in the soil-dwelling *Acanthamoeba* (Drainville and Gagnon, 1973).

Betaine (glycine betaine, + −) abounds in many marine animals (Awapara, 1962; Beers, 1967). It averages 38–149 mmol/kg tissue water in muscle of *Nephrops*, *Parastichopus*, cephalopods, *Squalus*, *Chimaera* and *Myxine* (Robertson, 1961, 1965, 1975, 1976, 1980) and 74 and 119 mmol/kg axoplasm, respectively, in *Loligo* and *Dosidicus* (Deffner, 1961).

Carnitine (+ −) was found by Beers (1967) in a wide range of animals, mostly in concentrations of a few mmol/kg wet tissue. Fraenkel (1954) assayed it in examples of most of the major metazoan phyla, finding most in *Limulus* muscle, i.e. 55–217 mmol/kg dry weight. In mammalian skeletal muscle and liver, he found at most 7 mmol/kg dry weight, i.e. 2 mmol/kg tissue water (Fraenkel, 1953). Sheep muscle contains 10 mmol/kg wet weight (Williamson and Brosnan, 1974).

Sarcosine (*N*-methylglycine, + −) occurs at 44 mmol/kg wet tissue in muscle of *Raja erinacea* (Boyd *et al.*, 1977), but only at 6 mmol/kg of muscle in *Squalus* (Vyncke, 1970). In *Otala* (category III) it averages 2.6 mmol/kg wet tissue in the hepatopancreas, where it is the most concentrated of the amino acids measured (Campbell and Speeg, 1968).

Homarine (+ −) is widespread in marine crustaceans and molluscs and also occurs in some annelids, echinoderms, urochordates and coelenterates, but it seems not to have been found in vertebrates and freshwater invertebrates (Welsh and Prock, 1958; Gasteiger *et al.*, 1960; Beers, 1967). Concentrations in muscle and other tissues may be high, reaching 75 and 55 mmol/kg wet tissue, respectively, in *Limulus* ventral nerve cord and *Loligo* cerebral ganglia (Gasteiger *et al.*, 1960). Dall (1971) found that homarine in crustacean haemolymph is linked to a tetrapeptide, so the possibility of intracellular binding must also be considered. In squid axoplasm there can be about 20 mmol/kg (Deffner, 1961), but Gasteiger *et al.* (1960) found that the concentration decreased progressively with distance from the cell body, from 15 down to 3 mmol/kg axoplasm.

Trigonelline (+ −), differing structurally from homarine only in the placing of the carboxyl group, seems generally less abundant (Beers, 1967). It occurs in some marine crustaceans, annelids (Beers, 1967) and coelenterates (Welsh and Prock, 1958). Beers found most in muscle of *Homarus* (about 10 mmol/kg wet tissue).

Taurine (+ −), widespread in animals, has been reviewed by Awapara (1962), by Jacobsen and Smith (1968) and by Allen and Garrett (1971). In mammalian tissues there may be little, or up to 38 mmol/kg wet tissue, as in rat heart, but it is in some marine invertebrates that it most abounds, sometimes exceeding 100 mmol/kg wet tissue. Florkin and Schoffeniels (1965), Severin *et al.* (1972) and Gilles (1975) tabulated concentrations (all less than that) in muscle of various vertebrates and invertebrates. Large amounts occur in axoplasm of squids and crustaceans (Lewis, 1952; Koechlin, 1955; Deffner, 1961) but not of *Myxicola*

(Gilbert, 1975), and also in the brain of *Dasyatis* and the heart and erythrocytes of *Raja* (Boyd *et al.*, 1977). Hypotaurine (+ −) has been found in sponges, coelenterates, annelids, molluscs, and crustaceans but, when quantified, always in lesser amounts than taurine (Amende and Pierce, 1978). Various derivatives of taurine and hypotaurine have occasionally been found in substantial amounts, including hypotaurocyamine (+ −) (see above), taurobetaine (+ − −) in a sponge and a gorgonian and monomethyltaurine (+ −) in a sponge (Beers, 1967; Jacobsen and Smith, 1968).

Isethionate (−) is an anionic derivative of taurine. Little is present in mammals (Jacobsen and Smith, 1968) and it rarely features in analyses of invertebrates, but at 165 (Deffner, 1961) or 220 mmol/kg (Koechlin, 1955) it is a major anion in squid axoplasm. Gilbert could not detect it in *Myxicola* axoplasm, but this contained the related cysteate (+ − −) at 116 mmol/kg. Squid axoplasm contains a little cysteic acid amide (Deffner, 1961).

Trimethylamine oxide (+ −) is another major osmolyte in some marine animals including teleosts, though it is negligible in others (Norris and Benoit, 1945; Ronold and Jakobsen, 1949; Groninger, 1959). It averages 34–189 mmol/kg tissue water in muscle of *Nephrops*, cephalopods, *Squalus* and *Chimaera* (Robertson, 1961, 1965, 1975, 1976). Published averages for muscle of *Myxine glutinosa* have varied from 100 to 230 mmol/kg cell water, varying inversely with concentrations of free amino acids and so, presumably, having a similar role (Cholette and Gagnon, 1973). In muscle of *Dasyatis* trimethylamine oxide can reach 260 mmol/kg cell water (Forster and Goldstein, 1976).

The polyamines are potentially of special interest in view of their high positive charge. However, they do not seem to be generally abundant and their interactions with membranes and nucleic acids must reduce their osmotic significance. They include spermine (+ + + +) which is derived from spermidine (+ + +), itself derived from putrescine (+ +). Data cited by Raina (1963) indicate that concentrations are generally low in mammalian tissues, though rat pancreas may contain 8.6 mmol of spermidine/kg wet tissue (25 m-equiv of charge/kg).

Urea, unusual amongst major osmolytes in being uncharged, passes easily through cell membranes, so that its concentration tends to be similar inside and out. Outside of the renal medulla, mammalian tissues contain only a few mmol/kg (Long, 1961) and urea contributes most to osmolality where plasma levels are high, as in the elasmobranchs. Forster and Goldstein (1976) found more than 600 mmol/kg cell water in erythrocytes and muscle of *Dasyatis*, but 300–400 mmol/kg cell water is perhaps commoner in elasmobranchs (Robertson, 1975, 1976; Forster and Goldstein, 1976; Boyd *et al.*, 1977). Urea occurs in muscle of *Bufo viridis* and *B. boreas* (Gordon, 1965) and of *Rana cancrivora* (Gordon and Tucker, 1968) and becomes concentrated when these amphibians are adapted to dilute sea water. Aestivating snails (*Bulimulus*) can also build up tissue urea, even to 380 mmol/kg wet tissue (Horne, 1971).

Concentrations of dissolved uric acid (uncharged) and urate (−) are limited to a few mmol/kg water by their low solubility. However, Na and K urate may be stored in cells, as in insect fat body.

Four imidazole dipeptides have been found in animal tissues, namely carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-methylhistidine), ophidine (β-alanyl-L-3-methylhistidine) and, less abundantly, homocarnosine (γ-aminobutyrylhistidine). The extensive literature has been reviewed by Lukton and Olcott (1958) and by Crush (1970). Lukton and Olcott did not distinguish ophidine from anserine. Of the first three dipeptides, one or two occur in skeletal muscle of most vertebrates examined, though rather little in some fish. The highest individual concentration tabulated by Crush is 47 mmol/kg wet tissue for *Balaenoptera* psoas, the highest total being 53 mmol/kg wet tissue for anserine plus carnosine, in *Gallus* pectoral muscle. Little or none occurs in most vertebrate and invertebrate tissues other than muscle. Since they have pK values close to intracellular pH, these dipeptides can be important buffers in vertebrate muscle (Burton, 1978), with about half the molecules having a positive net charge. The tripeptide glutathione (+ − −) is widespread in the animal kingdom. Rat tissues contain 0.7–10 mmol/kg tissue (Davidson and Hird, 1964).

Polyols and ascorbate

Glucose and trehalose average 9.4 and 3.4 mmol/kg wet tissue, respectively, in the hepatopancreas of *Carcinus* (Johnston and Spencer Davies, 1972). Glucose occurs at low levels in mammalian tissues and in tissues of *Raja*, but in kidney of *Raja* scyllo-inositol and myo-inositol average 13.5 and 5.7 mmol/kg wet tissue, respectively, with lesser amounts occurring in other organs and very little in muscle (Sherman *et al.*, 1978). In the rabbit myo-inositol abounds in the brain (13 mmol/kg wet tissue) and in some other tissues, but there is little scyllo-inositol (Sherman *et al.*, 1968).

Various sugars and other polyols (uncharged) occur in high concentrations in terrestrial arthropods, where they serve as antifreeze agents (see reviews of Duman *et al.*, 1982 and Sømme, 1982). These include glucose, trehalose, sucrose, glycerol, sorbitol, threitol, mannitol and erythritol. Data are mostly for whole animals, haemolymph and eggs. Some hibernating anurans become tolerant to freezing by accumulating glycerol. Thus, muscle of *Hyla* can contain about 300 mmol/kg tissue water (Schmid, 1982).

Ascorbate (−) is particularly concentrated in the vertebrate adrenal cortex; Zbiegieni (1979) found up to 17 mmol/kg of whole adrenal gland in mice.

DISCUSSION

Apart from Na, K, Cl and HCO_3, free inorganic ions generally contribute little to cellular osmolality and charge balance. The organic phosphates can be much more significant, especially MgATP and phosphagens in muscle. The phosphagens co-exist with their respective unphosphorylated guanidine compounds. Typically, most of the remaining osmolality is contributed by other nitrogenous solutes, though lactate or other anions may accumulate during anaerobic metabolism. Most of the organic solutes are of zero or negative net charge. Exceptions include arginine, the lysine that accumulates in K-deficient mammalian muscle and about half of the imidazole dipeptides present in some vertebrate muscle.

Polyamines rarely seem to be significant. Of the small water-soluble molecules of no net charge, most are zwitterions. Davis (1958) emphasized the importance of ionization for retention within the cell. The uncharged solutes that sometimes contribute significantly to osmotic pressure are those that pervade both intracellular and extracellular fluids, such as urea and antifreeze agents.

Comparing animals with dilute and concentrated body fluids, we may begin with skeletal muscle of the rat, as an example of category II. Table 1 shows representative concentrations of the major solutes. It represents a concensus of the literature, including references given above, but actual concentrations vary with such factors as fibre type (Edström *et al.*, 1982) and the corrections made for extracellular fluid. The total concentration of 325 mmol/kg cell water is close to the osmolality of mammalian plasma (about 300 mOsm/kg water), but should not be too closely compared with it since it is based on diverse and incomplete data and does not refer to an ideal solution. The excess of cationic over anionic charge of 89 m-equiv/kg water on the solutes tabulated must be largely balanced by the net negative charge on proteins, membranes and organelles (Conway, 1950). The contribution of these is not easy to assess directly. Gary-Bobo and Solomon (1968) estimated that human erythrocytes contain 7 mmol of haemoglobin/l of cell water, representing 24 m-equiv/l and 17 mOsm/l.

The muscles of marine invertebrates (category I) are like those of the rat with respect to K concentration and to the net anionic charge (m-equiv) on substances other than K, Na and Cl (see Introduction). They differ most in the large contribution to osmotic pressure of substances of no net charge. These vary with the species, but include notably glycine, proline, alanine, betaine, taurine, trimethylamine oxide and, in lesser amounts, carnitine and homarine. In high concentrations these seem mainly to have an osmotic rather than a metabolic role. The extra osmotic pressure is not obviously contributed to any great extent by buffers (Burton, 1978), fuel reserves or extra metabolic machinery, though various substances (e.g. phosphagens) are sometimes more concentrated than in animals of lower osmolality. Skeletal muscle of *Myxine* differs from rat muscle in the same sort of way, containing much more free amino acid, trimethylamine oxide and betaine (Robertson, 1976). Skeletal muscle of elasmobranchs differs most from that of other animals of category I in its high content of urea, but other major contributors to osmolality can include trimethylamine oxide and betaine (Robertson, 1975, 1976), sarcosine and β-alanine (Boyd *et al.*, 1977). In *Raja erinacea*, the heart differs from wing muscle in containing little sarcosine and β-alanine, but much more taurine (Boyd *et al.*, 1977).

The last example is a reminder that tissues differ within one animal. In marine invertebrates, it is particularly notable that axoplasm differs from muscle in containing much more K. This is accompanied by organic anions such as aspartate, glutamate, cysteate and isethionate.

Reduction in intracellular osmolality, whether in euryhaline animals at low salinity or in the evolutionary colonization of fresh water, mainly involves losing osmolytes least essential to metabolism. At present one can only speculate how far the lowest osmolalities of vertebrates represent, for such active animals, the limits of this process. On the more dilute, and generally less active, invertebrates of category III there is much less information, but the general dilution or loss of organic solutes is apparent.

A variety of solutes have been found to occur in cells at high concentrations, depending on the species and tissue. Even within one tissue, i.e. muscle of *Myxine*, it seems that trimethylamine oxide and amino acids can be to some extent interchangeable (Cholette and Gagnon, 1973). On the other hand, many other common metabolites, including some amino acids, are never very concentrated. Some of the factors determining which solutes are most suitable as major osmolytes have been reviewed by Yancey *et al.* (1982). In brief, some solutes, such as arginine, lysine, urea and excess K, have deleterious effects on certain enzymes, directly or on their substrates or other ligands, whereas other solutes do not, including octopine, glycine, alanine and proline. Solutes having opposite effects may occur together and so counteract each other's effects. In elasmobranch tissues, the deleterious effects of the high level of urea are counteracted by trimethylamine oxide, betaine, sarcosine and β-alanine; the ratio of urea to the sum of these methylamines that is optimal for some enzymes is 2 : 1 and ratios close to this have been found *in vivo* (Yancey and Somero, 1980). In skinned muscle fibres of the dogfish, urea (330 mM) and trimethylamine oxide (180 mM) have opposite effects on tension generation (Altringham *et al.*, 1982). Arginine interacts strongly with phosphate groups and may exert much of its disruptive effect by tying up phosphate-containing substrates and co-factors (Bowlus and Somero, 1979). Bowlus and Somero stress the value of the conversion of arginine released from phosphorylarginine to the less perturbing octopine and propose that the effects of normal levels of arginine (which can reach 100 mmol/kg cell water) may be offset by those of trimethylamine oxide, betaine and glutamate. The solutes most suited to a role in intracellular

Table 1. Representative concentrations of some solutes in rat skeletal muscle fibres

	Concentration, mmol/kg water	Charge, m-equiv/kg water
Na	18	+18
K	165	+165
free Mg	3	+6
Cl	6	-6
HCO_3	10	-10
inorganic phosphate	2	-2
MgATP	9	-18
phosphorylcreatine	34	-68
creatine	13	-
free amino acids (protein-forming)	24	-
taurine	18	-
anserine + carnosine	15	+8
urea	5	-
lactate	3	-3
TOTAL	325	+89

isomotic regulation in muscle are those of no net charge (Burton, in press).

REFERENCES

Abe H. (1981) Determination of L-histidine compounds in fish muscles using high-performance liquid chromatography. *Bull. Jap. Soc. scient. Fish.* **47,** 139.

Allen J. A. and Garrett M. R. (1971) Taurine in marine invertebrates. *Adv. mar. Biol.* **9,** 205–253.

Altringham J. D., Yancey P. H. and Johnston I. A. (1982) The effects of osmoregulatory solutes on tension generation by dogfish skinned muscle fibres. *J. exp. Biol.* **96,** 443–445.

Amende L. M. and Pierce S. K. (1978) Hypotaurine: the identity of an unknown ninhydrin-positive compound co-eluting with urea in amino acid extracts of bivalve tissue. *Comp. Biochem. Physiol.* **59B,** 257–261.

Awapara J. (1962) Free amino acids in invertebrates: a comparative study of their distribution and metabolism. In *Amino Acid Pools* (Edited by Holden J. T.), pp. 158–175. Elsevier, Amsterdam.

Awapara J., Landua A. J. and Fuerst R. (1950) Distribution of free amino acids and related substances in organs of the rat. *Biochim. biophys. Acta.* **5,** 457–462.

Bartlett G. R. (1976) Phosphate compounds in red cells of reptiles, amphibians and fish. *Comp. Biochem. Physiol.* **55A,** 211–214.

Bartlett G. R. (1978) Water-soluble phosphates of fish red cells. *Can. J. Zool.* **56,** 870–877.

Bartlett G. R. (1982a) Developmental changes of phosphates in red cells of the emu and the rhea. *Comp. Biochem. Physiol.* **73A,** 129–134.

Bartlett G. R. (1982b) Phosphate compounds in red cells of two dogfish sharks: *Squalus acanthias* and *Mustelus canis*. *Comp. Biochem. Physiol.* **73A,** 135–140.

Bartlett G. R. and Borgese T. A. (1976) Phosphate compounds in red cells of the chicken and duck embryo and hatchling. *Comp. Biochem. Physiol.* **55A,** 207–210.

Beers J. R. (1967) The species distribution of some naturally-occurring quarternary ammonium compounds. *Comp. Biochem. Physiol.* **21,** 11–21.

Beis I. and Newsholme E. A. (1975) The contents of adenine nucleotides, phosphagens and some glycolytic intermediates in resting muscles from vertebrates and invertebrates. *Biochem. J.* **152,** 23–32.

Bowlus R. D. and Somero G. N. (1979) Solute compatibility with enzyme function and structure: rationales for the selection of osmotic agents and end products of anaerobic metabolism in marine invertebrates. *J. exp. Zool.* **208,** 137–152.

Boyd T. A., Cha C-J., Forster R. P. and Goldstein L. (1977) Free amino acids in tissues of the skate *Raja erinacea* and the stingray *Dasyatis sabina*: effects of environmental dilution. *J. exp. Zool.* **199,** 435–442.

Burton R. F. (1978) Intracellular buffering. *Respir. Physiol.* **33,** 51–58.

Burton R. F. (1980) Adenosine triphosphate as a determinant of magnesium levels in cytoplasm. *Comp. Biochem. Physiol.* **65A,** 1–4.

Burton R. F. (1983) Cell composition as assessed from osmolality and concentrations of sodium, potassium and chloride: total contributions of other substances to osmolality and charge balance. *Comp. Biochem. Physiol.* **76A,** 161–165.

Caldwell P. C. (1960) The phosphorus metabolism of squid axons and its relationship to the active transport of sodium. *J. Physiol., Lond.* **152,** 545–560.

Campbell J. W. and Speeg K. V. (1968) Arginine biosynthesis and metabolism in terrestrial snails. *Comp. Biochem. Physiol.* **25,** 3–32.

Cholette C. and Gagnon A. (1973) Isosmotic adaptation in *Myxine glutinosa* L.—II. Variations of the free amino acids, trimethylamine oxide and potassium of the blood and muscle cells. *Comp. Biochem. Physiol.* **45A,** 1009–1021.

Conway E. J. (1945) The physiological significance of inorganic levels in the internal medium of animals. *Biol. Rev.* **20,** 56–72.

Conway E. J. (1950) Calculation of the idiomolar value and its electrostatic equivalent in normal mammalian skeletal muscle. *Ir. J. Med Sci.* 6th Series, 216–224.

Costa C. J., Pierce S. K. and Warren M. K. (1980) The intracellular mechanism of salinity tolerance in polychaetes: volume regulation by isolated *Glycera dibranchiata* red coelomocytes. *Biol. Bull. mar. biol. Lab., Woods Hole* **159,** 626–638.

Crush K. G. (1970) Carnosine and related substances in animal tissues. *Comp. Biochem. Physiol.* **34,** 3–30.

Dall W. (1971) The role of homarine in decapod crustacea. *Comp. Biochem. Physiol.* **39B,** 31–44.

Davidson B. E. and Hird F. J. R. (1964) The estimation of glutathione in rat tissues. *Biochem. J.* **93,** 232–236.

Davies L. P. and Dowe G. H. C. (1977) High levels of β-alanine associated with the cholinergic neurones of *Torpedo marmorata* electric lobe. *Comp. Biochem. Physiol.* **58C,** 111–112.

Davis B. D. (1958) On the importance of being ionized. *Archs Biochem. Biophys.* **78,** 497–509.

Dawson M. J., Gadian D. G. and Wilkie D. R. (1977) Contraction and recovery of living muscles studied by ^{31}P nuclear magnetic resonance. *J. Physiol., Lond.* **267,** 703–735.

Deffner G. G. J. (1961) The dialyzable free organic constituents of squid blood; a comparison with nerve axoplasm. *Biochim. biophys. Acta* **47,** 378–388.

Drainville G. and Gagnon A. (1973) Osmoregulation in *Acanthamoeba castellani*—I. Variations of the concentrations of free intracellular amino acids and of the water content. *Comp. Biochem. Physiol.* **45A,** 379–388.

Dubreuil R. and Timiras P. S. (1953) Effect of cortisone on free amino acids in the serum and organs of the rabbit. *Am. J. Physiol.* **174,** 20–26.

Duman J. G., Horwarth K. L., Tomchaney A. and Patterson J. L. (1982) Antifreeze agents of terrestrial arthropods. *Comp. Biochem. Physiol.* **73A,** 545–555.

Eckel R. E., Norris J. E. C. and Pope C. E. (1958) Basic amino acids as intracellular cations in K deficiency. *Am. J. Physiol.* **193,** 644–652.

Edström L., Hultman E., Sahlin K. and Sjöholm H. (1982) The contents of high-energy phosphates in different fibre types in skeletal muscles from rat, guinea-pig and man. *J. Physiol., Lond.* **332,** 47–58.

Ennor A. H. and Morrison J. F. (1958) Biochemistry of the phosphagens and related guanidines. *Physiol. Rev.* **38,** 631–674.

Flock E. V. and Bollman J. L. (1962) Free amino acids in plasma, brain and muscle following hepatectomy. In *Amino Acid Pools* (Edited by Holden J. T.), pp. 449–460. Elsevier, Amsterdam.

Florkin M. and Schoffeniels E. (1965) Euryhalinity and the concept of physiological radiation. In *Studies in Comparative Biochemistry* (Edited by Munday K. A.), pp. 6–40. Pergamon Press, Oxford.

Forster R. P. and Goldstein L. (1976) Intracellular osmoregulatory role of amino acids and urea in marine elasmobranchs. *Am. J. Physiol.* **230,** 925–931.

Fraenkel G. (1953) The distribution of vitamin B_T (carnitine). *Biol. Bull. mar. biol. Lab., Woods Hole* **104,** 359–371.

Fraenkel G. (1954) Studies on the distribution of vitamin B_T (carnitine) throughout the animal kingdom. *Archs Biochem. Biophys.* **50,** 486–495.

Friedberg F. and Greenberg D. M. (1947) Endocrine regulation of amino acid levels in blood and tissues. *J. biol. Chem.* **168,** 405–409.

Fugelli K. and Zachariassen K. E. (1976) The distribution of taurine, gamma-aminobutyric acid and inorganic ions between plasma and erythrocytes in flounder (*Platichthys*

flesus) at different plasma osmolalities. *Comp. Biochem. Physiol.* **55A,** 173–177.

Gary-Bobo C. M. and Solomon A. K. (1968) Properties of hemoglobin solutions in red cells. *J. gen. Physiol.* **52,** 825–853.

Gasteiger E. L., Haake P. C. and Gergen J. A. (1960) An investigation of the distribution and function of homarine (*N*-methyl picolinic acid). *Ann. N.Y. Acad. Sci.* **90,** 622–636.

Gilbert D. S. (1975) Axoplasm chemical composition in *Myxicola* and solubility properties of its structural proteins. *J. Physiol., Lond.* **253,** 303–319.

Gilles R. (1975) Mechanisms of ion and osmoregulation. In *Marine Ecology* (Edited by Kinne O.). Vol. II, Part 1, pp. 259–347. John Wiley & Sons, London.

Gordon M. S. (1965) Intracellular osmoregulation in skeletal muscle during salinity adaptation in two species of toads. *Biol. Bull. mar. biol. Lab., Woods Hole* **128,** 218–229.

Gordon M. S. and Tucker V. A. (1968) Further observations on the physiology of salinity adaptation in the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.* **49,** 185–193.

Grieshaber M. K. (1982) Metabolic regulation of energy metabolism. In *Exogenous and Endogenous Influences on Metabolic and Neural Control.* (Edited by Addink A. D. F. and Spronk N.), Vol. I, pp. 225–242. Pergamon Press, Oxford.

Grieshaber M. and Gäde G. (1976) The biological role of octopine in the squid, *Loligo vulgaris* (Lamarck). *J. comp. Physiol.* **108,** 225–232.

Grieshaber M. and Gäde G. (1977) Energy supply and the formation of octopine in the adductor muscle of the scallop, *Pecten jacobaeus* (Lamarck). *Comp. Biochem. Physiol.* **58B,** 249–252.

Groninger H. S. (1959) The occurrence and significance of trimethylamine oxide in marine animals. U.S. Fish and Wildlife Service Special Scientific Report. Fisheries. Part 333, 1–22.

Hanig R. C. and Freeman A. R. (1980) Relationship between potassium, aspartate and several amino acids in the circumoesophageal connectives of the lobster. *Comp. Biochem. Physiol.* **66A,** 513–515.

Herbert J. D., Coulson R. A. and Hernandez T. (1966) Free amino acids in the caiman and rat. *Comp. Biochem. Physiol.* **17,** 583–598.

Hertelendy F. and Taylor T. G. (1964) The citric acid content of blood plasma and tissues of the domestic fowl. *Comp. Biochem. Physiol.* **11,** 173–182.

Horne F. R. (1971) Accumulation of urea by a pulmonate snail during aestivation. *Comp. Biochem. Physiol.* **38A,** 565–570.

Jacobsen J. G. and Smith L. H. (1968) Biochemistry and physiology of taurine and taurine derivatives. *Physiol. Rev.* **48,** 424–511.

Johnston M. A. and Spencer Davies P. (1972) Carbohydrates of the hepatopancreas and blood tissues of *Carcinus*. *Comp. Biochem. Physiol.* **41B,** 433–443.

Kaneshiro E. S., Holz G. G. and Dunham P. B. (1969) Osmoregulation in a marine ciliate, *Miamiensis avidus*. II. Regulation of intracellular free amino acids. *Biol. Bull. mar. biol. Lab., Woods Hole* **137,** 161–169.

Khuri R. N., Agulian S. K. and Bogharian K. K. (1976) Intracellular bicarbonate of skeletal muscle under different metabolic states. *Am. J. Physiol.* **230,** 228–232.

Koechlin B. A. (1955) On the chemical composition of the axoplasm of squid giant nerve fibers with particular reference to its ion pattern. *J. Biophys. Biochem. Cytol.* **1,** 511–529.

Kubišta V. (1957) Accumulation of a stable phosphorus compound in glycolysing insect muscle. *Nature, Lond.* **180,** 549.

Lewis P. R. (1952) The free amino-acids of invertebrate nerve. *Biochem. J.* **52,** 330–338.

Livingstone D. R. (1982) Energy production in the muscle tissues of different kinds of molluscs. In *Exogenous and Endogenous Influences on Metabolic and Neural Control* (Edited by Addink A. D. F and Spronk N.), Vol. 1, pp. 257–274. Pergamon Press, Oxford.

Long C. (1961) *Biochemist's Handbook*. Spon, London.

Lukton A. and Olcott H. S. (1958) Content of free imidazole compounds in the muscle tissue of aquatic animals. *Fd Res.* **23,** 611–618.

Norris E. R. and Benoit G. T. (1945) Studies on trimethylamine oxide I. Occurrence of trimethylamine oxide in marine organisms. *J. biol. Chem.* **158,** 433–438.

Pandian T. J. (1957) Mechanisms of heterotrophy. In *Marine Ecology* (Edited by Kinne O.), Vol. II, Part 1, pp. 61–249. John Wiley & Sons, London.

Porcellati G., Floridi A. and Ciammarughi A. (1965) The distribution and the biological significance of L-serine ethanolamine and L-threonine ethanolamine phosphates. *Comp. Biochem. Physiol.* **14,** 413–418.

Potts W. T. W. (1958) The inorganic and amino acid composition of some lamellibranch muscles. *J. exp. Biol.* **35,** 749–764.

Raina A. (1963) Studies on the determination of spermidine and spermine and their metabolism in the developing chick embryo. *Acta physiol. scand.* **60,** Suppl. 218, 1–81.

Rapoport S. and Guest G. M. (1941) Distribution of acid-soluble phosphorus in the blood cells of various vertebrates. *J. biol. Chem.* **138,** 269–282.

Robertson J. D. (1961) Studies on the chemical composition of muscle tissue. II. The abdominal flexor muscles of the lobster *Nephrops norvegicus* (L.). *J. exp. Biol.* **38,** 707–728.

Robertson J. D. (1965) Studies on the chemical composition of muscle tissue. III. The mantle muscle of cephalopod molluscs. *J. exp. Biol.* **42,** 153–175.

Robertson J. D. (1970) Osmotic and ionic regulation in the horseshoe crab *Limulus polyphemus* (Linnaeus). *Biol. Bull. mar. biol. Lab., Woods Hole* **138,** 157–183.

Robertson J. D. (1975) Osmotic constituents of the blood plasma and parietal muscle of *Squalus acanthias* L. *Biol. Bull. mar. biol. Lab., Woods Hole* **148,** 303–319.

Robertson J. D. (1976) Chemical composition of the body fluids and muscle of the hagfish *Myxine glutinosa* and the rabbit-fish *Chimaera monstrosa*. *J. Zool., Lond.* **178,** 261–277.

Robertson J. D. (1980) Osmotic constituents of some echinoderm muscles. *Comp. Biochem. Physiol.* **67A,** 535–543.

Robin Y. (1964) Biological distribution of guanidines and phosphagens in marine Annelida and related phyla from California, with a note on pluriphosphagens. *Comp. Biochem. Physiol.* **12,** 347–367.

Ronold O. A. and Jakobsen F. (1947) Trimethylamine oxide in marine products. *J. Soc. chem. Ind.* **66,** 160–166.

Sassenrath E. N. and Greenberg D. M. (1954) Tumor host relationships 1. Effects on free amino acid concentrations of certain tissues. *Cancer Res.* **14,** 563–569.

Schmid W. D. (1982) Survival of frogs in low temperature. *Science, N.Y.* **215,** 697–698.

Seraydarian K., Mommaerts W. F. H. M. and Wallner A. (1962) The amount and compartmentalization of adenosine diphosphate in muscle. *Biochim. biophys. Acta* **65,** 443–460.

Severin S. E., Boldyrev A. A. and Lebedev A. V. (1972) Nitrogenous extractive compounds of muscle tissue of invertebrates. *Comp. Biochem. Physiol.* **43B,** 369–381.

Sherman W. R., Simpson P. C. and Goodwin S. L. (1978) *Scyllo*-inositol and *myo*-inositol levels in tissues of the skate *Raja erinacea*. *Comp. Biochem. Physiol.* **59B,** 201–202.

Sherman W. R., Stewart M. A., Kurien M. M. and Goodwin S. L. (1968) The measurement of *myo*-inositol, *myo*-inosose-2 and *Scyllo*-inositol in mammalian tissues. *Biochim. biophys. Acta* **158,** 197–205.

Sømme L. (1982) Supercooling and winter survival in terrestrial arthropods. *Comp. Biochem. Physiol.* **73A,** 519–543.

Tallan H. H. (1962) A survey of the amino acids and related

compounds in nervous tissue. In *Amino Acid Pools* (Edited by Holden J. T.), pp 471–485. Elsevier, Amsterdam.

Thoai N. van and Roche J. (1961) Phosphagens of marine animals. *Ann. N.Y. Acad. Sci.* **90,** 923–928.

Thomas R. C. (1976) The effect of carbon dioxide on the intracellular pH and buffering power of snail neurones. *J. Physiol., Lond.* **255,** 715–735.

Vyncke W. (1970) Influence of biological and environmental factors on nitrogenous extractives of the spurdog *Squalus acanthias*. *Mar. Biol.* **6,** 248–255.

Welsh J. H. and Prock P. B. (1958) Quarternary ammonium bases in the coelenterates. *Biol. Bull. mar. biol. Lab., Woods Hole* **115,** 551–561.

Williamson D. H. and Brosnan J. T. (1974) Concentrations of metabolites in animal tissues. In *Methods of Enzymatic Analysis*, 2nd Edition (Edited by Bergmeyer H-U.), Vol. 4, pp. 2266–2302. Academic Press, New York.

Yancey P. H., Clark M. E. H. and S. C., Bowlus R. D. and Somero G. N. (1982) Living with water stress: evolution of osmolyte systems. *Science, N.Y.* **217,** 1214–1222.

Yancey P. H. and Somero G. N. (1980) Methylamine osmoregulatory solutes of elasmobranch fishes counteract urea inhibition of enzymes. *J. exp. Zool.* **212,** 205–213.

Zbiegieni B. (1979) Level of ascorbic acid in adrenal glands of laboratory mice bred in various population densities. *Comp. Biochem. Physiol.* **62A,** 851–854.