ELECTROLYTE AND MINERAL METABOLISM[1]

By L. G. Welt, J. R. Sachs, and H. J. Gitelman
Department of Medicine, School of Medicine,
University of North Carolina, Chapel Hill, North Carolina

This review does not pretend to cover the entire field encompassed by the title. Special attention is given to certain aspects of the literature dealing with the alkali metal ions: sodium and potassium, and the alkaline earth metals: magnesium and calcium.

In the past several years there have been several reviews and symposia which discuss many of these areas (1–10). There are many articles that will not be cited specifically but will be found in the bibliographies of those papers that are noted. The only intention of this device has been to save space.

Drugs

Aldosterone has been shown to increase sodium transport in the toad bladder (11, 12) and the frog skin (13). Crabbé (11) has presented evidence that aldosterone increases the passive permeability of the mucosal surface to sodium. Porter & Edelman (12) have examined the relationships between activity and structure of various steroids on the toad bladder. Edelman and his group (14, 15) have demonstrated binding of aldosterone to the cell nuclei of the toad bladder; and, in addition, that actinomycin D (an inhibitor of DNA-mediated RNA synthesis) and puromycin (an inhibitor of protein synthesis) inhibit the influence of aldosterone. The effect of aldosterone is associated with elevated levels of adenosinetriphosphate (ATP) in tissue. The effect of actinomycin D and puromycin has also been demonstrated by Crabbé (16). However, Spach & Streeten (17) have found that aldosterone in physiological concentrations appears to inhibit significantly the transport of sodium in the dog erythrocyte. The red blood cell of this particular species, however, has unusual characteristics.

Oligomycin, a drug which blocks oxidative phosphorylation, was found to inhibit an $Na^+ + K^+$ activated ATPase preparation from brain microsomes (18, 19), erythrocyte membranes, and the kidney cortex (20). Whittam, Wheeler & Blake (20) have demonstrated that the drug also inhibits active potassium influx and sodium efflux in human erythrocytes. Van Rossum (21), however, was unable to find an effect of the drug on the transport of sodium and potassium in the erythrocyte, although it did inhibit cation transport in liver slices. In this respect, it is noteworthy that Skou has found a microsomal preparation from rat brain which displays an $Na^+ + K^+$ ATPase activity, an NADH-cytochrome C reductase activity,

[1] The survey of the literature pertaining to this review was concluded in July 1965.

and a diaphorase activity. N-Ethyl maleimide (NEM) inhibits ATPase and cytochrome C reductase activities; its inhibition of the ATPase activity is modified by ATP and to a lesser extent by NADH; its effect on the cytochrome C reductase is antagonized by NADH, and to a lesser extent by ATP.

The application of thyroxin in physiological concentrations to the serosal surface of toad skin or bladder was found by Green & Matty (22) to stimulate the active transport of sodium. Marusic & Torretti (23) observed the same effect in the isolated toad bladder; however, in the presence of vasopressin there was no further increase in sodium transport upon exhibition of thyroxin. Several other drugs were reported to have interesting effects on electrolyte metabolism or the components of the so-called "pump." Taylor (24) has found that mersalyl, Mercloran (chlormerodrin), esidron, and parachloromercuric benzoate inhibited the $Na^+ + K^+$ stimulated preparations of ATPase from the rabbit kidney, whereas caffeine, theobromine, theophylline, and chlorothiazide did not. Mersalyl, however, did not inhibit the sodium-stimulated respiration of kidney slices as did ouabain (25). Kahn (26) studied two erythrophleum alkaloids, cassaine and coumingine, which are structurally dissimilar from the cardiac glycosides but have similar cardiotonic properties. He noted that their influence was quite similar to cardiac glycosides in their effect on the transport of potassium across the erythrocyte membrane. Epinephrine has been found to increase sodium efflux in the frog heart, and this effect can be eliminated by exposure to dinitrophenol or iodoacetic acid (27). Procaine, dibucaine, cocaine, lidocaine, and tetracaine inhibit active uptake of potassium and the extrusion of sodium in cold stored erythrocytes (28). Rummell, Seifen & Baldauf (29) have found that calcium within the erythrocyte depresses the active influx of potassium and diminishes the fraction of that influx which is sensitive to ouabain. A dialyzable extract of suspensions of vibrio cholera has been found, which diminishes sodium influx, water movement, and thiourea transport when applied to the mucosal surface of the toad bladder (30).

ATPase

An enormous amount of literature has appeared dealing with the $Na^+ + K^+$ activated ATPase of cell membrane preparations, and this has been superbly reviewed by Skou(6). Judah & Ahmed (31) have prepared a detailed and provocative review of recent investigations on the biochemical correlates of active cation transport. Hokin & Hokin (32) have reviewed much of their evidence for the role of phosphatidic acid in ion transport.

Whittam & Ager (33, 34) have found that in human erythrocyte ghosts, stimulation of the $Na^+ + K^+$ activated ATPase requires internal sodium or lithium and external lithium, rubidium, cesium, or potassium in the order $Rb^+ = K^+ > Cs^+ > Li^+$ (35). External sodium inhibits the activation of potassium from the outside. This was also noted by Schatzmann (36). The

hydrolysis of ATP liberates inorganic phosphorus on the inside. Attempts to solubilize the ATPase have proved futile (37).

Squires (38) has found that the $Na^+ + K^+$ activated ATPase from the rat brain does not follow classical Michaelis-Menten kinetics, but behaves like an allosteric enzyme, i.e., one in which the binding of a substrate results in a conformational change in the protein. The protection from chlorpromazine inhibition by potassium also appears to behave as if it were an allosteric effect. Hokin & Yoda (39) also invoke the concept of allosterism to explain a complex interaction between diisopropyl fluorophosphate, ATP, potassium, strophanthidin, and the $Na^+ + K^+$ stimulated ATPase of beef kidney.

Charnock et al., using an $Na^+ + K^+$ activated ATPase system obtained from guinea pig kidney (40, 41), have found that, when γ-labeled ATP^{32} was used, it was possible to obtain evidence for the formation of an intermediate, and potassium in the presence of sodium leads to hydrolysis of the intermediate with release of P^{32}. Ouabain inhibits the effect of potassium but not that of sodium. The phosphorylated intermediate is thought to be a lipoprotein.

The role of phospholipids in the $Na^+ + K^+$ activated ATPase system of electrophorus and torpedo has been investigated by Glynn et al. (42); under no circumstances could P^{32} from γ-labeled P^{32} be found in phosphatidic, triphosphoinositide, or any other phospholipid. Kirschner & Barker (43) have presented evidence that the rate of turnover of phosphatidic acid is insufficient to permit its use as a sodium carrier in the erythrocytes of swine and the ox.

Sen & Post (44) have proposed that, in the human erythrocyte a "transport cycle" consists of the transport of three molecules of sodium out of the cells, two molecules of potassium into the cells, with the hydrolysis of one molecule of ATP.

Ion Interactions with Other Processes

Csáky (45, 46) has published discussions of some interactions of alkali metal ions with active transport systems for hexoses in the intestine. Using various preparations of hamster intestine, Bihler & Crane (47) have found that sodium is required at the mucosal surface for the active transport of sugar. Sodium cannot be replaced by potassium, lithium, ammonium, Tris, magnesium, calcium, choline, or guanidine. The transport process could be divided into two phases, one of which is a sodium-dependent accumulation (48). Harrison & Harrison (49) have found that transport of glucose, L-tyrosine, and inorganic phosphate by rat intestinal loops required sodium and that potassium was inhibitory. Sodium is not required for the transport of calcium. In muscle, sodium was required for the active transport of α-aminoisobutyric acid and its stimulation by insulin (50). Ouabain inhibits amino acid transport, but at concentrations higher than those neces-

sary to inhibit completely the transport of cations. In lymphocytes and granulocytes, the transport of α-aminoisobutyric acid, glycine, proline, alanine, and glutamic acid required both sodium and potassium for optimal activity, but high concentrations of potassium were inhibitory (51). Strophanthidin inhibited transport and calcium enhanced it. Schultz & Zalusky (52) found that the addition of sugars, which are transported actively, to preparations of rabbit ileum enhanced sodium transport, and, furthermore, the relation of increased sodium transport to the concentration of sugar followed Michaelis-Menten kinetics. Addition of amino acids similarly enhanced sodium transport (53). Others (54) have found a new member of the ATPase family which is magnesium-dependent and activated by sodium alone. Furthermore, this activation is glycoside-sensitive and, importantly, is not stimulated but inhibited by potassium. The possible role of this enzyme system in the inter-relationships alluded to above is provocative.

Interactions of calcium with sodium and water transport in epithelial membranes have been reported. Curran and his co-workers have found that calcium at the mucosal surface of the frog skin decreases the active transport of sodium (55), that the effect of serosal vasopressin seems to be different and additive to mucosal calcium (56), and that both agents exert their effect by increasing the permeability of the mucosal barrier to sodium (57). Petersen & Edelman (58) have found that calcium competes with vasopressin on water and urea transport in the toad bladder, but has no effect on the vasopressin-induced increase in sodium transport. Andersen & Tomlinson (59) have reported that low calcium concentrations on both sides of the toad bladder inhibit sodium transport, whereas high concentrations of calcium had no effect; in both cases the response to oxytocin was normal.

Sodium and Potassium Transport

Erythrocyte.—A review of the genetic and physiological implications of the existence of two classes of sheep which differ in their erythrocyte-electrolyte composition has appeared (60). Hoffman (61) has investigated the characteristics of sodium efflux in human erythrocyte ghosts. Sorenson, Kirschnez & Barker (62) report that the efflux of sodium from the erythrocytes of several species is proportional to their intracellular sodium content. Using human erythrocytes whose intracellular cation content had been altered by exposure to electrolyte free solution, McConaghey & Maizels (35) found that transport does not occur in the absence of intracellular sodium, and that the efflux of sodium depends on intracellular sodium in a complex manner (which may be interpreted as indicating that more than one sodium atom is required at the active transport site); and, furthermore, potassium, rubidium, cesium, lithium, or ammonium is necessary in the external solution for sodium efflux. Cook (63) has found that ultraviolet hemolysis may be explained as colloid osmotic swelling as a consequence of increased permeability of the erythrocyte to cations.

Several disease states have been found to be associated with abnormalities in the transport systems for sodium and potassium in erythrocytes. Jacobs & Jandl (64) observed that erythrocytes of patients with hereditary spherocytosis have an increase in the passive permeability to sodium. Welt, Sachs & McManus (65) have demonstrated a defect in the erythrocyte transport system for potassium, and in the $Na^+ + K^+$ activated ATPase system of erythrocyte ghosts from patients with uremia. The search for transport defects in other diseases deserves considerable attention since it may lead to insights into the molecular pathology of the disorder, and a more rational search for remedial measures.

Toad bladder and frog skin.—The concept of tight coupling of sodium and potassium transport has become less supportable in these structures. Frazier & Leaf (66) have found that the electrical potential across the serosal border of the toad bladder is too high to be explained as a potassium diffusion potential, or as a diffusion potential of any anion species. They conclude that the transport of sodium is electrogenic, i.e., that a net charge is transported. Essig (67) has shown that sodium transport may occur against an electrochemical gradient even in the absence of potassium on the mucosal surface. Bricker, Biber & Ussing (68) and Klahr & Bricker (69) have demonstrated that active sodium transport occurs in the frog skin even when the potassium concentrations bathing the serosal surfaces are higher than that within the cells; this is inconsistent with the Kofoed-Johnson-Ussing model, since potassium cannot diffuse passively into the serosal solution. This again supports the existence of an electrogenic pump. Essig & Leaf (70) report that the stimulation of sodium transport by potassium bathing the serosal surface of the toad bladder is not associated with K^{42} uptake, and does not appear to result from an increase in the active phase of sodium transport, but rather from an effect of potassium in increasing the permeability of the mucosal border. Exposure of frog skin to high concentrations of potassium results in swelling and a loss of response to vasopressin. Ussing (71) provides a critique in his Harvey lecture.

Klahr & Bricker (72, 73) have studied the transport of sodium in the freshwater turtle bladder. In this species, energy from anaerobic glycolysis may be used to drive sodium transport. The authors conclude that 15 molecules of sodium are transported per molecule of ATP hydrolyzed.

Frazier & Hammer (74) have demonstrated that vasopressin markedly increases sodium efflux across the mucosal surface of the toad bladder.

Bowers (75) has demonstrated that ouabain inhibits sodium transport when applied to the serosal surface but not the mucosal; high serosal concentrations of potassium inhibited the effect of ouabain.

Hays & Leaf (76, 77, 78) have investigated the action of vasopressin on water permeability of the toad bladder; they suggest that vasopressin exerts its effect by increasing the radius of pores in the membrane, thus permitting the enclosed water to assume the properties of bulk water; in the unstimulated state water within the pores behaves as though it were struc-

tured. Vasopressin also increases the permeability of the bladder to urea and a small group of amides and small alcohols, although penetration by other small molecules is unaffected. Whittembury (79), using probing molecules, has found that in the frog skin, the equivalent pore radius of the serosal membrane is 7 Å and that of the mucosal membrane 4.5 Å; vasopressin increases the mucosal pore radius to 6.5 Å.

Gall bladder.—In the mammalian gall bladder, the mechanism of isosmotic water transport and the transport of water against an osmotic gradient has been investigated. It is concluded by Diamond (80, 81) that the process occurs by "local osmosis," i.e., solute (NaCl) is transported across a membrane into a restricted area where its concentration increases; water follows as a result of osmosis. As a consequence of these events, the hydrostatic pressure rises in the restricted area and the solution is forced out of that area. Whitlock & Wheeler (82) have presented evidence that the process occurs as a result of a serial membrane system which differs from the "local osmosis" process in that the restricted area is enclosed within two membranes.

Muscle.—Horowicz & Gerber (83, 84) have found that the extrusion of sodium from muscle was increased by external potassium but only at concentrations of potassium higher than 7.5 m*M* per liter; the efflux was also stimulated by sodium azide at concentrations of 2 and 5 m*M* per liter. Both effects are explained as a result of depolarization of the membrane which then stimulates the active transport of sodium. Mullins & Frumento (85) have found that the rate of extrusion of sodium from muscle was a complex function of the intracellular concentration of sodium.

Kidney.—Burg, Grollman & Orloff (86) have examined sodium and potassium fluxes in isolated renal tubules; cardiac glycosides are found to inhibit potassium influx and a part of the sodium efflux. There does not appear to be a one to one coupling of sodium efflux and potassium influx. Burg & Orloff (87) have found active sodium and potassium transport in these preparations at 0° C. The techniques introduced by Burg & Orloff offer superb opportunities for direct observations of transtubular transport, and will no doubt provide an important approach to an understanding of the actions of drugs on renal tubular activities. Whittembury (88) has investigated sodium and potassium transport in guinea pig kidney cortex slices. Sodium is extruded against an electrochemical gradient and requires external potassium. The uptake of potassium at low levels of this cation is secondary to the extrusion of sodium. Whittam & Willis (25) have found that a portion of the oxygen consumption of rabbit kidney slices is dependent on the presence of sodium and is inhibited by ouabain.

Renal Sodium Excretion

Some of the problems relating to renal excretion of electrolytes have been reviewed by Wesson (89). In recent years, two new influences on the rate of excretion of sodium by the kidney have been noted, which are in-

dependent of the filtered load of this cation. One of these relates to the volume of the extracellular fluid (90, 91, 92), and it has been demonstrated that an increase in this fluid volume augments the rate of excretion of sodium independently of filtered load and of mineralocorticoid secretion. In addition, it has been demonstrated that the concentration of sodium in the plasma per se may also modify the rate of excretion of this ion. Hypernatremia and hyponatremia augment and diminish the rate of excretion of sodium respectively and independently of all the other factors which are known to play a role (93–96).

Magnesium

Growing interest in magnesium metabolism has particularly emphasized the biological consequences of magnesium deficiency. This literature has recently been reviewed (*7*, 97).

Magnesium-deficient rats characteristically develop hypercalcemia. The possibility that this might be related to an increase in parathyroid activity was first suggested by MacIntyre, Boss & Troughton (98). Although the mechanisms involved remain to be elucidated, it is clear that the parathyroid gland is involved, since hypercalcemia does not occur in the absence of this gland even when parathyroid extract is given to parathyroidectomized rats in amounts sufficient to restore the plasma calcium of appropriate control animals to normal levels (99). Moreover, an alteration in the parathyroid regulation of calcium homeostasis has been suggested, following the demonstration of elevated ionic levels of calcium in intact hypomagnesemic animals (100). Although a diminished quantity of magnesium in the diet is accompanied by an increase in calcium absorption from the gut (101), possibly caused by competition for a common absorptive mechanism, dietary calcium is not necessary for the hypercalcemia of this disorder (102).

Other alterations include loss of muscle potassium *in vivo,* and this occurs *in vitro* as well, following incubation of intact rat diaphragm in solutions free of magnesium (103). Hence, a possible relationship to membrane ATPase (102) and alteration in permeability (103) have been suggested. Hypophosphatemia and hyperphosphaturia occur. The hyperphosphaturia cannot be completely explained by the hyperparathyroid state since it is also seen in parathyroidectomized animals with magnesium deficiency (99). The chemical response to magnesium deficiency shows species variability. A recent report of experimental magnesium deficiency in two men demonstrated a marked hypocalcemia rather than hypercalcemia (104).

The renal lesion has been ascribed to the formation of a lamellated microlith in the broad ascending limb of Henle's loop which, with enlargement, induces secondary lesions by pressure on adjacent cells and proximal obstruction (105). In similar experimental circumstances, degenerative lesions of the proximal convoluted tubule have been reported to be prominent (106–108); and in one instance, the ingestion of a magnesium and calcium-deficient diet by intact rats has been associated with the renal deposition of

calcium in the presence of a minimally depressed level of calcium (107). Nevertheless, the degree of renal calcification was less than in the animals that were magnesium-deficient along with concomitant hypercalcemia. It would appear that the level of calcium in plasma plays some role, albeit not the sole factor, in the development of nephrocalcinosis (108). It is also of great interest to note that magnesium-deficient rats do not have a urinary concentrating defect despite the coincidental hypercalcemia (109).

Regulation of Plasma Calcium Concentration

The responses of the parathyroid gland to hypocalcemia and this aspect of its function in calcium homeostasis are well established. A possible additional role of the gland in the control of hypercalcemia was initially suggested by Copp et al. (110) who postulated the presence of a fast-acting hypocalcemic factor, calcitonin. They demonstrated that a fall in the concentration of calcium in plasma could be induced by the perfusion of dog parathyroid tissue with a fluid with a high concentration of calcium. The observations have been independently confirmed (111) and corroborated in sheep (112). Although the latter experiment, involving isolated perfusion of thyroid and parathyroid tissue, suggested that the parathyroid gland was the site of origin of the hypocalcemic factor, the demonstration of a potent hypocalcemic factor extractable from thyroid, thyrocalcitonin (113), and the observation that the calcitonin effect did not occur in the absence of thyroid tissue (114) strongly implicated the thyroid gland in this response. It has been subsequently shown in rats that both glands are necessary for a normal response to the hypercalcemia induced by exogenous parathyroid hormone administration (115). Moreover, a resolution of the apparent paradox has been proposed (115) with the suggestion that the parathyroids secrete a factor in response to hypercalcemia which, in turn, releases thyrocalcitonin from the thyroid. Preparations of material from parathyroid glands which induce hypocalcemia have been described (116), but it is not known whether their action is mediated by thyroid tissue.

Thyrocalcitonin is a polypeptide (117, 118), with a molecular weight of 8500, which has been isolated using a separation technique similar to that described for parathyroid hormone (119). Preliminary histochemical studies of thyroid glands perfused with blood with high and low concentrations of calcium have indicated that the parafollicular thyroid cells may be responsible for the secretion of thyrocalcitonin (120).

The site of action of this material is not yet established, although the possibility of a direct effect on bone has been raised in the absence of alternative explanations involving the kidney and the gastrointestinal tract (117). It is active in parathyroidectomized rats (117), excluding a direct inhibition of parathyroid hormone or the parathyroid gland as its primary mechanism.

A potent hypocalcemic factor has been isolated from porcine and human pituitaries that is active in the rabbit (121). Its relationship, if any, to the parathyroid-thyroid system is not known.

Mitochondria.—The uptake and translocation of ions in subcellular systems have received increasing attention in the past few years (122). The role of calcium in muscle contraction has been extensively investigated in the whole muscle and its individual components (i.e., actomyosin systems and the sarcoplasmic reticulum). Two reviews summarize recent studies in this area (123, 124).

The possibility that mitochondrial systems might be utilized as *in vitro* models for the study of parathyroid hormone and vitamin D influences was first suggested by DeLuca, Engstrom & Rasmussen (125) who noted that parathyroid hormone stimulated a specific vitamin D-dependent calcium release from rat kidney mitochondria. Subsequent investigations defined a highly specific influence of parathyroid hormone on the stimulation of phosphate uptake in an oligomycin-blocked mitochondrial system requiring the presence of ATP and magnesium (126). The uptake of phosphate is independent of vitamin D, in contrast to the vitamin D dependence of parathyroid hormone on mitochondrial calcium release. A similar relationship has been observed *in vivo* (127) since exogenous parathyroid hormone is able to diminish the level of serum phosphate in vitamin D-deficient rats without significant alteration in serum calcium levels. (However, the observations made with regard to the effect of acute parathyroidectomy on serum calcium and phosphorous levels must be interpreted with caution since parathyroidectomy was accomplished by cautery, a procedure which may release thyrocalcitonin into the circulation.) Nevertheless, others have demonstrated a persistence of the calcium mobilizing effect of parathyroid hormone *in vivo* (128) and *in vitro* (129) in rachitic animals.

A specific stimulation by parathyroid hormone of a liver mitochondrial ATPase preparation has been observed (130), which does not have the characteristics of Skou's enzyme (6). The significance of this observation is unknown. Since vitamin D-treated animals bind significantly less calcium to cellular debris than homogenates prepared from rachitic animals, vitamin D-stimulated "release" phenomena have also been noted in studies of calcium binding utilizing homogenates of intestinal mucosa (131). The relationship of this observation to vitamin D stimulation of calcium absorption is unclear.

The effect of vitamin D on absorptive mechanisms in the intestine has been shown to involve bidirectional alterations in mucosal permeability (132, 133) as well as stimulation of the previously described active transport process. Surprisingly, an effect of vitamin D deficiency on isolated intestinal villi was not demonstrated (134), although an effect of parathyroid hormone on the release of calcium at low temperature was observed.

Vitamin D_3 is localized in subcellular membranes (131); the major component is recoverable as D_3 (135) and can stimulate calcium transport in the intestine by direct application to the mucosa (136). Increased phospholipid turnover, independent of increased calcium transport, has been described (137). The relationship of these observations to the overall effect of vitamin D on gastrointestinal absorption remains to be elucidated.

HeLa cells have been demonstrated to respond to parathyroid hormone with increased mitoses, diminished adhesiveness to glass, and increased resorption of bone powder. This may provide a convenient model system for subsequent *in vitro* studies (138).

LITERATURE CITED

1. Comar, C. L., and Bronner, F., *Mineral Metab.*, **1** (Parts A and B), **2** (Parts A and B) (1964)
2. Wasserman, R. H., Ed., *The Transfer of Calcium and Strontium Across Biological Membranes* (Academic Press, New York, 123 pp., 1963)
3. Hoffman, J. F., Ed., *The Cellular Functions of Membrane Transport* (Prentice-Hall Inc., Englewood Cliffs, N.J., 291 pp., 1964)
4. De Graeff, J., and Leijnse, B., Eds., *Water and Electrolyte Metabolism II* (Elsevier, Amsterdam, 244 pp., 1964)
5. Frazier, H. S., and Leaf, A., *Medicine*, **43**, 281–89 (1964)
6. Skou, J. C., *Physiol. Rev.*, **45**, 596–617 (1965)
7. Welt, L. G., and Gitelman, H., *Disease-A-Month* (May 1965)
8. Glynn, I. M., *Pharmacol. Rev.*, **16**, 381–407 (1964)
9. Orloff, J., and Handler, J. S., *Am. J. Med.*, **36**, 687–97 (1964)
10. Forland, M., and Pullman, T. N., *Med. Clin. N. Am.*, **47**, 113–29 (1963)
11. Crabbé, J., *Nature*, **200**, 787–88 (1963)
12. Porter, G. A., and Edelman, I. S., *J. Clin. Invest.*, **43**, 611–20 (1964)
13. Crabbé, J., *Endocrinology*, **75**, 809–11 (1964)
14. Edelman, I. S., Bogoroch, R., and Porter, G. A., *Proc. Natl. Acad. Sci.*, **50**, 1169–77 (1963)
15. Edelman, I. S., Bogoroch, R., and Porter, G. A., *Trans. Assoc. Am. Physicians*, **67**, 307–16 (1964)
16. Crabbé, J., and DeWeer, P., *Nature*, **202**, 298–99 (1964)
17. Spach, C., and Streeten, D. H. P., *J. Clin. Invest.*, **43**, 217–27 (1964)
18. Van Groningen, H. E. M., and Slater, E. C., *Biochim. Biophys. Acta*, **73**, 527–30 (1963)
19. Jobsis, F. F., and Vreman, H. J., *Biochim. Biophys. Acta*, **73**, 346–48 (1963)
20. Whittam, R., Wheeler, P., and Blake, A., *Nature*, **203**, 720–24 (1964)
21. Van Rossum, G. D. V., *Biochim. Biophys. Acta*, **82**, 556–71 (1964)
22. Green, K., and Matty, A. J., *Gen. Comp. Endocrinol.*, **3**, 244–52 (1963)
23. Marusic, E., and Torretti, J., *Nature*, **202**, 1118–19 (1964)
24. Taylor, C. B., *Biochem. Pharmacol.*, **12**, 539–50 (1963)
25. Whittam, R., and Willis, J. S., *J. Physiol. (London)*, **168**, 158–77 (1963)
26. Kahn, J. B., *Proc. Soc. Exptl. Biol. Med.*, **110**, 412–14 (1962)
27. Haas, H. G., and Trautwein, W., *Nature*, **197**, 80–81 (1963)
28. Andersen, N. B., and Gravenstein, J. S., *J. Pharmacol. Exptl. Therap.*, **147**, 40–47 (1965)
29. Rummel, W., Seifen, E., and Baldauf, J., *Biochem. Pharmacol.*, **12**, 557–63 (1963)
30. Fuhrman, F. A., Fuhrman, G. J., and Burrows, W., *J. Infect. Diseases*, **111**, 225–32 (1962)
31. Judah, J. A., and Ahmed, K., *Biol. Rev. Cambridge Phil. Soc.*, **39**, 160–93 (1964)
32. Hokin, L. E., and Hokin, M. R., *Federation Proc.*, **22**, 8–18 (1963)
33. Whittam, R., and Ager, M. E., *Biochim. Biophys. Acta*, **65**, 383–85 (1962)
34. Whittam, R., and Ager, M. E., *Biochem. J.*, **93**, 337–48 (1964)
35. McConaghey, P. D., and Maizels, M., *J. Physiol. (London)*, **162**, 485–509 (1962)
36. Schatzmann, H. J., *Biochim. Biophys. Acta*, **94**, 89–96 (1965)
37. Wheeler, K. P., and Whittam, R., *Biochem. J.*, **93**, 349–63 (1964)
38. Squires, R. F., *Biochem. Biophys. Res. Commun.*, **19**, 27–32 (1965)
39. Hokin, L. E., and Yoda, A., *Biochim. Biophys. Acta*, **97**, 594–95 (1965)
40. Charnock, J. S., and Post, R. L., *Australian J. Exptl. Biol. Med. Sci.*, **41**, 547–60 (1963)
41. Charnock, J. S., Rosenthal, A. S., and Post, R. L., *Australian J. Exptl. Biol. Med. Sci.*, **41**, 675–86 (1963)

42. Glynn, I. M., Slayman, C. W., Eichberg, J., and Dawson, R. M. C., *Biochem. J.*, **94,** 692–99 (1964)
43. Kirschner, L. B., and Barker, J., *J. Gen. Physiol.*, **47,** 1061–78 (1964)
44. Sen, A. K., and Post, R. L., *J. Biol. Chem.*, **239,** 345–52 (1964)
45. Csáky, T. Z., *Federation Proc.*, **22,** 3–7 (1963)
46. Csáky, T. Z., *Biochim. Biophys. Acta*, **74,** 160–62 (1963)
47. Bihler, I., and Crane, R. K., *Biochim. Biophys. Acta*, **59,** 78–93 (1962)
48. Bihler, I., Hawkins, K. A., and Crane, R. K., *Biochim. Biophys. Acta*, **59,** 94–102 (1962)
49. Harrison, H. E., and Harrison, H. C., *Am. J. Physiol.*, **205,** 107–11 (1963)
50. Parrish, J. E., and Kipnis, D. M., *J. Clin. Invest.*, **43,** 1994–2002 (1964)
51. Yunis, A. A., Arimura, G. K., and Kipnis, D. M., *J. Lab. Clin. Med.*, **62,** 465–76 (1963)
52. Schultz, S. G., and Zalusky, R., *J. Gen. Physiol.*, **47,** 1043–59 (1964)
53. Schultz, S. G., and Zalusky, R., *Nature*, **205,** 292–94 (1965)
54. Czerwinski, A. W., Gitelman, H. J., and Welt, L. G., *J. Clin. Invest.*, **44,** 1039 (1965)
55. Curran, P. F., and Gill, J. R., Jr., *J. Gen. Physiol.*, **45,** 625–41 (1962)
56. Herrera, F. C., and Curran, P. F., *J. Gen. Physiol.*, **46,** 999–1010 (1963)
57. Curran, P. F., Herrera, F. C., and Flanigan, W. J., *J. Gen. Physiol.*, **46,** 1011–27 (1963)
58. Petersen, M. H., and Edelman, I. S., *J. Clin. Invest.*, **43,** 583–94 (1964)
59. Andersen, J., and Tomlinson, W. S., *J. Physiol. (London)*, **177,** 133–39 (1965)
60. Tosteson, D. C., *Federation Proc.*, **22,** 19–26 (1963)
61. Hoffman, J. F., *J. Gen. Physiol.*, **45,** 837–59 (1962)
62. Sorenson, A. L., Kirschner, L. B., and Barker, J., *J. Gen. Physiol.*, **45,** 1031–47 (1962)
63. Cook, J. S., *J. Gen. Physiol.*, **48,** 719–34 (1965)
64. Jacobs, H. S., and Jandl, J. H., *J. Clin. Invest.*, **43,** 1704–20 (1964)
65. Welt, L. G., Sachs, J. R., and McManus, T. J., *Trans. Assoc. Am. Physicians*, **77,** 169–81 (1964)
66. Frazier, H. S., and Leaf, A., *J. Gen. Physiol.*, **46,** 491–503 (1963)
67. Essig, A., *Am. J. Physiol.*, **208,** 401–6 (1965)
68. Bricker, N. S., Biber, T., and Ussing, H. H., *J. Clin. Invest.*, **42,** 88–99 (1963)
69. Klahr, S., and Bricker, N. S., *J. Clin. Invest.*, **43,** 922–30 (1964)
70. Essig, A., and Leaf, A., *J. Gen. Physiol.*, **46,** 505–15 (1963)
71. Ussing, H. H., *Harvey Lectures*, **59,** 1–30 (1963–64)
72. Klahr, S., and Bricker, N. S., *Am. J. Physiol.*, **206,** 1333–39 (1964)
73. Klahr, S., and Bricker, N. S., *J. Gen. Physiol.*, **48,** 571–80 (1965)
74. Frazier, H. S., and Hammer, E. I., *Am. J. Physiol.*, **205,** 718–22 (1963)
75. Bowers, B. F., *Nature*, **204,** 786–87 (1964)
76. Hays, R. M., and Leaf, A., *J. Gen. Physiol.*, **45,** 905–19 (1962)
77. Hays, R. M., and Leaf, A., *ibid.*, 921–32
78. Hays, R. M., and Leaf, A., *ibid.*, 933–48
79. Whittembury, G., *J. Gen. Physiol.*, **46,** 117–30 (1962)
80. Diamond, J. M., *J. Gen. Physiol.*, **48,** 1–14 (1964)
81. Diamond, J. M., *ibid.*, 15–42
82. Whitlock, R. T., and Wheeler, H. O., *J. Clin. Invest.*, **13,** 2249–65 (1964)
83. Horowicz, R., and Gerber, C. J., *J. Gen. Physiol.*, **48,** 489–514 (1964)
84. Horowicz, R., and Gerber, C. J., *ibid.*, 515–25
85. Mullins, L. J., and Frumento, A. S., *J. Gen. Physiol.*, **46,** 629–54 (1963)
86. Burg, M. B., Grollman, E. F., and Orloff, J., *Am. J. Physiol.*, **206,** 483–91 (1964)
87. Burg, M. B., and Orloff, J., *Am. J. Physiol.*, **207,** 983–88 (1964)
88. Whittembury, G., *J. Gen. Physiol.*, **48,** 699–717 (1965)
89. Wesson, L. G., Jr., *Medicine*, **43,** 547–92 (1964)
90. De Wardener, H. E., Mills, I. H., Clapham, W. F., and Hayter, C. J., *Clin. Sci.*, **21,** 249–58 (1961)
91. Levinsky, N. G., and Lalone, R. C., *J. Clin. Invest.*, **42,** 1261–76 (1963)
92. Rector, F. C., Jr., van Giesen, G., Kiil, F., and Seldin, D. W., *J. Clin. Invest.*, **43,** 341–48 (1964)
93. Goldsmith, C., Rector, F. C., Jr., and Seldin, D. W., *J. Clin. Invest.*, **41,** 850–59 (1962)

94. Blythe, W. B., and Welt, L. G., *J. Clin. Invest.*, **42,** 1491–96 (1963)
95. Kamm, D. E., and Levinsky, N. G., *J. Clin. Invest.*, **44,** 1144–50 (1965)
96. Blythe, W. B., and Welt, L. G., *Trans. Assoc. Am. Physicians,* **78** (1965)
97. MacIntyre, I., *J. Chronic Diseases,* **16,** 201–15 (1963)
98. MacIntyre, I., Boss, S., and Troughton, V. A., *Nature,* **198,** 1058–60 (1963)
99. Kukolj, S., Gitelman, H., and Welt, L. G., *Clin. Res.*, **13,** 80 (1965)
100. Gitelman, H., Kukolj, S., and Welt, L. G., *Clin. Res.*, **13,** 323 (1965)
101. Alcock, N., and MacIntyre, I., *Clin. Sci.*, **22,** 185–93 (1962)
102. Welt, L. G., *Yale J. Biol. Med.*, **36,** 325–49 (1964)
103. Whang, R., and Welt, L. G., *J. Clin. Invest.*, **42,** 305–13 (1963)
104. Shils, M. E., *Am. J. Clin. Nutr.*, **15,** 133–43 (1964)
105. Whang, R., Oliver, J., McDowell, M., and Welt, L. G., *Clin. Res.*, **10,** 257 (1962)
106. Ko, K. W., Fellers, F. X., and Craig, J. M., *Lab. Invest.*, **11,** 294–305 (1962)
107. Heaton, F. W., and Anderson, C. K., *Clin. Sci.*, **28,** 99–106 (1965)
108. Sabour, M. S., Hanna, S., and MacDonald, M. K., *Quart. J. Exptl. Physiol.*, **49,** 314–23 (1964)
109. Manitius, A., and Epstein, F. H., *J. Clin. Invest.*, **42,** 208–15 (1963)
110. Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G., and Henze, K. G., *Endocrinology,* **70,** 638–49 (1962)
111. Kumar, M. A., Foster, G. V., and MacIntyre, I., *Lancet,* **II,** 480–82 (1963)
112. Copp, D. H., and Henze, K. G., *Endocrinology,* **75,** 49–55 (1964)
113. Hirsch, P. F., Gauthier, G. F., and Munson, P. L., *Endocrinology,* **73,** 244–52 (1963)
114. Foster, G. V., Baghdiantz, A., Kumar, M. A., Slack, E., Soliman, H. A., and MacIntyre, I., *Nature,* **202,** 1303–5 (1964)
115. Gittes, R. F., and Irvin, G. L., *Science,* **148,** 1737–39 (1965)
116. Copp, D. H., *Recent Progr. Hormone Res.*, **20,** 59–88 (1964)
117. Hirsch, P. F., Voelkel, E. F., and Munson, P. L., *Science,* **146,** 412–13 (1964)
118. Baghdiantz, A., Foster, G. V., Edwards, A., Kumar, M. A., Slack, E., Soliman, H. A., and MacIntyre, I., *Nature,* **203,** 1027–28 (1964)
119. Arnaud, C. D., Tenenhouse, A., and Rasmussen, H., *Federation Proc.*, **24,** 322 (1965)
120. Foster, G. V., MacIntyre, I., and Pearse, A. G. E., *Nature,* **203,** 1029–30 (1964)
121. Natelson, S., Pincus, J. B., and Rannazzisi, G., *Clin. Chem.*, **9,** 631–36 (1963)
122. Lehninger, A. L., *Physiol. Rev.*, **42,** 467–517 (1962)
123. Huxley, A. F., *Ann. Rev. Physiol.*, **26,** 131–52 (1964)
124. Kielley, W. W., *Ann. Rev. Biochem.*, **33,** 403–30 (1964)
125. DeLuca, H. F., Engstrom, G. W., and Rasmussen, H., *Proc. Natl. Acad. Sci.*, **48,** 1604–9 (1962)
126. Sallis, J. D., DeLuca, H. F., and Rasmussen, H., *J. Biol. Chem.*, **238,** 4098–4102 (1963)
127. Rasmussen, H., DeLuca, H. F., Arnaud, C. D., Hawker, C., and von Steding, R. M., *J. Clin. Invest.*, **42,** 1940–46 (1963)
128. Toverud, S. U., *Acta Physiol. Scand.*, **62,** 391–406 (1964)
129. Nichols, G., Jr., Schartum, S., and Vaes, G. M., *Acta Physiol. Scand.*, **57,** 51–60 (1963)
130. Sallis, J. D., and DeLuca, H. F., *J. Biol. Chem.*, **239,** 4304–7 (1964)
131. Norman, A. W., and DeLuca, H. F., *Arch. Biochem. Biophys.*, **107,** 69–77 (1964)
132. Wasserman, R. H., and Kallfelz, F. A., *Am. J. Physiol.*, **203,** 221–24 (1962)
133. Harrison, H. E., and Harrison, H. C., *Am. J. Physiol.*, **208,** 370–74 (1965)
134. Rasmussen, H., Waldorf, A., Dziewiatkowski, D. D., and DeLuca, H. F., *Biochim. Biophys. Acta,* **75,** 250–56 (1963)
135. Norman, A. W., Lund, J., and DeLuca, H. F., *Arch. Biochem. Biophys.*, **108,** 12–21 (1964)
136. Schachter, D., Kowarski, S., and Finkelstein, J. D., *Science,* **143,** 143–44 (1964)
137. Thompson, V. W., and DeLuca, H. F., *J. Biol. Chem.*, **239,** 984–89 (1965)
138. Borle, A. B., and Neuman, W. F., *J. Cell Biol.*, **24,** 316–23 (1965)

CONTENTS