THE MOVEMENT OF WATER IN TISSUES REMOVED FROM THE BODY AND ITS RELATION TO MOVEMENT OF WATER DURING LIFE*

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The purpose of the present study is to determine under what conditions water enters or leaves tissues immediately after their removal from the body and to learn whether by this means information can be obtained concerning the movement of water between blood, interstitial tissue spaces, and parenchymatous cells during life. This study has been suggested by changes which occur in tumors; and in a publication which follows the movement of water in normal and tumor tissues will be compared.

Tissues immersed in water or in solutions of various substances undergo swelling which can be measured by weighing the tissue at intervals after immersion. The early studies of Overton (1) were undertaken to determine the nature of the surface of cells and in these and in subsequent studies the muscle of frogs was used. It was evident that the quantity of water which entered the tissue when approximate equilibrium was established was considerably less than that which might be expected to pass through a semipermeable membrane as the result of osmosis. In accordance with present usage the term "membrane" will be used to designate a superficial layer of cytoplasm defined by its physical properties and not necessarily recognizable by histological methods.

The penetration of water into the muscle of frogs has been studied repeatedly with the hope that it might give information concerning the contraction of muscle (Meigs, 2). Seibeck (3) measured at widely spaced intervals the entrance of water into the whole kidney of frogs kept at a temperature just above freezing. More exact studies of the penetration of water into the muscle and skin of frogs have been made in recent years by Adolph (4). Those who have studied the permeability of cells to water have regarded mammalian tissues as ill-adapted to experiment because it has been assumed that injury profoundly alters the osmotic changes that ensue.

Water with dissolved crystalloid substances passes freely from blood vessels into the interstitial tissue but the vessel walls oppose a more or less effective obstacle to the passage of the proteins of the plasma. The extracellular fluid of the interstitial tissue is the medium which surrounds the cells of the body. There has been wide difference of opinion concerning the movement of water to and from the interstitial tissue with reference especially to the significance of the protein content of the tissue fluid.

The term imbibition has long been applied to the hydration of colloidal substances such as gelatin or starch often with the formation of a jelly-like material. The physical conditions associated with this change were studied by Loeb (5) and for a time much attention was directed by Fischer (6) to the possible pathological significance of related changes in the proteins of the body. Schade and Menschel (7) have studied conditions which influence the swelling of connective tissues of the umbilical cord and of tendons but their observations at intervals of 24 hours after immersion were made upon tissues presumably injured severely by the immersion fluids.

When water enters into the parenchymal cells of liver, kidney, or pancreas the changes that ensue are doubtless dependent upon the passage of water by osmosis through cell surfaces which resemble though they are not identical with semipermeable membranes. When fibrous tissue assumes an edematous or gelatinous appearance. following immersion in hypotonic fluids the change is analogous to the hydration of colloids. The associated physical changes are as yet not clearly definable, but it is probable that hydration of the colloids is caused by the same forces that are concerned in the passage of fluids through membranes (Brooks and Brooks, 8).

Methods

In an earlier study (9) specific gravity was used to measure the entrance of water into tissues removed from white rats or guinea pigs and immersed in water or in various solutions. In the present study tissues of the white rat have been weighed at intervals after immersion in order to determine directly the quantity of water that has entered them. Animals used in the experiments have been maintained on a uniform diet of bread and milk with access to water. They have been killed by bleeding and blood has been removed from thin slices of tissue by placing them in contact with cotton gauze. A torsion balance has been used to weigh slices of liver, kidney, and other tissue. These slices have been cut by a razor blade, held by a dressing forceps with a clamp catch, to an approximate thickness of 0.5 mm., the aim being to obtain slices weighing from 50 to 100 mg. As it is difficult or impossible to obtain evenly cut slices of some tissues such as pancreas, thymus, or omentum, small pieces of approximately the same weight and thickness as the slices have been used. The percentage increase of weight has been used as an index of the movement of water into or from the tissue. As the size of the immersed particle and the area of its exposed surface doubtless modify the rate of movement of water, it has seemed desirable to multiply experiments in order to learn whether variation in the size of the immersed particle modifies significantly the changes that ensue (see Table I).

The Penetration of Water into Liver Tissue Removed from the Body

The penetration of distilled water into liver tissue has been studied as a basis for comparison with other fluids which more closely approximate normal relations. The rate of penetration is rapid immediately after immersion (Fig. 1) but later diminishes, a maximum intake of water being reached after 2 hours. Table I shows that variations in weight and thickness of the slices of liver tissue used in ten experiments have had insignificant influence on the rate of penetration of water. After 2 hours of immersion the weight of the particle diminishes and with increasing injury to the tissue, removal of soluble substances, and actual disintegration of the cytoplasm, the dry weight of the tissue may be reduced as much as one-third.

TABLE I

Change of Weight of Liver Tissue Immersed in Water

| Experiment	Weight of slice of liver	Period of immersion, *min.*						
		15	30	60	120	180	240	300
	mg.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	108.0	138.7	154.4	178.3	201.0	198.0		
2	143.6	140.0	157.5	180.0	193.3	188.9	184.7	
3	118.0	148.1	167.1	191.7	201.0	198.8	193.4	181.2
4	82.8	146.0	166.0	195.0	202.0	218.0	205.0	203.0
5	105.2	139.1	158.1	178.5	196.3	193.9		
6	42.8	168.2	200.0	238.2	236.9	225.7		
7	73.8	144.0	166.0	178.0	189.0	180.8		
8	96.6	157.0	180.0	194.5	195.5	186.0	180.0	169.3
9	70.2	158.9	177.2	202.0	200.0	186.5	180.4	
10	76.4	160.0	174.0	189.3	189.0	177.3	172.8	164.0
Average....		150.0	174.0	192.6	200.4	195.4	186.1	179.4
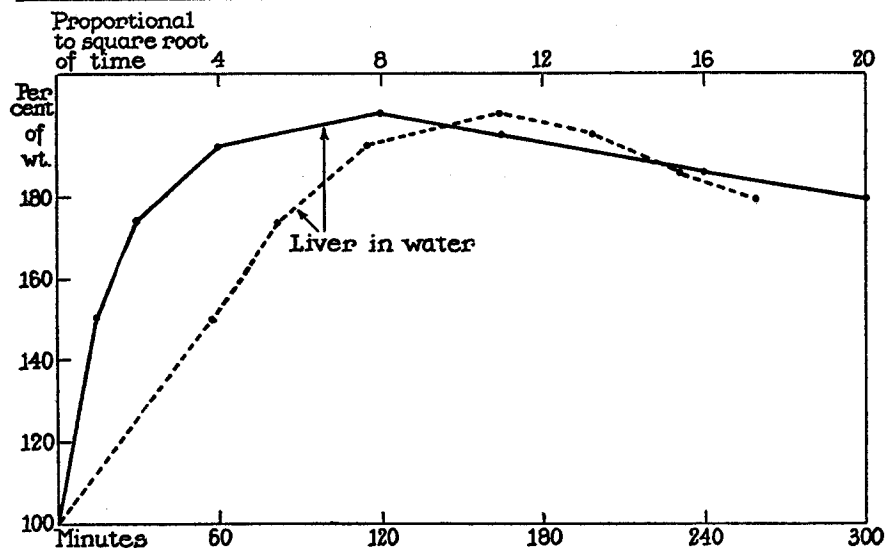
FIG. 1. A composite graph from ten experiments (see Table I) showing the percent increase of weight of slices of liver tissue immersed in distilled water. The same observations are plotted as a broken line in accordance with a scale (at the top of the graph) which measures the square root of the time of immersion. It is noteworthy that the two scales (at the top and at the bottom of the graph) record the same observation independently. Three of the experiments from which this composite graph has been prepared are shown in Fig. 2, Experiments 1, 2, and 3.

Experiments of Eggleston, Eggleston, and Hill (10) showed that diffusion of lactic acid from frog's muscle into Ringer's solution maintained a constant

ratio to the square root of the time during which diffusion occurred. Adolph (4) found that the increase in weight of frog's muscle during the period im-
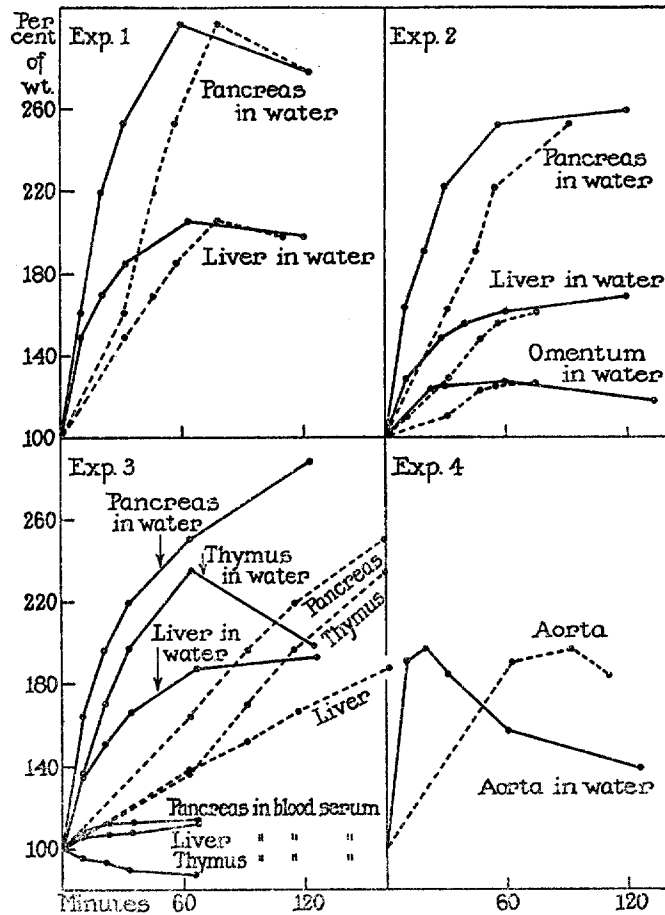

FIG. 2. Four experiments in which tissues immersed in water or other fluid have been weighed at intervals of 10 minutes during the first half hour of immersion. In each of these experiments the tissues that have been used are from the same animal. The broken lines as in Fig. 1 are plotted in relation to the square root of the time of immersion for comparison with the solid lines plotted in relation to the time.

mediately after immersion in distilled water and in some solutions of sodium chloride is directly proportional to the square root of the time of immersion. Parry (11) states that mammalian muscle under similar conditions maintains the same relation.

Increase of weight of liver tissue immersed in water takes a linear course

when plotted against the square root of the elapsed time during an initial period of at least one-half hour (Fig. 1), and this relation indicates that an existing osmotic system remains unimpaired. The deviation from it after more prolonged immersion gives evidence that other factors, doubtless including injury to the tissue by the immersion fluid, have interposed.

The relation between the initial rate of water penetration and the square root of the elapsed time can be shown by weighing of tissue at intervals of 10 minutes during the first half hour after immersion (see Fig. 2, Experiments 1, 2, and 3). Removal of the tissue from the fluid at more frequent intervals would materially diminish the time of immersion. After it was learned that
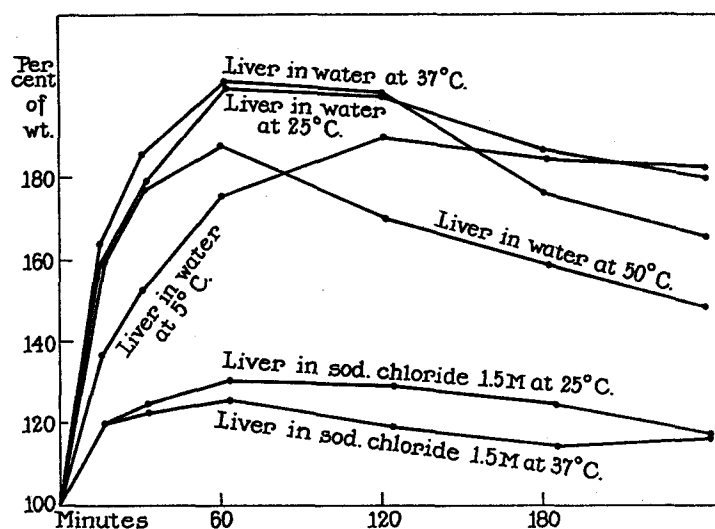
FIG. 3. Changes in the per cent of weight of slices of liver following immersion in distilled water and in normal salt solution maintained at temperatures indicated on the graphs.

the rate of water penetration pursued as plotted a linear course during one half hour, many of the initial readings were made at the more convenient intervals of 15 minutes (Fig. 1).

Intake of water by immersed tissue is modified by temperature. Fig. 3 shows the per cent increase of weight of liver immersed in water at 5°, 25°, 37°, and 50°C. during 4 hours. A low temperature, 5°C., diminishes the rapidity with which water enters the tissue and also the quantity of water intake. At temperatures of 25°, 37°, and 50°C. the per cent of water that has penetrated into the tissue during the first 15 minutes is almost identical. The loss of weight which occurs after a maximum is reached is presumbly the result of injury to the tissue and occurs earliest and is greatest in liver kept at a temperature of 50°C. It is noteworthy that during a period of 3 hours water intake at

body temperature, approximately 37°C., and at room temperature of 25°C., usual in these experiments, differed very little. Subsequent diminution in weight of the tissue is probably the result of more or less severe injury and is greater as the temperature increases. After 3 hours of immersion weight is almost the same at 37°C., at room temperature (23°C.), and at 5°C. When liver has been immersed in normal salt solution (sodium chloride 0.15 molar), initial changes in weight have differed little at 25°C. and at 37°C. (Fig. 3).

Intake of water by liver is not conspicuously impaired by preservation during 24 hours at a temperature just above freezing (in ice box at 5°C.). In Fig.
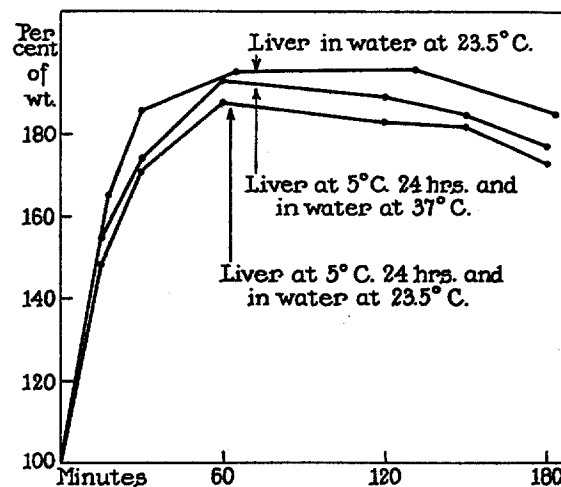

FIG. 4. Changes in the per cent of weight of slices of liver maintained at a low temperature (5°C.) during 24 hours and then immersed in water at the temperatures indicated on the graph. These changes are compared with those which follow immediate immersion in distilled water.

4 intake of liver tissue immersed in water at 23.5°C. shortly after removal from the body is compared with that of liver kept for 24 hours at 5°C. and then immersed in water (a) at 23.5° and (b) at 37°C. It it noteworthy that increase in weight is little greater at 37°C. (body temperature) than at 23.5°C. (approximate room temperature). In the experiments that will be described, unless otherwise stated, tissues have been introduced into the immersion fluid as soon as possible after removal from the body.

Changes in the Water Content of Liver Tissue in Solutions of Sodium and of Potassium Chloride

When liver tissue is immersed in solutions of sodium chloride of gradually increasing concentration a level is reached at which it loses instead of gains

water. In the experiment shown in Fig. 5 the concentration in which no movement of water occurs is between 0.26 and 0.34 molar. Osmotic changes that occur during the initial period of immersion, that is, during the first 10 or 15 minutes, are least affected by injury to the tissue caused by the immersion fluid. Experiments 1, 2, 3, 4, 5, 6, 7, and 8 of Fig. 6 show that the quantity of water taken up by liver in solutions of sodium chloride varies with the concentration of the solution and when plotted against the molar concentration, maintains an approximately linear relation. This movement of water is in accord with that caused by osmosis through a semipermeable membrane. The point
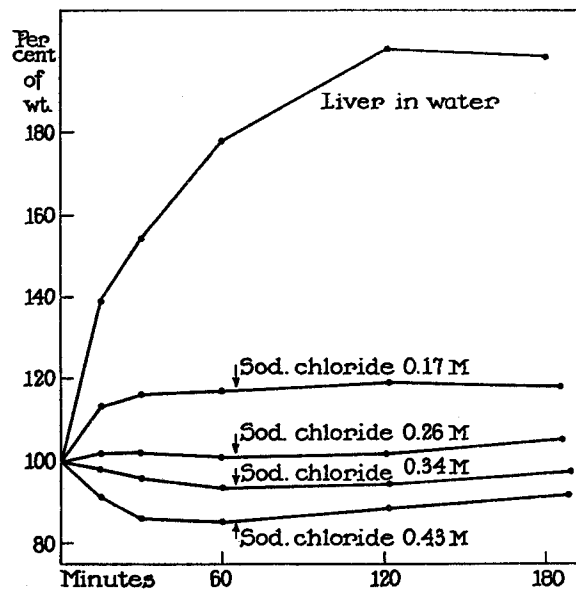

FIG. 5. Changes in per cent of weight of liver slices following immersion in distilled water and in solutions of sodium chloride varying in concentration from 0.17 to 0.43 molar.

at which the plotted line crosses the abscissa represents with close approximation the concentration of sodium chloride which is in osmotic equilibrium with the immersed tissue (see Table II). The concentration that has been found by this procedure isotonic for liver tissue has been with three exceptions greater than 0.3 molar, the average being 0.34 molar (see Table II). In the one instance (Experiment 7) in which isotonicity was at the level of 0.5 molar, the liver was the site of slight, irregularly distributed fatty degeneration but otherwise the animal appeared to be normal.

When liver has been kept immersed in normal salt solution (Fig. 3), the initial intake of water during 15 minutes has been the same at 23°C. and at 37°C., but later the ability to take in water had been better maintained at the
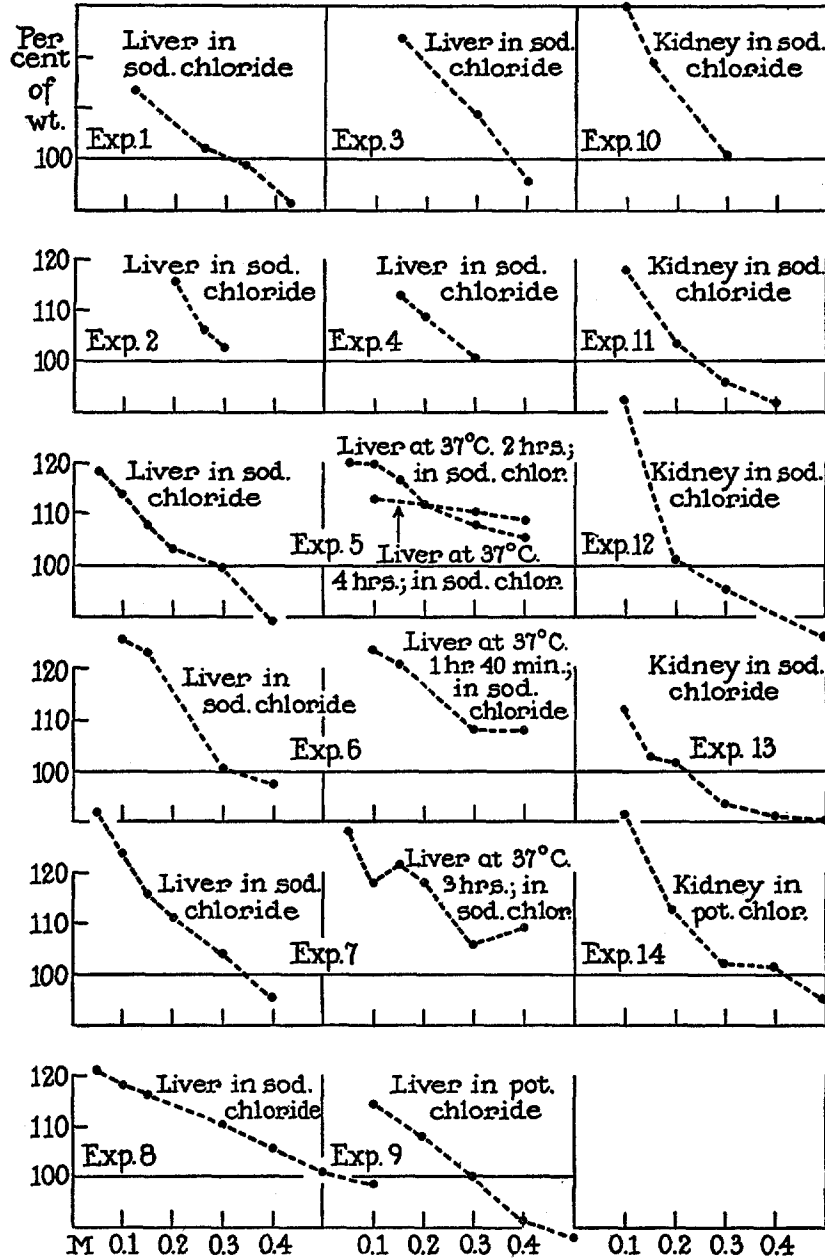
FIG. 6. Initial changes in the per cent of weight of slices of liver or of kidney tissue when immersed in solutions of sodium or of potassium chloride varying in concentration from 0.05 to 0.6 molar. In Experiments 5, 8, and 13 the initial period of immersion was 10 minutes and in the remaining experiments 15 minutes. In Experiments 5, 6, and 7 slices of the liver of the same animal were immersed in sodium chloride (a) immediately after removal from the body and (b) after maintenance at 37°C. during 1 hour and 40 minutes, 2, 3, or 4 hours.

lower temperature, perhaps because the tissue has been less injured. To determine the changes presumably referable to autolysis, liver tissue has been kept at 37°C. during different periods following removal from the body, that is 1 hour and 40 minutes (Fig. 6, Experiment 6), 2 hours (Experiment 5), 3 hours (Experiment 7), and 4 hours (Experiment 5), and then immersed in solutions of sodium chloride of varied concentration. The intake of water has been compared with that of liver immersed in similar solutions of sodium chloride immediately after removal from the body. The graphs show that the rate of water intake is no longer proportional to the concentration of the immersion fluid, the linear relation being lost and none of the solutions that

TABLE II

Solutions of Sodium or of Potassium Chloride Isotonic for Liver and Kidney Tissue

Experiment	Molar concentration of sodium chloride isotonic for liver	Molar concentration of potassium chloride isotonic for liver	Molar concentration of sodium chloride isotonic for kidney
1	0.31		
2	0.33	0.32	
3	0.31		
4	0.37	0.46	
5	0.3	0.36	
6	0.36		
7	0.5		
8	0.3		
9	0.34		
10	0.3	0.3	
11			0.22
12			0.31
13			0.22
14			0.25
Average..........	0.34	0.36	0.25

have been used are isotonic with the altered liver. The increased penetration of water is perhaps the result of increased molecular concentration caused by the breaking down of protein within the cells of the autolyzed liver.

Solutions of potassium chloride, like those of sodium chloride, have maintained an approximately linear relation between concentration and initial increase of weight following immersion (Fig. 6, Experiment 9). Solutions of potassium chloride isotonic with liver tissue have differed little from those of sodium chloride, the average being 0.36 molar (Table II).

Changes in Water Content of Liver Tissue Immersed in Normal Salt Solution, Ringer's Solution, or Blood Serum

A solution of sodium chloride with twice the concentration of the sodium chloride in the blood plasma is isotonic for liver, and liver immersed in sodium
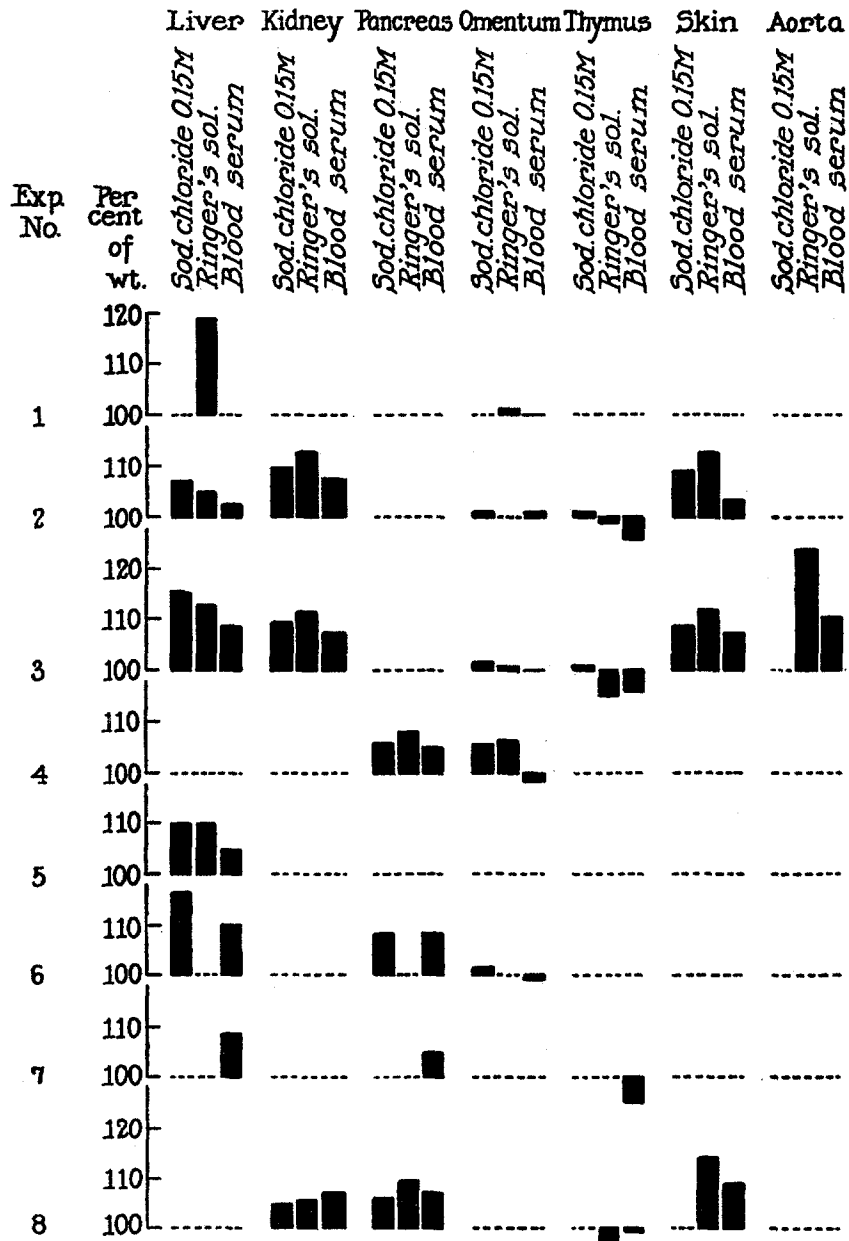
FIG. 7. Initial changes in the per cent of weight of liver, kidney, pancreas, omentum, thymus, skin, and aorta immersed in normal salt solution, Ringer's solution, or blood serum. This initial period of immersion is 10 minutes in Experiments 4, 5, 6, and 7 and 15 minutes in the other experiments.

chloride that is isotonic for red blood corpuscles takes up water in considerable quantity (Fig. 7). In Ringer's solution, which has approximately the electrolytic content of the plasma, liver tissue takes up water, measured by the per cent increase in weight, in quantity somewhat less than that taken up by liver in a solution of sodium chloride with concentration the same as that of the sodium chloride in the Ringer's solution (0.15 molar).

A modification of Ringer's solution known as Krebs-Ringer solution and much used in the manometric measurement of supravital oxygen consumption of tissues has been used in these experiments (9). When tissues have been immersed in this solution with a phosphate
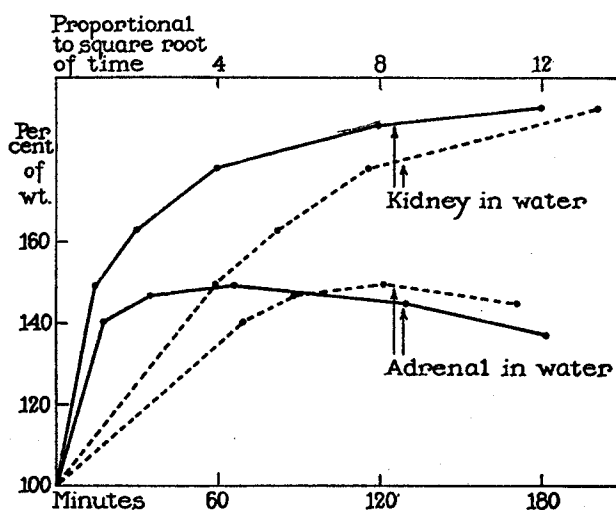

FIG. 8. (1) A composite graph from three experiments showing the per cent increase of weight of slices of kidney cortex immersed in distilled water. (2) A graph showing the per cent increase of weight of two adrenals of one animal under the same conditions. For explanation of the broken lines see legend of Fig. 1.

buffer of pH 7.4 in the presence of air, the changes that followed have not differed from those with a bicarbonate buffer in the presence of carbon dioxide and oxygen.

When immersed in blood serum of the rat, liver takes up water but the initial per cent of increase is less than that of liver in Ringer's solution (Fig. 7). This difference is doubtless explained, in part at least, by the osmotic pressure exerted by the proteins of the serum.

The Penetration of Water into the Cortex of the Kidney and into Adrenal Tissue

Slices of the cortex of the kidney about 0.5 mm. thick have been cut from the convex surface of the organ after removal of the capsule. Immersion of this tissue in distilled water is followed by increase of weight similar to that in the case of liver tissue (Fig. 8) and during the first half hour the relation when

plotted between intake of water and square root of the elapsed time is with slight deviation approximately linear. Solutions of sodium chloride cause movement of water which has not been proportional to their concentration. In two instances (Fig. 6, Experiments 11 and 12) the quantity of water lost in strong solutions has been less than that indicated by the plotted linear relation. Solutions isotonic for cortex of kidney determined by the procedure described for liver have varied from 0.22 to 0.31 molar (Table II), the average being 0.25 molar. Hence kidney immersed in normal salt solution or in Ringer's solution (Fig. 7) takes up water. Kidney cortex immersed in blood serum, like liver, takes up less water than in Ringer's solution, the difference being referable presumably to the protein content of the former.

In one instance in which kidney tissue has been immersed in solutions of potassium chloride (Fig. 6, Experiment 14), intake of water, as with sodium chloride, has not been proportional to the concentration of the solution but in the highest concentrations has been less than that represented by this relation. The concentration of potassium chloride isotonic for the same tissue is 0.42 molar.

When the two adrenals, carefully freed from surrounding fat, have been immersed in distilled water (Fig. 8), the changes in weight that followed have been similar to those of liver and of kidney under similar conditions, but the per cent intake of water has been less.

Penetration of Water into the Tissue of the Pancreas

Pancreas immersed in water undergoes changes conspicuously different from those of liver. The interstitial tissue surrounding the organ and separating its lobules becomes much swollen, translucent, and gelatinous in appearance as the result of water imbibition. The weight of the tissue increases rapidly (Fig. 9) and after 2 hours has reached a maximum about three times the original weight. Later the weight decreases considerably. During the 1st hour of water immersion the per cent increase of weight maintains a linear course when plotted in relation to the square root of the time of immersion (Fig. 9, and Fig. 2, Experiments 1, 2, and 3).

Histological examination shows that within 15 minutes after immersion in water acini in a broad zone below the surface of the tissue are swollen and the interstitial tissue is distended. The latter has begun to lose its fibrillation, collagen fibers being replaced by finely granular material. After 1 or 2 hours of water immersion there is widespread distention of interlobular septa so that the acini become much separated. Soon after immersion vacuole-like spaces appear at the base of gland cells, the cells become much swollen, and after 2 hours their cytoplasm is foam-like, though the zymogen granules are apparently little changed.

When pieces of pancreas are immersed in solutions of sodium chloride the initial rate of water intake is not, as with liver, proportional to the concentration, and when plotted in relation to the molar concentration (Fig. 10) it follows

a curved line which crosses the absicissa obliquely at a point representing approximate isotonicity. The concentration isotonic for pancreas is consider-
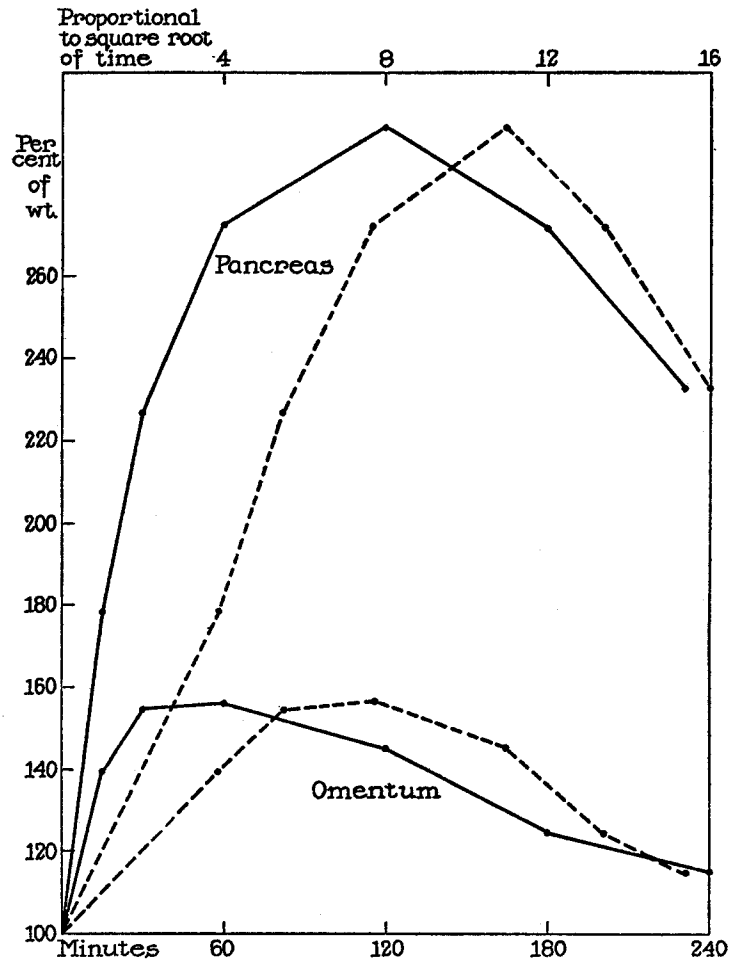

FIG. 9. (1) A composite graph from seven experiments showing the per cent increase of weight of small pieces of pancreas immersed in distilled water. Three of the experiments from which this graph is prepared are shown in Fig. 2. (2) A composite graph from five experiments showing the per cent increase of weight of pieces of omentum immersed in distilled water. For explanation of the broken lines see Fig. 1.

ably greater than that isotonic for liver or kidney and has varied from 0.44 to 0.5 molar; the average being 0.47 molar (Table III). In accord with this relation pancreas takes up water in 0.15 molar sodium chloride, in Ringer's solution, and in blood serum (Fig. 7). The quantity in per cent of the tissue weight has been less in blood serum than in Ringer's solution.
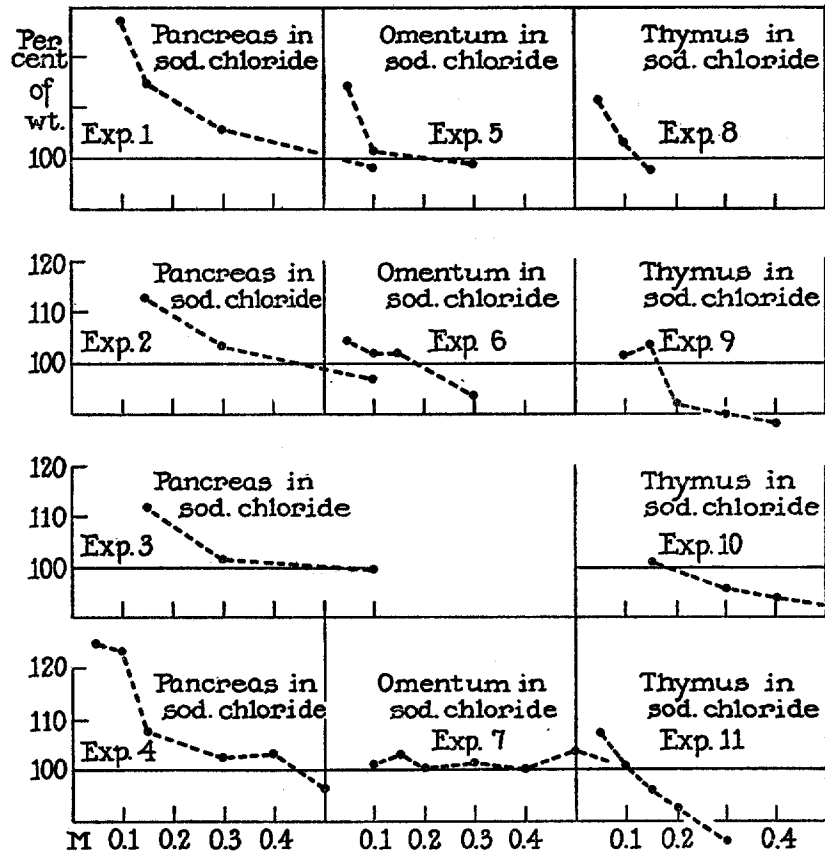
FIG. 10. Initial changes in the per cent of weight of small pieces of pancreas, omentum or thymus immersed in solutions of sodium chloride varying in concentration from 0.025 to 0.6 molar. In Experiments 2, 3, 4, 6, 9, and 11 this initial period of immersion is 10 minutes and in Experiments 1, 5, 7, 8, and 10, 15 minutes.

TABLE III

Solutions of Sodium Chloride Isotonic for Pancreas, Omentum, and Thymus

Experiment	Molar concentration isotonic for pancreas	Molar concentration isotonic for omentum	Molar concentration isotonic for thymus
1	0.5	0.2	
2	0.45		
3	0.49		
4	0.44	0.18	
5			0.13
6			0.17
7			0.17
8			0.11
9			0.21
Average..........	0.47	0.19	0.16

198

Entrance of Water into Omentum

Gross and histological examination of liver immersed in water shows that the scant interstitial tissue of the organ is not conspicuously changed whereas pancreas under similar conditions has a gelatinous appearance as the result of water imbibition. In the rat the characteristic cells of the pancreas are embedded in the mesentery which extends from the duodenum to the spleen and is continuous with the omentum. Changes in the omentum and splenic mesentery following immersion in various fluids have been compared with those which occur in pancreas under similar conditions (Fig. 9). The omentum contains much fat as adipose tissue (which cannot be removed) and its presence modifies figures representing the percentage intake of water. The pancreas on the contrary has been carefully separated from its surrounding fat and the tissue that has been used in these experiments contains very little.

The rate of penetration of water into omentum reaches a maximum after 1 hour and later the weight of the immersed tissue diminishes considerably. The intake of water during the first half hour is proportional to the square root of the elapsed time (Fig. 9). When omentum is immersed in solutions of sodium chloride initial movement of water (during the first 10 or 15 minutes, Fig. 10) has not varied in proportion to the concentration of the solution and has resembled that seen with the pancreas. The point at which the plotted line crosses the abscissa indicates isotonicity and has been in two instances 0.18 and 0.2 molar (Table III). In Experiment 7 of Fig. 10 the plotted line approaches the abscissa at a point representing 0.2 molar but does not cross it, being for this reason omitted from Table III. In higher concentrations water exchange has taken an anomalous course suggestive of the changes found more advanced in compact fibrous tissue such as that of the corium of the skin and the wall of the aorta (Fig. 13). Fig. 7 shows a delicate balance between the tissue of the omentum and solutions approximately isotonic with blood; namely, normal salt solution and Ringer's solution. In three experiments the omentum, immersed in the blood serum of the animals from which the tissue was obtained, has taken in water in small quantity once, remained unchanged in weight once, and lost water once.

Penetration of Water into Thymus

Penetration of water into thymus has been measured because its cellular elements are contained in loose areolar tissue which undergoes astonishing hydration. When immersed in water the tissue after about 30 minutes becomes greatly swollen and the interstitial tissue assumes a translucent gelatinous appearance. Microscopic examination shows that the interstitial tissue undergoes changes similar to those seen in the pancreas. In Fig. 11 measurements of the water intake of thymus of six animals have been included in one graph, and those of one of these animals have been plotted separately in order to show the

unusual rapidity and quantity of water intake in this instance.   The composite graph shows somewhat less rapid increase of weight, which nevertheless reaches a maximum within 1 hour and then diminishes considerably.   The initial in-
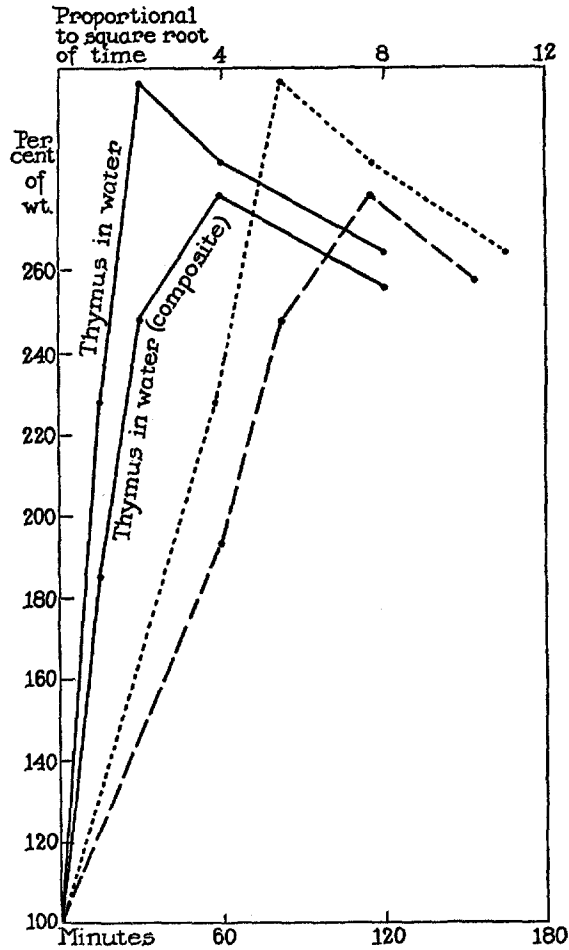
Fig. 11. (1) A composite graph from six experiments showing the per cent increase of weight of small pieces of thymus immersed in distilled water.   (2) A graph from one of these experiments in which the weight of the tissue increased to three times the original weight. For explanation of the broken lines see Fig. 1.

crease of weight, that is, during the first half hour, has been almost exactly proportional to the square root of the time of immersion.

Sodium chloride isotonic for thymus has varied from 0.11 to 0.21 molar, with an average of 0.16 molar (Fig. 11 and Table III).   In normal salt solution (Fig. 7) thymus has taken in water in very small quantity but in both Ringer's fluid

and blood serum thymus has lost weight. These relations are in accord with the low concentration of sodium chloride isotonic for thymus. In experiments on three animals the relation of Ringer's solution and blood serum has varied.

Penetration of Water into Skin

The skin of the white rat consists of epithelium, dense fibrous tissue of the corium, and loose subcutaneous fibrous tissue containing adipose tissue and striated muscle which over the trunk forms a thin layer firmly adherent to the corium. When fat and muscle are removed by careful dissection, the corium consisting of dense fibrous tissue remains and is covered on its outer surface by
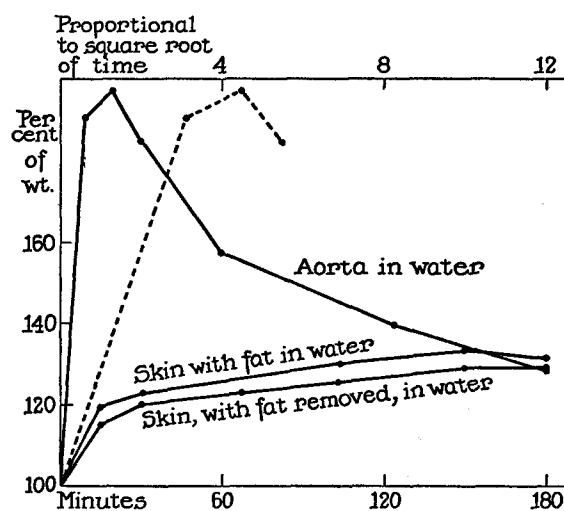

FIG. 12. (1) A graph showing the per cent increase of weight of tissue from the wall of the aorta carefully freed from fat and immersed in distilled water. (2) A graph showing the same changes in skin with some subcutaneous fat adherent and (3) in skin with fat carefully removed. For explanation of broken line see Fig. 1.

epithelium. In water the skin with or with no fat increases in weight about one-third and the quantity of water taken in is only slightly less when the fat and muscle have been carefully removed (Fig. 12). When the skin with some adherent fat (Fig. 13, Experiments 1 and 2) or with fat removed (Experiments 3, 4, and 5) is immersed in solutions of sodium chloride varying from 0.1 to 0.6 molar, in the gradations indicated in the figures, no solution is found in which the tissue neither takes in nor loses water. The loose connective tissue of the omentum or of the thymus is isotonic with solutions of concentration less than that of the sodium chloride of the blood serum but the dense fibrous tissue of the skin takes up water in concentrations up to 0.6 molar. In some of the experiments (Fig. 13, Experiments 3 and 4) the intake of water has been greater in solutions of sodium chloride than in water alone.
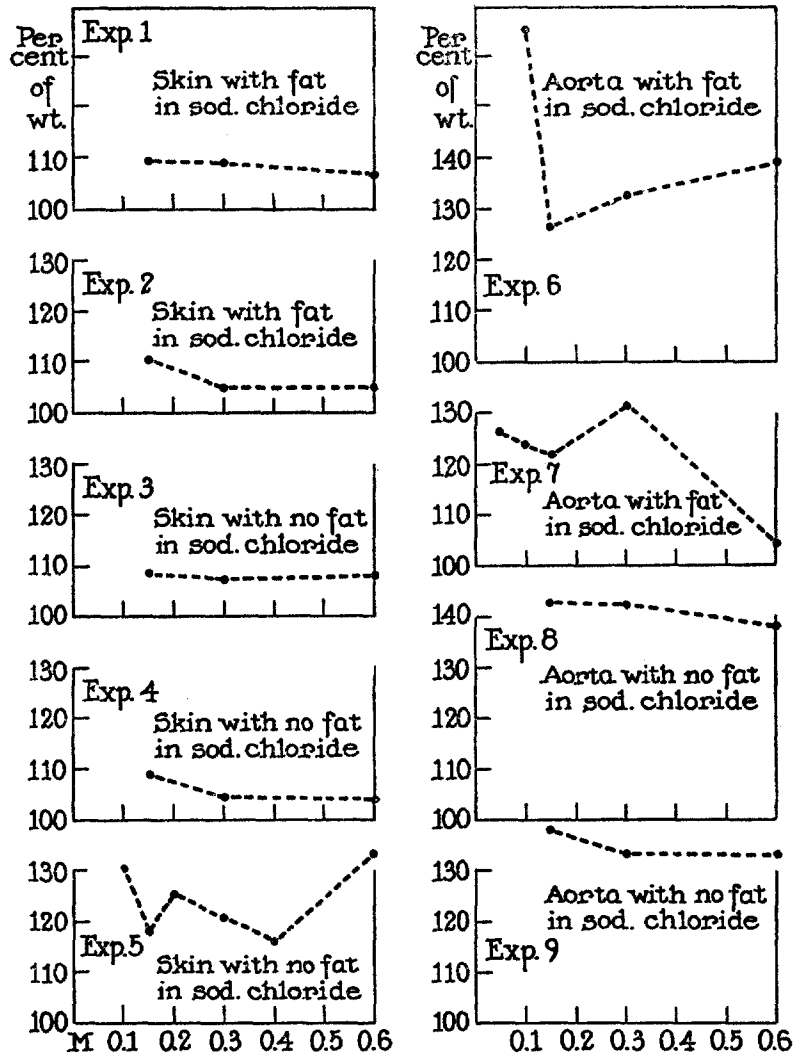
FIG. 13. Initial changes in the per cent of weight of small pieces of skin or of aorta with and with no fat attached immersed in solutions of sodium chloride varying in concentration from 0.1 to 0.6 molar. In Experiments 1, 2, 3, and 4 this initial period of immersion is 10 minutes and in Experiments 5, 6, 7, 8, and 9, 15 minutes.

In Ringer's solution and in blood serum the behavior of skin has been similarly anomalous. In contrast with the changes in omentum or thymus under similar conditions there has been conspicuous water intake but this has been considerably less in blood serum than in Ringer's solution.

Penetration of Water into Tissues of the Aorta

Changes following immersion of the wall of the aorta in different fluids have been studied because the vessel consists in large part of very dense fibrous tissue. They became more significant when it was learned that they resembled those of the dense connective tissue of the skin under similar conditions. The importance of removing all of the closely adherent fat tissue was not recognized at first and more characteristic changes were found after careful removal of it. Another difficulty is the small size of the pieces of the vessel wall that are available. When immersed in water the weight of the tissue increases very rapidly (Fig. 13) and may reach a maximum after 20 minutes. With subsequent rapid loss the original weight is approached. When pieces of aorta are immersed in concentrations of sodium chloride ranging from 0.15 to 0.6 molar, like skin, they take up water in all concentrations (Fig. 13). Adherent fat may modify the intake of water but when this has been removed the per cent increase of weight in different concentrations of the salt has been nearly uniform.

Changes of aorta immersed in Ringer's solution and in blood serum (Fig. 7) are similar to those of skin under similar conditions. Water enters into the tissue in both of these fluids but the quantity that enters in the first 15 minutes of immersion in blood serum has been only half of that in Ringer's solution.

RECAPITULATION AND DISCUSSION

Most of the changes that have been described here have been observed at the usual room temperature which has been from 23–25°C., but they differ little from those that occur at body temperature. When the immersion fluid is maintained at 25°C., at 37°C., or at 50°C., the initial intake of water measured by the per cent increase of weight during the first 15 minutes is almost the same (Fig. 3), but water enters more slowly at a temperature of 5°C. In normal salt solution the initial intake of water has been identical at 25°C. and at 37°C.

Very high and very low temperatures impair the permeability of the tissue when immersion is long continued. Changes at 23.5 to 25° and at 37° differ little (Figs. 3 and 4) but with much higher (50°) and much lower (5°) temperatures the maximum intake of water is less. Rapid-decrease of weight after 1 hour of immersion at 50° suggests that profound injury has occurred (Fig. 3) whereas tissue immersed at 5° during almost 4 hours maintains its increased weight. Further evidence that this low temperature under the conditions of the experiment tends to maintain almost unchanged the permeability of the tissue to water is obtained by keeping liver during 24 hours at 5°C. in an ice box followed by immersion at 23.5° or at 37° (Fig. 4). The changes after preliminary immersion at 5° differ very little from those that occur at room temperature immediately after removal from the body.

Conditions favorable to autolysis on the contrary profoundly alter permeability. If liver is kept during $1\frac{1}{2}$ or 3 hours at 37°C., with precautions to prevent evaporation, it takes up with subsequent immersion much less water than liver immersed immediately after removal from the body. If tissue subjected to the same conditions is put into solutions of sodium chloride, intake of water is no longer proportional to the concentration of the solution and in solutions isotonic for normal liver the altered liver takes up water (Fig. 6, Experiments 5, 6, and 7).

When liver or kidney cortex is immersed in water, the subsequent increase of weight during the early period of immersion is approximately proportional, as Adolph (4) found for voluntary muscle of the frog, to the square root of the time of immersion and follows a linear course when plotted against this factor (Figs. 1 and 8). The maintenance of this relation during the 1st half hour of immersion indicates that penetration of water into the tissue is proceeding as it would if it were brought about by osmosis. Later the intake of water is much below the quantity needed to maintain a linear relation. Actual disintegration of the cellular elements of the tissue recognizable by histological examination (9) may limit intake of water.

When other conditions remain unchanged osmotic pressure varies directly with the concentration of solutions (Boyle's law, 12) and the movement of water varies with it. When liver is immersed in solutions of sodium chloride of varying concentration the passage of water to or from the tissue bears when plotted a linear relation to the concentration of the solution (Fig. 6), whereas other tissues such as pancreas, thymus, and omentum (Fig. 10) deviate from it. The anomalous character of the changes found with certain tissues, such as corium of the skin and wall of the aorta (Fig. 13), is especially significant.

The water exchange that occurs in tissues in the initial period of immersion in water or in various solutions immediately after removal from the body pursues an orderly course in quantitative accord with changes explainable by osmosis. Autolysis (liver) or injury caused by the immersion fluid soon disturbs this relation. The movement of water in the initial period of immersion is evidently referable to the physical characteristics of the living tissue and is in this sense supravital.

The procedures that have been used in the present study are as follows: (1) Measurement of the quantity of water that enters the tissue when it is immersed in water alone; (2) measurement of the initial gain or loss of water when tissues are immersed in solutions of sodium chloride or other salt in various concentrations; (3) determination of the concentration of sodium chloride isotonic for each tissue; (4) comparison of the movement of water to or from tissues immersed in normal salt solution or Ringer's solution with that in blood serum.

The movement of water in tissues predominantly composed of parenchyma-

tous, that is, glandular cells differs when defined by the criteria just cited, from that in tissues chiefly formed by fibrous tissue including blood vessels and lymphatics. Though liver and kidney consist of both parenchymatous cells and interstitial tissue, the former are predominant and movement of water under the conditions of these experiments is evidently determined chiefly by them. When tissue from these organs is immersed in water, its weight increases until it reaches a maximum after about 2 hours and is approximately doubled (Figs. 1 and 8). When liver tissue is placed in solutions of sodium chloride entrance or loss of water varies proportionally with the concentration of the solution (Fig. 6). Movement of water in kidney tissue pursues a similar course less exactly and deviates from this relation when immersed in solutions more concentrated than 0.3 molar. Solutions of sodium chloride that are isotonic for liver or kidney have a molecular concentration that is approximately twice that of solutions isotonic for red blood corpuscles (Table II). These tissues take up water when immersed in blood serum but the water that enters is less than that which enters the same tissue in Ringer's solution (Fig. 7). As both blood serum and Ringer's solution are hypotonic for liver and kidney and have approximately the same electrolytic content, the osmotic pressure exerted by the serum proteins presumably diminishes the quantity of water that in their absence would enter the tissue.

Histological examination of liver or kidney immersed in water or in hypotonic solutions shows that the parenchymatous cells become swollen whereas changes in the interstitial tissue though doubtless present are inconspicuous. Thymus has a supporting framework of collagenous tissue with blood vessels and lymphatics and contains cells of lymphoid type in great number. Immersed in water this fibrous tissue becomes swollen and assumes an edematous appearance. Intake of water is much more rapid (Fig. 11) than with liver or kidney and reaches a maximum which is much greater.

Similar changes in the omentum are somewhat obscured by its large content of adipose tissue (Fig. 9). The hydration of both thymus (Fig. 11) and omentum proceeds in direct relation to the square root of the elapsed time. In solutions of sodium chloride increasing in concentration from 0.05 to 0.6 molar the initial intake of water fails to decrease in proportion to the increase of concentration (Fig. 10) so that when plotted it does not maintain the linear relation observed with liver under the same conditions. Solutions of sodium chloride with concentration, 0.15 molar, approximating that of the blood are isotonic for omentum and for thymus (Fig. 7) but isotonicity is at a slightly higher concentration for the former (Table III). In Ringer's solution omentum takes in water in small quantity and thymus loses water. Blood serum has caused a small loss of water from both omentum and thymus (Fig. 7) and in this respect they differ from the other tissues that have been examined.

Movement of water in tissue of pancreas following immersion in different fluids has characters like those of parenchymatous or secreting cells, represented by liver or kidney, on the one hand, and of interstitial tissue, represented by thymus and omentum, on the other. The interstitial tissue of the pancreas like that of the thymus becomes distended by fluid and edematous in appearance. This change corresponds with a per cent increase in the weight which is greater than that of thymus (Fig. 9). The subsequent changes are like those of liver and kidney; weight reaches a maximum after 2 hours and is later well sustained. The initial intake of water during 1 hour maintains an approximately linear relation to the square root of the elapsed time.

In solutions of sodium chloride the movement of water to and from the pancreas (Fig. 10) is not proportional to the concentration of the solution and when plotted is represented by a line which is curved with concavity upward because loss of water becomes scant as the concentration of solutions increases. Solutions of sodium chloride isotonic for pancreas have varied from 0.44 to 0.5 molar, this concentration being greater than that isotonic for liver or kidney and far in excess of that for thymus or omentum. In correspondence with this relation pancreas takes up water from Ringer's solution and in less quantity from blood serum (Fig. 7).

Dense fibrous tissue such as the corium of the skin or the wall of the aorta does not take up water rapidly and in large quantity like the loose fibrous tissue of the thymus or pancreas (Fig. 12). The intake of water by the skin or aortic wall in concentrated solutions of sodium chloride is anomalous; it does not diminish in linear relation to the concentration of the salt and no solution is isotonic for the tissue (Fig. 13). Sodium chloride in strong solution causes the fibrous tissue to take up and hold water. Occasionally under conditions that are not evident omentum may take up water in strong solutions of sodium chloride (Fig. 10, Experiment 7). In Ringer's solution and in blood serum, in conspicuous contrast with omentum and thymus, skin and aorta take up water in considerable quantity (Fig. 7).

The fluid within the spaces of the interstitial tissue is in contact with the cells of the part and is the medium through which substances, including water, enter and leave the cells. The osmotic pressure of the interstitial tissue of omentum and thymus is evidently nearly the same as that of the blood. Omentum is in approximate equilibrium with blood serum and thymus immersed in it loses a small quantity of water. A solution of sodium chloride isotonic with liver has a molar concentration more than twice that of the same salt in blood serum and liver immersed in blood serum takes in water. A solution of sodium chloride isotonic for kidney has a concentration slightly less than twice that of blood serum whereas a solution isotonic for pancreas has three times that of blood serum. Metabolic changes associated with the peculiar function of these cells may increase the molecular concentration within them.

CONCLUSIONS

During the initial period following immersion of parenchymatous cells of liver, kidney, or pancreas in various fluids immediately after their removal from the body water exchange is like that which occurs when water passes by osmosis through a semipermeable membrane; intake of water is proportional to the square root of the elapsed time and when liver tissue is immersed in solutions of sodium chloride movement of water is approximately proportional to the concentration of the solution.

Solutions of sodium chloride isotonic for parenchymatous cells of liver have twice the molar concentration of sodium chloride in the blood serum; for those of the kidney slightly less than twice and for those of the pancreas three times this concentration.

When interstitial tissue of thymus, omentum, or pancreas is immersed in water, it undergoes edema-like swelling caused by hydration of the colloids of the fibrous tissue; quantitative water exchange in an initial period accords with water movement by osmosis and is proportional to the square root of the elapsed time.

Solutions of sodium chloride isotonic for fibrous tissue of the omentum have slightly greater molar concentration than the sodium chloride in the blood serum and for that of the thymus approximately the same as that of blood serum.

Sodium chloride produces changes in fibrous tissue which increase with increasing concentration its power to hold water; the dense fibrous tissue of the corium of the skin and of the wall of the aorta takes up water in both weak an strong solutions of sodium chloride.

The initial movement of water induced in tissues in the period immediately following removal from the body is dependent upon forces which are active during life but soon impaired by injury to the tissues.

The molar concentration of the contents of secreting cells is greater than that of the blood serum and of the fluid surrounding them. These conditions are favorable to the passage of water from the tissue spaces to the cells.

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