THE DISTRIBUTION AND MOVEMENT OF WATER AND SOLUTES IN THE HUMAN BODY*

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In the course of analysis of data dealing with water and salt balances and serum electrolyte equilibria, it became apparent that certain assumptions and deductions concerning the distribution of electrolytes within the body and the forces which determined this distribution, as well as the exchanges of salt within the body and between the body and its environment, required examination. It seemed necessary, first, not only to inquire into the reasonableness of the assumptions, but also to see how far they were supported by the available factual knowledge. In the second place, it seemed advisable to learn how far these assumptions, emanating from various sources, could be welded into a reasonable hypothesis. Third, there was good reason to hope that collection and arrangement of the assumptions and deductions would reveal implications of importance which had hitherto escaped attention and perhaps render more intelligible phenomena which seemed incomprehensible. Finally, by identifying the gaps in present knowledge in proper relation to the more or less completed chapters, future efforts might be directed more intelligently. The present paper embodies the main features of such an examination conducted by the author for his own edification and submitted for publication because it has intrigued certain others who have seen it. It is obviously not a review, because it begins with assumptions and the material with which it deals is largely speculative. Although the attempt has been made not to allow preconceptions to eliminate critical treatment entirely, objections to the general theoretical structure have been recognized only when they seemed of compelling importance. The bibliography is incomplete and the works cited are not selected so much for their importance as for their relevance to certain points, usually of a controversial nature.

The alimentary canal

There is ever increasing evidence that the gastrointestinal secretions, at least in the stomach and upper portion of the small intes-

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tines, although varying greatly in composition, are isotonic with blood and body fluids. This has been demonstrated most unequivocally for the gastric, duodenal and pancreatic secretions. It follows, almost as a corollary, that materials introduced into the alimentary canal are brought into osmotic equilibrium with the body fluids either before or during the process of absorption, by passage into the gut of water or salts. This offers an explanation, perhaps, of the fact that the rate of absorption of a material such as glucose seems to be independent of the amount or concentration in which the material is given. The character of the salt mixture which passes into the fluids seems to partake of the nature of the normal secretions and, therefore, like these secretions, varies in different portions of the alimentary canal. Apparently this secretion proceeds *pari passu* with the absorption from the alimentary canal medium of the materials which are not natural components of this medium. The result suggests a process of diffusion through membranes of highly selective permeability, at least as far as soluble nutrient materials, water and inorganic ions, are concerned. This does not signify that the intermediate reactions by which this result is achieved may not be as complex as the highly differentiated nature of the lining wall of the gut would lead one to expect.

Bidder and Schmidt[4], in their classic work on the nature of the gastrointestinal secretions, first pointed out the enormous quantities of water and solutes which pass into the alimentary canal daily, only to be reabsorbed. Fecal matter was formerly believed to consist largely of food substances which had escaped absorption in their passage through the gastrointestinal tract. It is becoming ever more doubtful whether in the normal individual this is true of anything except indigestible or insoluble materials, such as cellulose. The constancy of fecal fat and protein when the dietary content of fat and protein is varied from extremely high values to zero is quite incompatible with the conception that the excretory material originates in the food. That the partition of calcium and phosphorus between stools and urine depends on certain characteristics of the host and not on the route of administration of these elements likewise becomes comprehensible only if fecal calcium and phosphorus are regarded as products of excretion.

The relative insolubility of these elements, especially calcium, and their high concentration in feces, seems to present a serious objection to an absorption-reexcretion theory, especially if these

processes are related to diffusion. The objection is, however, more apparent than real. If the gut wall is permeable to calcium, the latter will diffuse into the lumen until equilibrium is reached. If the nature of the gut contents causes the calcium to be precipitated, the process of diffusion can continue until a large amount of calcium is excreted, because only that fraction of the element which remains in solution is involved in the diffusion equilibrium. Precipitation will continuously remove calcium from solution, affording an opportunity for further diffusion until precipitation has ceased and the calcium still in solution has come into equilibrium with that of the serum and body fluids.

These exchanges between the alimentary canal and the body fluids can not fail to have a considerable influence upon the composition of the latter. It complicates the interpretation of the phenomena which follow peroral administration of fluids. For example, after the ingestion of water, reduction of the salt concentration of the serum occurs earlier and exceeds in magnitude actual dilution of the serum, because the salt reduction is due quite as much to passage of salt into the alimentary contents as it is to absorption of water from the alimentary contents. To differentiate the two processes is, as yet, beyond our analytical powers. In addition to the disturbances of body media which occur as the result of exchanges with fluids introduced into the alimentary canal, the secretory activity of the digestive glands may become great enough, at times, to alter the composition of the internal environment. This is well illustrated by the experiments of Gamble and McIver[11], Miller[32] and others on pyloric obstruction in rabbits. These animals are unable to vomit. They will, nevertheless, excrete into the stomach sufficient amounts of water and salt to cause fatal dehydration and salt depletion. The difficulties involved in the attempt to evaluate changes in the concentrations of solutes in the serum without any knowledge of the quantity or nature of the gastrointestinal contents are only enhanced by the ingestion of fluids and food.

It seems paradoxical that, although sodium is the predominant base in all gastrointestinal secretions, normal feces contain less of this element than of potassium. This would appear to indicate some irreversible permeability of the intestinal wall to potassium. It is, however, possible that the potassium is chiefly within the bacteria and other cells which make up so large a proportion of stool solids. In this case it is removed from the field of free diffusion just as

effectively as if it had been precipitated in solid form in the gut. In diarrhea, when the fecal fluid excretion is great, sodium losses increase. It would be interesting to learn whether in diarrhea fecal potassium parallels stool solids or proteins, while excretion of sodium is proportional to that of water. Except for Schmidt's[41] investigations of cholera and dysentery the total composition of diarrheal stools seems to have received scanty attention. Whether the isotonic nature of secretions, which is so characteristic of the upper portion of the gastrointestinal tract, is maintained in the lower small intestine and colon, has not, apparently, been determined. The high solid content of feces makes such determinations difficult.

Because of the variation of electrolyte patterns in the secretions at different levels of the alimentary tract, the effects of physiological activity, disordered function or loss of secretions upon the serum will depend upon the point at which these disturbances are maximum. During the active secretion of gastric juice after a fast, for example, in normal individuals, a distinct reduction of serum chloride associated with a comparable elevation of bicarbonate can be detected, which can be ascribed to the loss of free hydrochloric acid into the stomach. It is impossible to demonstrate a similar reaction after subsequent meals of the day, probably because the effects of gastric activity are obscured by activity of the intestinal tract, in which chloride is absorbed and replaced by bicarbonate. Gamble and McIver[11] and others have shown that losses of fluid from any point in the alimentary canal have, in common, the effect of withdrawing water and salts from the body; but the results on serum electrolyte patterns will vary, depending upon the nature of the salt mixture in the fluids sacrificed. Vomiting sacrifices chiefly chloride and sodium, in proportions which vary according to the concentration of free hydrochloric acid in the vomitus. Diarrhea, on the other hand, tends to deplete bicarbonate more than it does chloride because the feces contain more base than acid.

Equilibria with parenteral fluids

Fluid introduced into the subcutaneous tissues or into one of the serous cavities, before or during absorption, is also transformed by processes of diffusion[40]. In this case, however, not only the total concentration of salt, but the whole pattern of the fluid become identical with those of a serum ultrafiltrate. The effect on the serum

is comparable to that which would be produced by diluting the serum with a solution of the electrolyte composition of the injected fluid. However, this effect may be evident before any of the fluid is absorbed, because the simultaneous diffusion of materials from the serum to the fluid represents, to all intents and purposes, dilution of the fluid with serum. Again it is evident that changes in the serum composition can not be ascribed entirely to absorption of fluid.

After intravenous administration of fluid it is generally believed that equilibrium is almost instantaneously attained. This impression is based largely on the fact that the original volume of blood in circulation is so rapidly restored. However, adjustment of blood volume is brought about chiefly by interplay of colloid osmotic pressure and capillary blood pressure, which are almost independent of the diffusion forces which effect even distribution of electrolytes. Although intravenous injection, because it establishes more rapid and universal contact between the foreign and native fluids, probably leads to earlier establishment of equilibrium; it is conceivable, and indeed likely, that local circulatory variations may lead to temporary unequal distributions of fluid. If there are such temporary repositories the fluid in them must behave much like the subcutaneous fluids discussed above.

The distribution of body fluids

In general, the fluids within the body may be divided into several large categories more or less separated from one another into compartments: serum, interstitial fluids, serous fluids, lymph and cell contents. The only characteristic which all these fluids share is isotonicity,—equality of osmotic pressure. The first four are further sharply distinguished from the last by a general similarity of electrolyte pattern. In fact the distinctions between them seem to be entirely referable to variations in content of undiffusible colloids, chiefly proteins. The evidence is strong that interstitial fluids, serous fluids and lymph are simple filtrates of serum.

The propriety of discussing the nature of *interstitial and serous fluids* when their very existence is a matter of inference, may well be questioned. The discontinuous appearance of tissues under the microscope and the separation of actively functioning cells from blood capillaries suggests that there are spaces about these cells

and between them and the capillaries which are filled with some fluid medium which is poor in protein. Some such fluid would seem almost essential for the conveyance of solutes to and from the blood stream. Furthermore the accumulation of lymph, a fluid so similar to blood serum and so different from intracellular fluid, would be incomprehensible were there not, in the tissue spaces between the capillaries and the lymph spaces, some fluid similar to both serum and lymph. There must be some fluid reservoir larger than the blood serum mass from which the fluids and salts can be drawn to provide gastrointestinal secretions without disorganizing the constitution of actively functioning cells. In keeping with the concept of such an extracellular reservoir is the observation that the chief base in these secretions is, like that of serum and lymph, sodium.

Few question the fact that in pathological conditions with edema, the fluid accumulates in extracellular or interstitial spaces rather than in the cells. Such transudates have been proved by *in vitro* experiments to be identical with serum dialyzates. It is a reasonable inference that accumulation of edema represents nothing more than an augmentation of the normal tissue fluids. Lesser accumulations can be produced and demonstrated when the normal balance between the colloid osmotic and hydrostatic pressures in the capillary blood stream of a part are disturbed. When such accumulations are produced rapidly reduction of the serum volume occurs without any change of composition other than an increase of the concentration of serum colloids, a clear indication that a colloid-free filtrate of serum has been lost from the circulation. It is unbelievable that the cells themselves are continually being expanded or contracted by the accession or delivery of such an undifferentiated fluid, thereby suffering continual changes of highly distinctive chemical patterns which presumably have peculiar functional significance. Finally, changes which have been described as occurring in fluids which are introduced subcutaneously or into serous cavities can hardly be explained unless there is a reservoir of fluid resembling serum filtrate which is contiguous with both serum and foreign fluid. It may seem almost superfluous to insist unduly upon the existence of something which is so generally accepted. But, in the absence of direct evidence, it is worth while to examine the soundness of the inferences upon which the existence of interstitial fluids is predicted, more especially since concepts concerning the nature of these fluids are

based upon the same inferences. What has been said of the interstitial fluids holds equally well for the fluids in serous cavities.

Lymph appears also to partake of the nature of serum and to reflect rapidly in its own composition changes in the composition of serum[1]. Field and Drinker[9], in fact, adhere to the theory that lymph and interstitial fluids are identical. There are reasons, however, for believing that lymph has a higher colloidal content than the interstitial fluid from which it is presumably chiefly derived. It seems to have been rather clearly proved that the lymph channels are not continuous with the interstitial spaces[30, 37], but are closed at their distal ends, forming a completely self-contained system lined by endothelial cells. When injected from within they appear to be relatively impervious to particulate matter of larger size. On the other hand, particulate matter injected into the interstitial tissues or serous cavities rapidly finds its way into the lymphatics[7, 30], although the same material appears to be unable to penetrate the blood capillary wall to enter the circulation[27]. By what means these large particles find access to the lymphatics is still obscure. Some seem to be conveyed to the lymph vessels by extraneous phagocytic cells, others may be taken up by phagocytic activity of the lymphatic endothelial cells themselves. It may be relevant to point out that lipoids, to which capillary walls seem to be relatively impervious are differentiated from the other products of digestion in that they pass from the gut into the lymph and by this means are conveyed to the blood stream, whereas the other readily soluble products are absorbed directly into the portal capillaries. Iversen[22] has shown that during reabsorption, the proteins in transudates become more concentrated, suggesting that fluid and proteins are removed by different channels. According to Drinker the protein of lymph comes from that of the interstitial fluids with which it is identical. In this case it is hard to envisage the physical and chemical forces which control transudates, because the difference in colloidal osmotic pressure between interstitial fluids and capillary serum must be smaller than the Starling theory demands. If the protein concentration in normal interstitial fluid is as high as Drinker suggests, the transudates found in nephrosis, malnutrition edema and plasmapheresis edema should not contain such minute amounts of protein. Landis[26] has, by indirect methods, estimated the protein lost from the blood serum when transudation is accelerated in the extremities by increase of hydro-

static pressure. Unless the hydrostatic pressure is greatly augmented there is no demonstrable leakage of protein. Finally, the protein concentration in injected fluids which are allowed, during absorption, to come into diffusion equilibrium with serum and interstitial fluids, does not increase to the extent that one would expect if the latter contained a high concentration of protein[25, 40].

On the whole, it seems more consonant with the available evidence to believe that proteins and other colloids which gain access to the interstitial fluid spaces through the irreducibly minimal leakage from blood capillaries or the unavoidable attrition of cells, are selectively removed into the lymph stream. If they can not return directly through the sound capillary wall into the blood stream some alternative channel for their escape would have to be provided to prevent their continuous accumulation in the interstitial fluid with a consequent steady increase of colloid osmotic pressure. Such an explanation seems more satisfactory than the proposition that lymph represents an admixture of fluids of varying protein content and that there are constantly parts of the circulation in which protein is leaking through the capillary walls because of local capillary stasis or some other disturbance. To explain the high protein content of lymph on this basis would demand that over a fair proportion of the capillary bed a fluid of extremely high protein content was continually escaping. It would follow that, unless this protein were immediately removed, the colloid osmotic force resisting transudation in these high protein regions would be reduced to a negligible point.

It can not be denied, of course, that there are local or temporary variations in the protein content of interstitial fluids, depending upon differences of vascular permeability. Starling[45] himself explains on this basis the fact that hepatic lymph regularly contains more protein than intestinal lymph, while the protein in the latter exceeds that of lymph from the extremities. Even in such areas of high vascular permeability the lymph may contain more protein than the original capillary transudate. Even Drinker does not deny that protein-free fluid is, as Starling[46] and others[42] have demonstrated, absorbed directly into the blood stream from the interstitial spaces through the capillary walls.

It is hard to see how protein which is freely diffusible would find its way out of the interstitial fluid or lymph according to Drinker's theory, instead of coming into equilibrium with the protein in the

blood stream. The selective absorption of proteins and other colloids into the lymph vessels would, among other things, afford a reasonable explanation for the passage of fluid into and along these vessels from the interstitial spaces, because it would produce in the lymphatic system an osmotic pressure above that of the interstitial fluids from which lymph is derived. To the author the present explanations for the collection and flow of lymph have seemed hopelessly unsatisfying. The suggested additions to these explanations lack the support of direct experimental evidence, but are not at variance with the body of ascertained facts. It is, of course, possible that the proteins in lymph may be elaborated by the cells of the lymphatics; but one hesitates to attribute to endothelial cells such a highly specialized metabolic function.

The general character of body fluids

The general isotonicity of all body media despite their extreme diversity of composition demands particular consideration. The foundation of the Starling theory is the well known fact that if two fluids containing different concentrations of protein are separated by a membrane which is permeable to water and salts, but not to protein, the osmotic pressure will be greater in the medium containing the higher concentration of protein. Yet the evidence is strong that in adjacent media in the body osmotic pressures may be identical in spite of a 25 to 30 per cent difference in protein concentration. Wu[49] has pointed out that this osmotic effect can be balanced if the membrane separating the two media is impermeable not only to the anion, protein, but also to cations. This offers a valid reason for the generally recognized impermeability of cell membranes to base, over and above the fact that only by such highly selective permeability is the free exchange of necessary water and solutes consistent with a high degree of cellular differentiation.

The nature of this differentiation requires examination on its own merits and because of the light it may throw upon the origin of media and the exchanges which take place between them. It has been stated above that interstitial fluids and lymph can be looked upon as simple ultrafiltrates of serum. If transudates are merely expansions of interstitial fluid this proposition has been unequivocally proved by *in vitro* dialysis experiments. Nevertheless, such a conclusion could hardly have been reached by chemical analyses of

serum and transudates because they differ quite appreciably in chemical composition. The total electrolyte concentration of transudates as well as the concentration of the two chief inorganic components, sodium and chloride, differ from those of serum. These differences are referable chiefly to differences in water content. The concentration of water in serum is less than that in transudates because the former contains greater amounts of protein and other colloids of large molecular size. It must be emphasized that the general equality of electrolyte concentration throughout the body, which was chosen as an approximate expression of osmotic pressure equality, refers to concentrations measured in relation to unit weight of water only. Corrected for water content the total electrolyte concentrations of serum and transudates do not differ significantly (the slight observed differences can be explained by the Gibbs-Donnan effect). There is, however, distinctly less calcium and potassium in transudates than in serum. The uneven distribution of calcium can certainly, and that of potassium can possibly, be ascribed to the greater affinity of proteins for these two bases than for sodium. Protein, at reactions of body fluids, combines with a certain amount of base to form salts which seem to be comparatively non-ionized. Presumably calcium, and possibly to a lesser extent potassium, make up a larger fraction of these protein salts than does sodium, in proportion to the relative concentrations of the three bases in solution. Certainly these ions maintain their unequal distributions when the two solutions are dialyzed across collodion membranes which are permeable to all the inorganic ions present. Non-colloidal organic solutes are distributed between the two media in proportion to water. Proteins and lipoids, compounds of larger molecular size, are found in lower concentration in transudates than in serum.

Lymph, it has already been said, is distinguished from transudates only in its higher content of colloids and in the differences which have been mentioned above as corollaries to the unequal distribution of protein.

Especial consideration must be given to the position in this system of spinal fluid. This is normally an almost protein-free fluid having most of the properties which have been described as characteristic of a serum ultrafiltrate, which many believe it to be. Like transudates it contains less potassium and calcium and more chloride than serum. Its urea content is practically identical with that of serum, but it contains far less reducing substances. If it is a filtrate of

serum one would expect its glucose concentration to equal that of serum. Part of the observed difference in concentrations appears to be referable to the fact that serum contains more reducing substances other than glucose; but this does not explain the entire discrepancy. Cerebrospinal fluid is probably elaborated over only a small area within the cranial cavity and, even if it is formed by diffusion, equilibrium with the serum can be secured only in this region where serum and spinal fluid are in contiguity. It has been demonstrated by Stewart[47] that there is layering in the spinal fluid, the concentration of glucose being higher in ventricular than in lumbar fluid. This suggests that a certain amount of the sugar is oxidized in the cerebrospinal canal. In diabetes the spinal fluid glucose is elevated, indicating that it is not unsusceptible to the influence of the serum. When the blood sugar is more rapidly and temporarily altered by the administration of glucose or other means, the spinal fluid sugar rises, but to a lesser extent and much later than that of the blood. This is again what one would expect if the glucose during a short interval diffused into a small portion of the fluid at a distance from the point of collection. There is, then, nothing in the facts concerning the distribution of glucose between serum and spinal fluid which is incompatible with the theory that the latter is a simple ultrafiltrate of serum. Nevertheless, irrefutable quantitative proof of its character has not been secured.

The concentration of calcium in spinal fluid is of the same order of magnitude as the fraction of serum calcium which is not combined with protein and, therefore, presumably diffusible. Moreover, the difference between serum and spinal fluid calcium seems to be related to the serum protein concentration, although there are too few data available, especially from subjects with abnormal serum proteins, to warrant definite conclusions on this point. An interesting contradiction is found between the observations of Arnold and Mendel[1] on lymph and Merritt and Bauer[31] on spinal fluid, after disturbances of parathyroid activity. According to Arnold and Mendel, parathyroidectomy and injections of parathyroid hormone cause parallel changes in the calcium of serum and thoracic duct lymph. At the same time the difference between serum and lymph calcium, which is proportional to the difference in protein concentrations of the two media and, presumably, represents non-diffusible calcium bound to protein, remains relatively constant. From this one would surmise, as is generally believed, that the parathyroid hormone influ-

ences only the active, diffusible fraction of serum calcium. On the other hand, Merritt and Bauer found that parathormone did not alter the spinal fluid calcium. The logical inference from this would be that spinal fluid is not a simple dialyzate of serum. The only alternative would be that lymph is not such a dialyzate, a conclusion against which there is an overwhelming body of evidence.

It can be inferred from studies of Arnold and Mendel[1], Salvesen and Linder[39] and others that the inorganic phosphate of serum is completely diffusible and distributes itself freely and equally throughout the water of serum, transudates and lymph. Brull[5] and others claim that a variable fraction of serum inorganic phosphate exists in the form of relatively non-diffusible complexes. Brull[5] found in viviultrafiltration experiments that serum phosphate was not completely filtrable. To offset this Green and Power[16], by vividiffusion, and Greenberg and Greenberg[14] by electrodialysis were unable to detect any evidence of non-diffusible or complex phosphate compounds. The data of Salvesen and Linder[39], Greene, Bollman, Keith and Wakefield[15], and analyses made in this laboratory show excellent agreement between phosphate concentrations in serum and transudates. In Arnold and Mendel's[1] comparisons of blood serum with thoracic duct lymph of the dog, changes of phosphate in the serum, regardless of magnitude or mode of production, were rapidly reflected by corresponding changes in lymph. On the other hand, all observers, including Merritt and Bauer[31], agree that the inorganic phosphorus concentration in spinal fluid is far lower than that of serum. If, then, spinal fluid is an ultrafiltrate of serum, the membrane separating it from the blood would seem to be more highly differentiated than capillary walls and endothelial membranes in the rest of the body.

The subject of the character of spinal fluid merits further investigation. Meanwhile, it is necessary to reserve judgment concerning its exact nature.

The exchange between blood and interstitial fluids

If interstitial fluids are merely ultrafiltrates of serum, it follows that they must be the product of filtration, by simple physical or physicochemical forces, through membranes which are impermeable only to the protein and lipoid molecules. This compelling argu-

ment for the Starling theory, which had to wait upon exact demonstration of the character of transudates, has been too little considered by both proponents and opponents of this theory. There can be no doubt that with its higher protein content serum must exert an osmotic pressure tending to draw water and salts from any fluid of lower protein content which is separated from it by a dialyzing membrane which is impermeable to protein alone. If interstitial fluid and lymph differ from serum only in their protein concentrations, it is reasonable to conclude that they are derived from serum by filtration through such a membrane. Physical pressure greater than the osmotic pressure of the serum proteins is required to produce such filtration.

Rous[36] has presented certain experiments with vital stains which, he believes, prove a serious objection to acceptance of the Starling theory. These dyes, with molecular sizes moderately large, but still within the limits which permit free diffusion through the capillary wall, instead of escaping from the blood stream chiefly at the arterial ends of certain capillaries, pass out in greater quantities at the venous ends, although the blood pressure must exceed the colloid osmotic pressure by a greater amount at the arterial end. This, Rous considers as evidence that a gradient of capillary permeability rather than any gradient of hydrostatic pressure must determine the fluid exchange between blood and tissue spaces. He seems to have overlooked the fact that Starling was dealing with the forces that determined the passage of protein-free filtrate as a whole and which held back protein selectively, not with the processes which determined the diffusion of single solutes to which the vessel wall was permeable. In later experiments Smith and MacDonald[44] found that the passage of water through the capillary walls was greatly altered by varying the protein concentration of the blood serum, but these variations did not alter the distribution of his dyes. This would seem to establish clearly the soundness of Starling's theory and the irrelevancy of Rous' experiments to the problem of water exchange. Exchange of solutes by diffusion need not follow the current of filtration. If it did, the establishment of electrolyte and osmotic equilibrium among body fluids would be a slower process than it is. Such gradients as Rous has demonstrated may equalize the distribution of nutrient materials of large molecular size to the tissues as he has suggested, without appreciably influencing the exchange of water or electrolytes.

The nature of cell differentiation, membrane permeability and the exchanges between cells and extracellular fluids

Exact knowledge of the composition of human tissue cells can not be secured because it is impossible to obtain such cells for analysis without admixture of interstitial fluid. Available tissue analyses do support the theory of uniform osmotic pressure. The only cells which can be isolated and directly analyzed are the red blood corpuscles. These have been subjected not only to chemical analysis, but also to *in vitro* experimental procedures aimed to test their reactions to variations of osmotic pressure and their permeability to various solutes. The red blood cells contain about 33 per cent of protein, almost all of which consists of hemoglobin. The concentration of water is proportionately reduced. Freely diffusible organic compounds such as glucose and urea are distributed between cells and serum in proportions approximately equivalent to the water content of the two phases. Alterations of the concentration of these constituents in the serum are reflected by rapid changes of their concentration within the cells, indicating that the cell membrane is freely permeable to these substances. The pattern of bases in the cells differs strikingly from that of serum. In the human red blood cell the chief base is potassium; calcium is not present in detectable quantities and sodium has been demonstrated in such low concentration as to make it doubtful whether this element is a normal constituent of the cell. In some mammals, for example, the dog, the segregation of sodium and potassium is by no means so complete as it appears to be in humans. If the osmotic equilibrium between cells and serum is disturbed by alteration of the pH of the serum or by the addition of salts, adjustment is effected, not by passage of base across the membrane, but by a transfer of water. The segregation of bases appears to be obligatory because the cell membrane is impervious to the alkaline metals. This impermeability has been demonstrated *in vitro* under the most varied conditions[8, 17, 48]. In the circulating blood the volume of the red cells changes detectably under the influence of similar disturbances if these are rapidly produced. For example, the cell volume of arterial blood differs appreciably from that of venous blood. This similarity of behavior *in vitro* and *intra vitam*, coupled with the fact that the absence of sodium from the cells is maintained regardless of the condition of the subject from whom the blood is drawn, seems to justify the

conclusion that the cell membrane is always impermeable to bases. This conclusion can not be put to the acid test of experiment, because it is not possible to subject the cell to such extremely abnormal conditions in the body as it is in the test-tube. Attempts to correlate the concentration of water in the cells statistically with the pH or the base concentration of the serum have failed. Theoretically these two factors are the chief determinants of the osmotic pressure of the cells. Variation of either causes cell volume to change *in vitro*. If cell volume is statistically related to neither pH nor base concentration, some other undetermined variables must, in the body, compensate for changes in these functions. In the absence of any disease of the hematopoietic system the concentration of hemoglobin per unit volume of cell, a measure of water concentration, may remain more constant than does the base concentration of the serum. At the same time, per unit of water the relation of the base concentration in cells to that in serum is maintained under the most diverse conditions[8]. If the cell could be adjusted to alterations of serum osmotic pressure only by exchanging water with its environment, constancy in the concentration of cell protein and in the distribution of base between cells and serum could not be simultaneously maintained against variations of osmotic pressure. The extreme abnormalities of serum base encountered in certain diseases would provoke equally large alterations of cell volume, involving complete disorganization of the cellular constitution with inevitable dislocation of function. Evidently the volume of intracellular fluids is an object of more solicitude than is that of the interstitial fluids. To prevent large fluctuations in volume, the cells would seem to be provided with the ability, in case of need, to eliminate or to take up base. Judging from the changes in cell volume that occur in the blood stream during the course of the circulation or after rapid changes in the reaction of the blood, this faculty is not exercised to resist small variations or sudden variations of cell volume; or perhaps it is not called into play to prevent variations due to alteration of pH, but only for those due to alteration of base concentration. The last seems the least likely hypothesis because it would draw a qualitative distinction between *in vitro* and *intra vitam* reactions. Any one of these hypotheses, however, demands recognition of a distinct difference between the reactions of cells to osmotic changes within and outside of the body.

It is, of course, imperative to recognize that in the living, func-

tioning organism the impermeability of the cell membrane to base must be only facultative; else how could base enter the cells initially. One could imagine that in the processes of growth, inanition and regeneration potassium entered or left the cells always in proportion to protein. Indeed, somewhat of this attitude has been taken by Gamble[12] and others in studies of salt balance. The data of Gamble, Ross and Tisdall[12] in their study of starvation, like those of Loeb, Atchley, Richards and Benedict[29], gathered during the development of diabetic acidosis, indicate that when protein is wasted more than the expected amount of potassium is excreted, and during the process of protein regeneration more than the expected potassium is retained. An ingenious explanation for this discrepancy was offered by Gamble, who suggested that the extra potassium went with the water held in the cells by glycogen. In Gamble's studies of starvation and Loeb's experiments with diabetic acidosis this explanation is at least plausible, although the quantities of potassium excreted seem far in excess of any possible glycogen losses. Gamble[13] and others have, however, detected similar losses of potassium in excess of protein under conditions which lead to dehydration and salt depletion without carbohydrate starvation. Under these circumstances there is no reason to believe that glycogen was sacrificed. This may be evidence that when interstitial fluids are depleted of their sodium the cells yield a certain proportion of potassium in behalf of the maintenance of osmotic equilibrium.

The rather sweeping generalization that the effects of changes in body water and salts are confined entirely to the interstitial fluids unless there is simultaneous alteration of the fixed constituents of cells, such as proteins, requires a certain amount of qualification. The experiments of Salkowski[38], Gamble, Ross and Tisdall[12], Benedict[3], etc. have given rise to the opinion that when cell protein is wasted a proportional amount of salt and water is sacrificed; form and size of cells are entirely neglected in behalf of chemical composition. The variability of the proportions of protein to water and electrolytes in reported tissue analyses would seem to refute this theory. Such arguments are always open to criticism because it is impossible to separate tissue cells from interstitial fluids and therefore to be assured that the reported variations are not due merely to differences in the quantities of intercellular diluent. In investigations of the proportions of protein to water in red blood cells, which will be published later, Eisenman and Wakeman have found no

constant relation. Even in normal subjects the protein concentration of cells varies by as much as ± 8 per cent (29.9 to 35.1 gm. per cent); in patients the range was increased greatly, extending from 23.1 to 36.2 gm. per cent, or 43 per cent of the average normal hemoglobin concentration. Nevertheless, the concentrations of base in these cells may be normal. That there may be no misunderstanding concerning the significance of these findings it may be added that the water content and the protein of these cells are inversely proportional, the protein making up the greater part of the solids. If these cells may be considered as analogous to tissue cells, and anemia as merely a specialized form of wasting, it follows that form and size are not always sacrificed for composition and that water, salts and protein do not move as units.

It may be emphasized here that, whether there is such a thing as "bound" water or not, the application of this term to the total diluting fluid of cells is entirely unwarranted. The conception that each gram of protein retained in or discharged from the body carries with it an inseparable 3 gm. of water and a moiety of salts is questionable on physiological grounds; the idea that any such association represents a chemical attachment has nothing to support it. This 3: 1 ratio, so widely used, represents approximately the proportions in which water and protein are supposed to occur in most cellular tissues. Any concept of chemical association in this ratio would, therefore, imply that all the water of cells was water of hydration of proteins. Proof that this is the case has not yet been adduced.

It is necessary to examine more carefully, then, the experiments in which close association between protein and water has been reported. In all those which have been cited wasting has been produced by measures which also caused loss of body water, usually by starvation with its attendant acidosis. Moreover, when the initial fluid and salt losses are compared with nitrogen balances they are found to exceed greatly the expected ratio. The administration of acidifying diuretics, on the other hand, without increasing nitrogen excretion, sweeps out of the body more potassium in proportion to sodium than can reasonably be credited to interstitial fluids[10, 13, 28]. Although the latter stand the chief burden of the diuresis the intracellular fluids do not remain untouched. In some instances the result of the acidosis or diuretic measure is not only to deplete body fluids, but also to reduce the electrolyte concentration in the serum. In this case, for the sake of maintaining osmotic equilibrium the

cells must either give up some base or else withdraw water from their surrounding medium which is already being taxed by the accelerated renal activity. In this case even the humble interstitial fluids may not be treated with complete indifference.

However, potassium losses of a similar kind have been reported during body water losses without tissue destruction even when serum base is not detectably lowered. To ascribe these losses to glycogen wastage can afford only specious satisfaction. In certain patients with edema, diuresis caused by calcium chloride[13] or hydrochloric acid[28] sweeps out potassium which would appear to come from cells. Must one then also admit that edema is not always confined to interstitial tissue? Before any satisfactory conclusions can be reached on this point, more exact knowledge of the concentrations of serum base in the course of such diuresis must be secured.

It appears to be a general principle of biology that no single function is maintained at the expense of all others. Although the volume and composition of the intercellular fluids seem to be favored in comparison with those of the interstitial fluids, the latter, upon which the cells depend for their nutriment and existence, receive some consideration. Under certain circumstances and within certain limits cell water and salts remain intact in the face of protein depletion. At other times water or salts or both pass across the cell membrane without change of protein. It has even been suggested that foreign ions may enter the cells under certain conditions; but the evidence on this point is far less satisfactory. The circumstances which determine such diverse reactions are completely unexplored. Certainly, a more systematic study of the volume changes of the circulating red blood cells in response to rapid and gradual alterations of pH and base concentration is needed and should yield physiological information of great importance, especially if such a study is accompanied by determinations of salt and water balances.

It is generally stated that the red blood cell membrane is freely permeable to acids. Certainly it is readily traversed by bicarbonate and chloride ions. Nevertheless, the distribution of these ions between cells and serum does not conform exactly to the requirements of the Gibbs-Donnan equilibrium. According to this theory the distribution coefficients of bicarbonate and chloride should be identical. In point of fact the ratio of chloride in cells to that in serum is far lower than the corresponding ratio for bicarbonate. Furthermore, the distribution of bicarbonate is more similar to that

of base than is the distribution of chloride. Hastings, Sendroy, McIntosh and Van Slyke[18] have ascribed the differences between the distributions of bicarbonate and chloride to differences in the activities of the two ions. As the actual activities of these ions in serum and red blood cells have not been measured, this conclusion bears with it the assumptions that the Gibbs-Donnan equilibrium equation in its simplest form is applicable to so complex a system as that of blood and that the red cell is freely permeable to the chloride and bicarbonate ions. Both these assumptions, but especially the first, may be questioned. Hastings and Van Dyke[19] have demonstrated that the bromide ion, when added to blood, penetrates the red cell. Within certain limits of concentration, the partition between red cells and serum, when equilibrium is finally attained, is the opposite of the chloride partition. The concentration of bromide in the cells exceeds that in the serum. Various explanations can be afforded for these departures from the Gibbs-Donnan law. It has been suggested, with some questionable evidence, that part of the bicarbonate in the cells is combined with hemoglobin. There is also some evidence that a fraction of the chloride in blood may be in combination with lipoids. Such organic combinations may be incompletely ionized or entirely unionized in which case they would not take part in the Gibbs-Donnan equilibrium.

It is, however, possible that the permeability of the cell membrane to chloride is conditioned, or that unknown biological forces determine the peculiar distribution of chloride, as they do that of sodium and potassium. It may be well, for the moment, to neglect questions of permeability and distribution and to turn attention to the electrolyte pattern of the red cell. In this cell in humans the base is almost entirely composed of potassium and the concentration of base per unit of water is the same as that in serum. To balance this, at a reaction so nearly neutral as that of blood, there must be an equivalent concentration of acids. Among these acids protein covers a far larger fraction of base in cells than it does in serum. To this acid the cell membranes are entirely impermeable. There is in cells also a far larger concentration of organic esters of phosphoric acid, all of which are supposed to have acid properties and to combine with base. These also seem to be unable to pass through the cell wall in ester form, although their hydrolytic product, orthophosphoric acid, can escape freely. At least this would appear to be a reasonable inference from observed facts. Inorganic phosphate is partitioned equally

between cells and serum, while the organic phosphate concentration in cells greatly exceeds that of serum. When blood is allowed to stand reciprocal changes occur in the organic phosphate of cells and the inorganic phosphate of serum. There are, then, two fractions of acid, to which the cell membranes are impermeable, which are found in higher concentration in cells. The magnitude of these fractions can be roughly estimated.

The total concentration of base in cells is about 111 m.eq. per liter. The water content is about 72.3 per cent. Therefore the concentration of base per liter of water is $\frac{111}{0.723} = 153.4$ m.eq. The concentration of chloride is about 54 m.eq., or 74.7 m.eq. per liter of water; that of bicarbonate is about 18.3 m.eq., or 25.3 m.eq. per liter of water. The concentration of the remaining acids is, therefore, about $153.4 - (74.7 + 25.3) = 53.4$ m.eq. per liter of water. Inorganic phosphate and sulfate make up only a small fraction of this remainder, probably not more than 2 or 3 m.eq., the major portion is composed of hemoglobin and organic phosphate. The concentration of hemoglobin in terms of oxygen-combining power is about 19.6 m.eq. per liter of water. If, as Hastings, Van Slyke et al.[20] have estimated, each oxygen equivalent of hemoglobin combines with 1.7 equivalents of base at average blood pH, hemoglobin accounts for 33.3 m.eq. per liter of water. This will leave about 18 m.eq. to combine with organic phosphate. From data of Kay and Byrom[24] the average red blood cell contains about 23 millimols of organic ester P per liter of water. If these esters are as strong acids as creatine phosphate and other esters which have been studied, each millimol of P should combine with more than one equivalent of base. The base actually available for combination with phosphate, according to the calculations above, falls somewhat short of this, but is of the proper order of magnitude. Considering the various sources of the data used the agreement is not unsatisfactory.*

The cells contain roughly 50 to 55 m.eq. more of protein and phosphates per liter of water than serum does, a difference which must be compensated by a proportionate reduction of Cl and HCO_3. If this reduction were shared by both ions in proportion to their serum concentrations Cl would suffer most. In point of fact Cl is reduced relatively far more than HCO_3. This would seem to be a useful provision, because HCO_3, being only a combination of CO_2

* Except for the phosphorus figures of Kay, the data used for these calculations are from unpublished determinations made in this laboratory by Anna J. Eisenman and Pauline M. Hald.

with base, is continuously produced in cell metabolism. A relatively high concentration of bicarbonate base affords a better buffered medium. Furthermore, it is essential for the continued function of the cell that CO_2 pass across the membrane more freely than other acids, in order that any excess may be eliminated as rapidly as possible. Cl may be looked upon merely as a biologically neutral or indifferent ion which fills up the acid complement,—only so much osmotic packing. This argument is, of course, highly teleological and waives entirely the question of the forces which determine or permit so advantageous a distribution of the load. In this instance the uneven partition can not be ascribed to impermeability of the membrane, because if, by change of pH or other means, the acid-base or osmotic equilibrium is disturbed, the compensatory reactions provoked involve a free exchange of chloride between cells and serum.

At least one of these reactions is of extraordinary interest because it is so closely concerned with the maintenance of acid-base equilibrium and respiration. If CO_2 is added to blood, the latter becomes more acid. The change of reaction is, however, enormously less than that which would be produced if the same amount of CO_2 were added to water, or even serum. The extremely high buffer value of blood is referable chiefly to the high protein content of the cells. The proteins of both blood and serum act as weak acids and yield their base, when the pH falls, to neutralize the added CO_2, forming new bicarbonate. As the protein concentration in cells is far greater than in serum, more bicarbonate is formed in the cells. This disturbs the equilibrium between chloride and bicarbonate distributions. To restore this a certain amount of the new bicarbonate from the cells is exchanged for chloride from the serum. In this process there is an increase of osmotic pressure in the cells which causes them to withdraw water from the serum, increasing in size. One result of this reaction, among others, is that the poorly buffered serum reaps the benefits of the more plentiful buffers in the cells. Presumably similar reactions involving exchange of acids and water occur between tissue cells and interstitial fluids.

One peculiar paradox requires mention. It was stated above that the cell membranes were permeable to free organic solutes of moderate molecular size, such as urea and glucose, and probably creatinine. If the concentration of these constituents in the serum is altered, either in the test-tube or in the blood stream, the concentration in

the cells changes in the same direction and to such an extent that the distribution per unit of water becomes always the same on the two sides of the membrane. If two solutions are separated from one another by a semipermeable membrane, the introduction into either of the media of a solute to which the membrane is permeable will cause no lasting change in the osmotic equilibrium between the solutions, which will depend upon the concentrations of only those solutes which can not traverse the membrane. Glucose and urea, to be sure, exert osmotic pressure in blood; but, when they are evenly distributed between the two phases of blood, this will not affect the osmotic pressure relations between these two phases. Of course, before the equilibrium state of even diffusion is reached, they will increase the osmotic pressure of and draw fluid into the phases in which they are momentarily more concentrated. The effect of glucose or urea solutions of any strength on red cells should, then, be the same as that of distilled water. They should cause the cells to swell and rupture. Hemolysis in hypotonic solutions is due to the passage of water into the cells under the influence of the osmotic pressure produced in the cells by the imprisoned base and protein. It is hard to see how there can be isotonic solutions of such materials as glucose or urea, if the term isotonic is used in its restricted sense to indicate a solution in which the red blood cells preserve their normal size and form. If, as is generally taught, such solutions of glucose can be prepared, it would be interesting to know how adjustment of osmotic pressure is effected. Is base freed from the cells by contact with glucose, and, if so, does this indicate that the membranes or cells have been injured by their strange environment?*

How far an analogy can be drawn between the red blood cells and tissue cells is uncertain because the latter can not be isolated for analysis. Available data on tissue analyses reveal far more potassium than sodium. The question may be raised whether the small amounts of sodium found in the tissues are not contributed entirely by interstitial fluid from which the cells can not be sepa-

* Some preliminary experiments made in this laboratory by Klinghoffer indicate that glucose and urea solutions do act like distilled water. Loeb (personal communication) states that this is true of solutions of urea and glycerol. Nevertheless, under certain circumstances, still to be defined, red blood cells, suspended in glucose solutions of proper strength, remain unchanged.

ated. In this case the analogy to the human red blood cell is extremely close. The table below gives Katz'[23] analysis of muscle obtained from the arm of a suicide shortly after death.

Water	*72.5 per cent by weight*	
Substance	*m.eq. per kilo.*	*m.eq. per kilo. of water*
Potassium	81.9	112.5
Sodium	34.7	48.0
Calcium	3.7	5.2
Magnesium	17.3	23.9
Chloride	19.8	27.2
	mM. per kilo.	*mM. per kilo. of water*
Phosphorus	65.6	90.4
Sulfur	64.8	89.4
Iron	0.147 gm. per kilo.	

The total base concentration per kilo of water in the tissue mass is 189.6 m.eq., of which 112.5 is composed of potassium, only 48.0 of sodium, 5.2 of calcium and 23.9 of magnesium. If this is compared with the composition of interstitial fluid it is at once apparent that muscle tissue contains enormously more potassium and magnesium, far less sodium, and about the same amount of calcium.* The total base concentration is extraordinarily high. Let it be assumed that the fluid represents an admixture of muscle cell juice with interstitial fluid, both having the same total base concentration, but the latter containing Na, K, Ca and Mg in the proportions usually found in transudates, and finally that all the Na is in the interstitial fluid, which contains a negligible quantity of solids. These are rather extreme conditions. In this fluid 93.6 per cent of the base should be Na, 1.6 per cent K, 3.0 per cent Ca and 1.8 per cent Mg. Therefore, of the base in the total fluid 48 m.eq. of Na, 0.8 of K, 1.5 of Ca and 0.9 of Mg come from interstitial fluid with a total concentration of 189.6 m.eq. That is,

$$\frac{100\ (48 + 0.8 + 1.5 + 0.9)}{189.6} =$$

the per cent of interstitial fluid in the mixture = 27 per cent. The remaining 73 per cent is actual muscle juice. As this fluid made up

* Collip[6] states that he has found, by analysis, in muscle and parenchymatous tissues only 2 to 3.5 m.eq. of Ca per liter, a far lower concentration than that reported by Katz, in fact, low enough to make its presence within the tissue cells themselves a matter for argument.

only 72.5 per cent of the total muscle tissue, there was actually only 19.6 per cent of interstitial fluid present. The remaining 80.4 per cent of muscle cell juice contained the 27.5 per cent of protein. Therefore, there was $\frac{27.5}{80.4} = 34.2$ per cent of solids in the muscle cells. These are not unreasonable estimations. They would credit the muscle cell with about the same concentrations of protein and solids which are found in the red blood cell. Nor does it seem incredible that as much as 20 per cent of the tissue should consist of interstitial substance. Certainly the sodium content of the muscle cell must be extremely low.

The potassium content of human heart muscle, secured by biopsy and at autopsy from patients suffering chiefly from cardiac and renal diseases with and without edema, has been determined by Harrison, Cullen, Calhoun et al.[35] Their figures are extremely variable, ranging from 122 to 361 mg. per 100 gm. of fresh tissue. In the same muscles the solids varied from 13.8 to 27.1 per cent. There is a general tendency for the concentration of potassium to vary directly with the total solids, that is, inversely with the water. It seems incredible that the solids within the heart muscle cells can vary 100 per cent; it is far more likely that the variations in observed solids were referable to differences in the amounts of interstitial fluid included in the materials analyzed. The parallel fluctuations of K and solids can, in this case, be attributed to dilution of an intracellular fluid rich in potassium with varying quantities of interstitial fluid containing chiefly sodium, with little potassium. The average potassium concentration per kilo of water, 71 m.eq., is far less than that found by Katz in skeletal muscle. This may indicate that heart muscle tissue contains a larger amount of interstitial substance than skeletal muscle does, or that the heart muscle cell is not so entirely devoid of sodium. Scott[43], in a more limited series of post-mortem examinations of human muscle tissue, found average solids only 20 per cent by weight. The potassium concentration per kilo of water was only 58 m.eq., that of sodium 95 m.eq. If the latter is all derived from intracellular fluids, these must make up a large proportion of the total tissue mass. By the methods applied to Katz' figures above, the interstitial fluids may be estimated as composing about 50 per cent of the tissue analyzed by Scott.

Some indirect evidence of an entirely different nature concerning

the distribution of, and the permeability of cell membranes to, bases within the body is found in experiments in which large amounts of one of the basic constituents are retained or lost. Most of the available information of this nature deals with sodium. In this department electrolyte studies have been made on patients with diabetes and other conditions at intervals while they have been receiving or have received large amounts of sodium chloride intravenously or subcutaneously. In some instances the excreta have been analyzed for base. In two cases complete anuria has simplified the problem. Evaluation of the data is difficult because of the complex reactions involved. Nevertheless, with few exceptions the changes in the level of serum base are far greater than one would expect if the administered sodium were distributed over the total mass of body fluids and more in agreement with the theory that the sodium is distributed over a more limited volume of fluid.

After administration of potassium salts the changes of serum potassium are extremely transitory. This may mean that the potassium is excreted with great rapidity or that it is immediately seized by the cells. Unfortunately it may indicate only that the amounts of potassium given and retained were too small to have a demonstrable effect on the serum. In conditions of edema, which presumably represent expansions of the interstitial fluids, potassium salts are usually well excreted, while sodium salts are retained with an equivalent amount of water. If the tendency to accumulation of fluid is confined to the interstitial spaces and the fluid has the characteristics of normal interstitial fluid, such a distinction between the reactions to the two ions is intelligible. Potassium could be retained only in the cells, and there only with an equivalent amount of water.

On the acid side of the balance-sheet information concerning any ions except bicarbonate and chloride is extremely scanty. The same figures of Katz, cited above, reveal less chloride in muscle tissue in proportion to sodium than is usually found in serum. If, then, the sodium is to be credited entirely to admixed interstitial fluid, the chloride must also have an extracellular origin. Other analyses of tissues from various animals including man have been made by Vladesco, Müller and Quincke, and Cameron and Walton (cited by Peters and Van Slyke[34]). These analyses reveal varying amounts of Cl in all tissues. The concentration of the ion seems, as Cameron and Walton have pointed out, to be inversely proportional to the

quantity of highly differentiated cellular structure in the material analyzed and directly proportional to the vascularity of the tissue and the amount of blood, lymph and interstitial substance which it contains. Nevertheless, the concentration of Cl is always lower than that in blood, lymph or interstitial fluid. This is what one would expect if all the chloride were contributed by these extracellular fluids. The enormous quantities of organic phosphorus esters and protein in muscle would, indeed, leave little room for chloride if an acid-base balance were to be maintained within the cells. Like all tissues, however, the muscle cell contains bicarbonate and exchanges CO_2 with the surrounding medium. Banus and Katz[2] found that when muscles of experimental animals were perfused with blood to which hydrochloric acid had been added, the Cl content of the perfusing fluid did not change; further evidence of the impermeability of the cell membrane to the Cl ion. That the distributions of Cl and Na are more or less coincident is suggested by studies of the effect of parenteral sodium chloride administration on serum base and chloride. A sodium chloride solution, as compared to interstitial fluid, contains a relative excess of Cl. If the two ions are distributed over the same body of fluid, then, the effects on serum sodium and chloride will differ in magnitude in a predictable manner. The alterations of the two ions in the serum after injections of sodium chloride not only follow predictions in a directional sense, but are also of the general order of magnitude that would be expected if both distributed themselves through fluid volumes of the same magnitude and much smaller than the estimated volume of the total body fluids.

Many investigators have studied the metabolism of potassium, sodium and chloride under various conditions which involve destruction of cells or dehydration or both. In all these investigations balances of chloride have agreed closely with those of sodium without much relation to the potassium. For example, in Benedict's[3] famous study of Levanzin's 31-day fast, 325 m.eq. of sodium and 345 of chloride were recovered in the urine. During the same time the urine potassium amounted to 724 m.eq. Furthermore, not only the total quantities of sodium and chloride excreted, but the courses of the curves of excretion agreed remarkably closely.

Taken together all these fragmentary bits of evidence point to the same end, that the differentiated cells of the body, with the exception of the red blood cell, contain little if any chloride and

possess membranes impermeable to this ion. Following the suggestion made in the discussion of the red blood cell, the guess might be hazarded that these cells, of which skeletal muscle cells have received most attention, contain a full complement of acids which have more important and specialized functions than chloride. In this case one is forced to wonder which, if any, of these acids play the rôle in the exchange of bicarbonate buffer between cells and their environmental fluid that chloride serves between red blood cells and serum. Can inorganic phosphate or lactate, for example, act in this capacity in the muscle cell? Can this possibly afford an explanation of the changes of blood lactic acid which occur independently of increased functional activity of the muscle cell, *e.g.*, when the pH of blood is shifted in an alkaline direction by forced ventilation or the administration of alkalinizing salts?

Inorganic phosphate resembles bicarbonate in its ubiquitous distribution. The two acids are analogous in another respect that may be connected with, or the reason for, their universal presence in cells and fluids. Organic esters of phosphoric acid, like bicarbonate, are almost as characteristic and essential features of animal protoplasm as are proteins. The phosphoric acid in these compounds may be looked upon as having the power to restrict, and in turn as being restricted by, the organic radicles with which it is combined. This restriction is exhibited in one dimension by the inability of the compounds as a whole to diffuse across the cell membrane which their components may traverse without difficulty. Functional activity is attended and presumably subserved by alternate hydrolytic cleavage and reformation of these esters, often with complex intermediary reactions involving transformation of the organic components. Both the organic esters and inorganic phosphate have large buffer powers, maximum in different ranges of reaction, which tend to minimize intracellular pH changes that would otherwise result from these complex transformations which have been mentioned. As all these esters have, as a hydrolytic product, orthophosphoric acid, and can be formed within the cells from this acid, it is essential that phosphate be always available in and have free access to the cell. Unfortunately the exact concentration of orthophosphoric acid in tissues, and consequently its partition through the various body media, can not be determined with certainty by analysis because of the extreme instability of the phosphoric esters.

Just how electrolytes and other nutrient materials gain access to

the interior of cells and there become immobilized, while waste products are selectively moved in the opposite direction is, for the most part, unknown and incomprehensible. The exchange of carbon dioxide and oxygen has commanded much attention. Knowledge of the properties of hemoglobin and the reciprocal effects of carbon dioxide and oxygen has afforded a more or less satisfactory explanation of the gas exchange in the blood. Hemoglobin within the cells enables the blood to carry a greater load of oxygen at low oxygen tensions than simple solubility in aqueous solutions would permit, in a combination so labile that a maximum load can be assumed or delivered with great rapidity; and to take on or discharge carbon dioxide with a minimal disturbance of pH; moreover, so automatically are the responses regulated, that accumulation of carbon dioxide accelerates discharge of oxygen and, vice versa, oxygenation of hemoglobin facilitates delivery of carbon dioxide. There is evidence that certain compounds in tissue cells play a rôle in aiding gas exchange similar to that which hemoglobin has in the red blood cell.

Similar mechanisms may exist for the fixation in the cells of other essential substances. Glucose, like urea, appears to diffuse freely into the cells, and the free glucose in cells is in diffusion equilibrium with that of serum and other body fluids. Free glucose, however, comprises only a small proportion of the carbohydrate available in cells; the remainder consists of glycogen and phosphate esters which are restrained by their non-diffusibility from escaping from the cells, but afford a readily available supply of carbohydrate for combustion. An analogous situation with regard to inorganic phosphorus and phosphate esters was pointed out above. Under certain conditions, when carbohydrate combustion is suddenly disturbed, the inorganic phosphate and glucose of blood are coincidently altered. This has been interpreted as evidence that glucose can traverse the tissue cell membranes only when it is combined with phosphorus. It seems more reasonable, in the face of the known diffusibility of glucose, to believe that glucose and inorganic phosphate diffuse to or from the cell when the concentrations of these components in the cell are altered by accelerated formation or hydrolysis of phosphate esters associated with the intracellular utilization of carbohydrate. The mechanisms for the exchange of gases and for the transfer of glucose and phosphate illustrate processes in which no other force than diffusion need be assumed to explain the

passage of solutes across cell membranes. Diffusion could not, however, by itself cause concentration of these solutes within the cells, where they are most needed. This is effected by the production of chemical compounds of these substances which are no longer diffusible, but which can be resolved into their component parts with great facility when these are required. Northrop[33] has succeeded in producing membrane systems in which heaping up of ions in one medium is achieved by similar principles.

Kidney function

It is impossible to dwell at length upon the function of the kidneys because this is too large a subject in itself for a single review. In spite of the frequency with which arguments with teleological implications have been used, the author is in general in hearty sympathy with that school which feels that the safest way to truth is through the attempt to interpret biological phenomena, as far as possible, in terms of known natural laws of physics and chemistry. This end can not, however, be forwarded by stretching or distorting either the laws or the facts to fit one another. In the constant struggle to bring one more phenomenon out of the obscurity of vitalism into the physicochemical order, every shade of failure must be clearly recognized through painful analysis. The extent of the remaining realm of vital force must be defined relentlessly. There is little danger in teleological arguments and concepts if the view is taken that these are merely temporary conveniences or expedients, perhaps even goads to the imagination, which is always trying to reach behind their implications of purpose to the impersonal natural reactions which are so orderly regulated as to give this impress of personality.

In the analysis of kidney function it is gratifying to see an ever increasing body of evidence favoring the filtration-reabsorption theory of Bowman and Ludwig because this seems to establish the function of the glomeruli upon a comprehensible basis. This satisfaction must, however, be tempered by the realization that mystery has been displaced from the glomeruli only to become more concentrated in the tubules. What controls the extent or selectivity of the reabsorption process is still completely obscure, and the situation is hardly improved by the introduction of such terms as threshold, threshold substances, etc. In fact, it may well be argued that such

terms merely add to the obscurity by veiling ignorance in a specious sense of knowledge. At best the conceptions embodied in these terms are only temporary working hypotheses to be subjected to experimental attack from every direction. Ultimately the kidneys seem to act as guardians and regulators of the internal environment of the body, under the combined influence of at least three controlling forces: the composition and volume of the blood flowing through the kidney; the condition of the renal circulation; and the influence of the nervous system or endocrine secretions (these last may express themselves only by altering the volume and composition of the blood or the state of the renal circulation). The responses to this complex control result in an uncannily exact regulation of the composition and volume of the body fluids in the face of the most adverse conditions. Foreign products which gain access to the blood may be completely eliminated with the greatest rapidity at all times. Other substances, though present in high concentration in the blood and so freely diffusible that they enter the glomeruli in the same concentration, are so exactly reabsorbed that no traces appear in the urine unless they accumulate to excess in the blood.

It seems inconceivable that osmotic pressure and fluid volume could be simultaneously maintained if the regulatory mechanism were not sensitive to changes of both functions. An attempt has been made with urine formation as it has with respiration to find the one factor that controls excretion of water, whereas the many dimensions in which constancy is maintained in the internal environment by the kidneys would seem to preclude the dominance of any single controlling force. The filtration-reabsorption theory has certain very definite implications concerning the forces that must control glomerular filtration, which have been excellently stated by Holten and Rehberg[21]. These forces must be quite similar to those which control the exchange of fluids between the serum and interstitial fluid in any capillary bed: (*1*) the osmotic pressure of nondiffusible solutes in the serum, which opposes filtration; (*2*) the blood pressure in the glomerular capillaries, promoting filtration; (*3*) the condition and surface area of the filtering surface; (*4*) the pressure within Bowman's capsule opposing filtration. The fluid which filters through the normal glomerular membrane must be a protein-free, lipoid-free ultrafiltrate of serum, quite similar to interstitial fluid. Injury and circulatory disturbances may increase the

permeability of the membranes, in which case proteins and lipoids will find access to the urine.

The process of reabsorption is obviously far more complex and no theories concerning the mechanisms by which it is effected can be considered as more than the vaguest working hypotheses. Nevertheless, certain general deductions follow naturally from what has been said of the nature of glomerular filtration. The concentrations of diffusible solutes in fully elaborated urine vary extremely from those in serum. Some, like creatinine and urea, are always found in far higher concentration in the urine; others like chloride, and especially glucose, are often less concentrated in the urine. In fact normal urine may be practically free from glucose when the concentration of this compound in serum is as great as 1 to 2 gm. per liter. Obviously no single simple process comparable to that of glomerular filtration can account for such diverse absorptive activities. It seems necessary, as Rehberg has pointed out, to hypothecate processes of at least two different kinds: (*1*) absorption of water with consequent concentration of solutes; (*2*) absorption of certain individual solutes. The latter process may, as Rehberg suggests, consist in part merely of back diffusion of diffusible solutes which have been concentrated in the tubules by absorption of water. Such a mechanism might serve to explain urea excretion. In addition the tubule cells must have a highly selective permeability if they will take up glucose and urea so freely and, as has been claimed, leave creatinine, xylose, etc. entirely unabsorbed. The absorption of glucose, chloride, etc., the solutes which Cushny termed threshold substances, could not possibly be effected by simple diffusion unless, after entry into the cells, these solutes are removed from the field, as glucose is in muscle cells, by conversion into non-diffusible inactive components. That Cl should be involved in such a reaction seems unlikely. The absorption of such components must be highly facultative. It has been stated that alterations of the concentration of freely diffusible solutes like glucose and urea cause no disturbance of the osmotic pressure relations between media in the body because their effect on osmotic pressure is evenly transmitted throughout all the media. The same substances seem to have a striking effect on the excretion of urine. This is to be expected if general opinion is correct in asserting that the osmotic pressure or total concentration of solute which can be attained in the urine is limited. Under these

circumstances if one or more solutes reached excessive concentration in the serum (and consequently in the glomerular filtrate) and were unabsorbed or little absorbed in the tubules, the absorption of water would be limited and diuresis would ensue. It must not be inferred that the limit of concentration is the osmotic pressure of body media. The osmotic pressure of urine except in the last stages of renal destruction may greatly exceed that of serum. The process must, therefore, involve the expenditure of energy.

It may be because the kidney is less indifferent to changes in total osmotic pressure that diuresis ensues when such freely diffusible solutes accumulate to excess in the blood. In the excretion of such substances and foreign materials there is almost always a loss of water and salts from the body. Why such losses should so often result in depletion of salt in excess of water is not entirely clear; but it is extremely common to find in states of dehydration a reduction of the total concentration of salt in the serum and body fluids; the reverse is seldom observed. Considering that besides the loss of both salts and water through the kidneys there is a continuous wastage of water without an equivalent amount of salt through the skin and lungs, one would expect the salt concentration in the body fluids to increase. The only adequate explanation for the apparent paradox is that in conditions that produce dehydration the fluids ingested usually contain little salt in proportion to water.*

It has been suggested that the kidney is responsive to changes in the volume of body fluids. It would be easier to conceive of the blood volume as the effective influence, perhaps securing its effect merely through secondary variations in the renal circulation. Inasmuch as the volumes of the circulating blood and of the body fluids or even the interstitial fluids may be entirely dissociated, the kidney might be exonerated entirely for the accumulation of edema on this score alone. These suggestions are only put forward tentatively as illustrations of possible reactions. They are advanced partly to call attention to the fallacy of referring all disorders of the internal environment to improper renal function when the influences which control the activities of the kidneys, and especially reabsorption, are so vaguely known; chiefly to point out the necessity of analyzing the responses of the kidney to alterations of the internal

* Whether the fluids are absorbed or vomited makes little difference in the end results. If water is taken into the stomach and vomited without absorption it carries away with it salts which have diffused or been secreted into it.

environment with a more thorough recognition of the other forces which influence the disposition and character of fluid and solutes within the body, many of which have been discussed above.

Excretion by the skin and lungs

It is generally held that the fluid continuously dissipated in what is called the insensible perspiration is almost devoid of solutes, and especially of inorganic constituents. Of the expired air this is almost certainly true. If it is equally true of the water lost by evaporation from the skin, a sharp line must be drawn between the character of sensible and insensible perspiration, with the necessary inference that the two are produced by different processes. Such a distinction is in keeping with what is known of the nature of ectodermal membranes which separate higher organisms from their environment. Such membranes appear to have a primarily protective function, the insulation of the internal media from encroachments by the external environment. They are also possessed of a limited secretory or eliminative function. In some respects this latter seems to aid in the regulation of the internal environment, especially in the thermal control. It seems, however, to play an insignificant rôle in the maintenance of the composition or volume of the body fluids.

Exact studies of salt and water balance are required to establish beyond peradventure the saltlessness of insensible perspiration and the influence of the internal environment on sensible perspiration. The statement that the insensible loss varies directly as the metabolism and is unrelated to the state of the body fluids has been challenged. It has been suggested that this loss also depends upon the state of the body water stores. The challenge forces a review of previous concepts and perhaps modification of current opinions about the nature and function of the insensible perspiration. Again Benedict's[3] study of fasting offers illuminating data. In this experiment the confusing element of salt and food ingestion was eliminated. During the first days of the fast the loss of both water and salts was far greater in proportion to the heat production than it was during the latter part of the study. Moreover, a significant quantity of chlorine, amounting to about one-tenth of the urinary chloride excretion, was recovered from the skin secretions. Of course, this may have come from sweating provoked by the moderate exercise in which Levanzin indulged.

Concentrations and volume changes

A final note is necessary concerning the greatest weakness of the chemical approach to all these problems, the inability to determine by analysis anything except concentrations of substances; which confines the description of a three dimensional world to two dimensions. Methods must be devised for the estimation of the volumes of at least two great fluid compartments, intracellular and extracellular. But before these are perfected reasonable attempts may be made to take into account the influence of expansions and contractions of these compartments in the evaluation of the results of determinations of concentrations of solutes in serum or blood. And in these estimations the peculiar differences of distribution of various solutes must be given due consideration.

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