CHANGES CAUSED BY INJURIOUS AGENTS IN THE
PERMEABILITY OF SURVIVING CELLS
OF LIVER AND OF KIDNEY

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The experiments which will be described were undertaken to determine whether the permeability of surviving tissues to water could be changed by extraneous agents. It was first necessary to find a procedure by which the osmotic activity of liver or kidney tissue could be maintained after their removal from the body.

When the specific gravity of small particles of liver or of kidney cortex was measured (1) by a method introduced by Philips and his associates (2) for determination of the specific gravity of blood plasma, this was found to diminish rapidly during the first 10 minutes of immersion in water, physiological salt solution, or Ringer's solution, but later an approximate equilibrium was established. Determination of the isotonicity of tissue slices by immersing them in solutions of sodium chloride of graded concentration has given more precise information concerning the initial water exchange which occurs after immersion. The changes found when tissues are immersed in solutions of sodium chloride, as in many of the preceding experiments, will be described and they will be compared with similar changes in Krebs-Ringer solution which under varied conditions has been the medium used in most of the present experiments.

Water Exchange of Tissues Immersed in Solutions of Sodium Chloride

In earlier publications (4, 5) the methods employed have been described in detail.

Slices of liver from white rats have been cut with a razor blade approximately 0.5 mm. thick and 1.5×0.5 cm. varying in weight from 50 to 100 mg., determined rapidly by means of a torsion balance. Tissues have usually been immersed during 10 minutes in solutions of sodium chloride of graded concentration from 0.1 to 0.5 molar. The plotted graph, which is linear in relation to concentration, marks on the base line the level of isotonicity of the tissue. The period of 10 minutes has been used because liver tissue immersed in a solution with which it is approximately isotonic maintains water equilibrium during a period of 15 or 20 minutes. The progress of the changes which follow immersion of slices of liver during longer periods in solutions of sodium chloride, hypotonic, approximately isotonic and hypertonic for them, are shown in Fig. 1.

In hypotonic solutions (0.15 molar) water intake has been almost continuous and reached a maximum after 50 minutes. In a solution approximately isotonic for the tissue (0.3 molar) there is little if any water exchange during about 15 minutes but later water intake has increased considerably and reached a maximum after 45 minutes. In a hypertonic solution (0.36 molar) loss of water has maintained an approximate level during 10 to 15 minutes; water intake at a comparatively low level has followed. In a stronger solution (0.4 molar) no

FIG. 1. Liver in solutions of sodium chloride 0.15 to 0.4 molar.

water intake may occur, during a period of almost 1 hour. Subsequent experiments will show that increasing intake of water is referable to injury causing increased permeability of the tissue.

Available evidence shows that the extracellular fluid of the tissue has the composition of an ultrafiltrate of plasma and with the same electrolyte components (Hastings (3)). It follows that pressure exerted by the tissue in excess of that of the blood and of the extravascular fluid is evidently referable to the cells of the part.

As earlier studies (4, 5) showed the level of isotonicity of liver tissue varied

within definable limits. In adult animals this level varied with unusual exceptions between 0.25 and 0.4 with a mean between 0.3 and 0.35 and an average of 0.34 molar. It is probable that water exchange of tissue slices may be modified by variations in their collagen content, which is dependent upon the size of portal spaces and of blood vessels included in the slice. Though the attempt has been made to remove blood from the slices by applying them on both sides to cotton gauze immediately after they are cut, their blood content doubtless varies within narrow limits.

FIG. 2. Liver in a solution of potassium chloride 0.3 molar.

FIG. 3. Liver in a solution of mannitol 0.66 molar.

The maintenance of water equilibrium by liver during 10 to 15 minutes still occurs when liver has been immersed in solutions of other substances that have been tested. This water equilibrium of liver in an isotonic solution of potassium chloride approximately 0.3 molar is seen in Fig. 2 and in an isotonic solution of mannitol, 0.6 molar, in Fig. 3.

Slices of the kidney have been prepared by section of the organ parallel to the capsular surface. Water exchange of kidney cortex immersed in solutions of sodium chloride undergoes a series of changes similar to those seen with liver (Fig. 4). In an hypotonic solution, 0.15 molar, water intake begins immediately and increases rapidly. With a solution approaching isotonicity for kidney cortex, 0.2 molar, water exchange remains at a low level during 10 minutes. With

a slightly hypertonic solution, 0.25 molar, withdrawal of water, as with liver, is followed by water intake. The significance of these changes will be discussed later.

Water Exchange of Tissues Immersed in Krebs-Ringer Solution with Oxygenation

The increasing water intake of liver and of kidney tissue during immersion suggests that the occurrence of injury may be diminished by a more favorable medium. With this aim tissues have been immersed in Ringer's solution as

FIG. 4. Kidney in solutions of sodium chloride 0.15 to 0.25 molar.

modified by Krebs for study of oxygen consumption (6) because its electrolytes are adjusted (0.154 osmolar) to those of the blood plasma. Oxygen, 95 per cent and carbon dioxide 5 per cent, have been allowed to pass through flasks assembled as in the Warburg manometric apparatus described by Umbreit and his associates (7). In some experiments the flasks have been agitated by rhythmic shaking. The weight of immersed liver slices has increased rapidly (as in Fig. 5) and reached a maximum after 15 to 20 minutes of immersion. It has then diminished so that after 60 minutes it does not greatly exceed the original weight. After shaking, the immersion fluid has contained fine particles and become turbid. The dry weight of tissue determined by heating to a constant weight at a temperature slightly above 100°C. has been found to decrease dur-

ing the period of immersion (Fig. 5), and diminution in weight of the immersed slices is referable to loss of tissue substance in greater part than to loss of water.

The dry weight of liver or kidney tissue determined by heating to a constant weight has given information about changes in the tissue during the progress of immersion under the conditions that have been described. The wet weight of the liver slices has been used as standard, and subsequent changes in wet or

FIG. 5. Liver in Krebs-Ringer solution with phosphate buffer and oxygen at 38°. Flasks containing liver slices have been shaken throughout the experiment. The dry weight of the tissues in per cent of the original wet weight is indicated by black columns.

dry weight with immersion are recorded as per cent of it. The dry weight of the liver tissue is slightly greater than 30 per cent of this weight. During the 1st hour of immersion dry weight diminishes from 3 to 5 per cent in terms of the original wet weight. In the next 2 hours there is usually little change but later the dry weight may diminish considerably, the loss being perhaps from 12 to 14 per cent. Diminution of the weight of tissue slices after 3 or 4 hours of immersion suggests that they have lost some of the water that has been taken in. It is, however, evident that loss of tissue substance in large part explains the loss of

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FIG. 6. Liver and kidney in Krebs-Ringer solution as in the experiment of Fig. 5 but with no shaking.

FIG. 7. Liver in Krebs-Ringer solution with molar concentration doubled by addition of sodium chloride.

weight, the quantity of water remaining being little diminished in proportion to the loss of tissue substance (Fig. 6).

The experiments show that liver tissue shortly after its removal from the living body when immersed in Krebs-Ringer solution approximately isotonic with blood serum, buffered with phosphate or bicarbonate, maintained at 38° and supplied with oxygen takes up water rapidly. When the molar concentration of the Krebs-Ringer solution has been increased twofold by addition of sodium chloride approximate water equilibrium may be maintained during 3 hours followed by rapid intake of water (as in the second graph of Fig. 7).

The experiments in which liver slices were immersed in approximately isotonic solutions of sodium chloride with no buffer, no stream of oxygen, and at

FIG. 8. Water exchange of liver in oxygenated Krebs-Ringer solution at 38° compared with that in sodium chloride 0.15 molar at 20° with no oxygen.

20°C (Fig. 1) show that the period of water equilibrium is about 15 minutes, whereas that with Ringer's solution, buffered and with oxygen, may be 2 or more hours (Fig. 7). A closer approach to physiological conditions delays the increased intake of water.

In previous experiments (4, 5) solutions of sodium chloride of varying concentration have been used to measure the isotonicity of tissues immersed during 10 minutes in them. Water intake of liver, kidney, and pancreas in physiological salt solution at 20°C . during 10 minutes has differed little from that in Krebs-Ringer solution (4). Comparison has been made (Fig. 8) between the water intake of liver in Krebs-Ringer solution with oxygenation at 38°C . and that in a solution of sodium chloride 0.15 molar with no oxygenation at 20°C . (Fig. 8). As with the earlier observations increase in weight after 10 minutes has been almost the same in salt solution and in Krebs-Ringer solution of nearly the same molar concentration. After longer immersion intake of water has been far

greater in sodium chloride alone. The relation of this greater intake to injury will be discussed later.

The Effect of Injurious Agents on the Movement of Water in Tissue Removed from the Body

The permeability of living cells of lower animals and of plants has been the subject of an extensive literature. In experiments on cells of the sea plant *Laminaria* Osterhout (8) defined by means of electric conductivity changes in permeability to water and salts that accompany the injury which follows transfer from sea water to a solution of sodium chloride. Lucké and McCutcheon (9) described changes in the permeability of sea urchin eggs following a variety of injuries. Knowledge of the subject was reviewed by Lucké and McCutcheon, by Osterhout, by Davson and Danielli (10), and by others.

Water exchange of mammalian tissues immersed in oxygenated buffered Krebs-Ringer solution during several hours has offered opportunity to determine whether increased permeability is caused by agents that are known to injure these tissues during life. Increased temperature, anoxia, simple chemical compounds, as ethyl alcohol and chloroform, bacteria and bacterial products will be considered. These agents have been selected because they are representative of the most frequent causes of injury.

Water exchange of tissue slices immersed at 38° in Krebs-Ringer solution buffered with sodium bicarbonate and gassed with oxygen, 95 per cent, and carbon dioxide, 5 per cent, has been regarded as a standard for comparison because it occurs under conditions that approximate those present during life. In each experiment, unless otherwise stated, tissue slices have been from one animal. Consistent results have not been obtained in periods longer than 3 hours.

Temperature.—Temperature has caused wider variation in permeability than any other agent that has been studied. In the first attempts to determine the effects of temperature on water exchange liver slices have been immersed continuously in oxygenated Krebs-Ringer solution maintained at a fixed temperature. During 1 hour water intake at 38° C. has been 16 per cent of the original weight (Fig. 9) whereas at 48° it has been 30 per cent and at 58°, 45 per cent. Similar increase of water intake has been found when liver slices have been temporarily brought to the chosen temperatures and then transferred to Krebs-Ringer solution at 38°. Flasks containing immersed liver slices have been left during 10 minutes with no gassing in water baths at 38, 42, 46, and 50°. They have then been transferred to a water bath at 38° and gassed by the usual procedure. Water intake after 1 hour at 38° has been 16 per cent of the original weight; after initial heating at 46°, 23 per cent and at 50°, 40 per cent. In the latter instance water intake has reached 55 per cent after 3 hours.

It has seemed desirable to learn how much water enters the tissue during the short period of heating. Liver slices have been weighed, exposed to different

temperatures, and again quickly weighed before their transfer to oxygenated Krebs-Ringer solution at 38°. In the experiments shown in Table I and Fig. 10 liver slices have been exposed for 10 minutes to temperatures of 38, 52, 55, and

FIG. 9. Water exchange of liver tissue maintained at 38, 48, and 58°.

FIG. 10. Water exchange of liver tissue heated during 10 minutes to 38, 52, 55, 59, and later kept at 38°.

59°. During the period of heating water enters the tissue rapidly in least quantity at 38° and in greatest, at 55°. In the subsequent 3 hours at 38° tissue previously heated to 52 has become much more permeable to water than that which had been at 38° throughout. Tissue heated to 55 and 59 has lost water continuously. The high level of water intake shown in Fig. 9 after 1 hour is presumably referable to the period of heating.

In the experiment shown in Table II and Fig. 11 slices have been heated as before but during a shorter period at 38, 50, 60, and 75°. During this period of heating water intake has not varied greatly at different temperatures. When for each slice the weight after heating is subtracted from the final reading the ability of the heated slices to take up water is measured (Table II, Fig. 11).

The experiments show that maximum water intake occurs when tissues have been heated at 50 to 52, the maximum per cent increase being about twice that at 38°. Ability of the tissue to take up water is lost at 55° and at this temperature slices have the appearance of coagulated tissue. Liver heated to temperatures above 38° has become more permeable to water than liver kept at this temperature under the same conditions.

TABLE I
Water Exchange of Liver Slices in Oxygenated Krebs-Ringer Solution at 38° after Heating for 10 Minutes at Different Temperatures

Temperatures of initial heating	Per cent of weight after heating	Per cent of weight 2 hrs. after heating	Per cent of weight 3 hrs. after heating	Per cent change of weight 3 hrs. after heating
°C.				
38	108.3 107.6	109.9	113.0	7.35
52	113.4 112.2	133.9	132.4	10.2
55	127.9 124.1	121.8	116.	-8.1
59	123.4 121.3	116.6	113.0	-8.3

Anoxia.—Anoxia of tissue slices in Krebs-Ringer solution buffered with sodium bicarbonate at 38° has been produced by gassing with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. Weight of liver slices (Fig. 12) increases more rapidly than with oxygenation, reaches a higher level after 1 hour and continues to increase at a time when with oxygenation a maximum has been reached. With the molar concentration of the Krebs-Ringer solution increased twofold by addition of sodium chloride, that is, to about 0.3 molar, water intake proceeds less rapidly (Fig. 12) but is considerably greater than that in the same medium gassed with oxygen. Anoxia has increased the permeability of liver tissue to water when tested in Krebs-Ringer solution, hypotonic, or isotonic for the tissue.

Ethyl Alcohol.—Ethyl alcohol in the proportion of 1/100, 1/200, and 1/1000, 1/5000 added to oxygenated Krebs-Ringer solution at 38° has increased the

FIG. 11. Water exchange of liver tissue heated during 5 minutes to 38, 50, 60, 75, and later kept at 38°.

TABLE II

Water Exchange of Liver Slices in Oxygenated Krebs-Ringer Solution at 38° after Heating for 5 Minutes at Different Temperatures

Temperature of initial heating	Per cent of weight after heating	Per cent increase of weight 1 hr. after heating	Per cent increase of weight 2 hrs. after heating	Per cent increase of weight 3 hrs. after heating	Per cent change of weight 4 hrs. after heating
°C.					
38	111.3	20.4	24.0	10.1	-4.0
	106.7				
	108.2				
	106.2				
50	110.8	18.0	49.3	37.4	30.0
	109.2				
	108.3				
	111.2				
60	116.6	-0.2	-2.9	-7.3	-5.4
	114.8				
	119.7				
	120.7				
75	111.8	-6.3	-4.2	-6.3	-7.0
	108.4				
	108.3				
	112.0				

permeability of liver tissue to water (Table III, Fig. 13). In each experiment with tissue from one animal water intake in the presence and in the absence of alcohol has been compared. Increased water intake with alcohol may be evident after 1 or 2 hours of immersion. Ethyl alcohol, 1/100 and 1/200, likewise increases the permeability of liver tissue when immersed in Krebs-Ringer solution

FIG. 12. Water exchange of liver tissue in Krebs-Ringer solution with oxygenation and with anoxia, and in Krebs-Ringer solution with double the usual molar concentration with oxygenation and with anoxia.

which has been made approximately isotonic with liver by doubling its concentration of sodium chloride (Table III).

Chloroform.—

Buffered Krebs-Ringer solution has been shaken with chloroform in the proportion of 0.5 cc. in 100 cc. and decanted from the undissolved chloroform. Oxygen has been passed through a series of nine flasks of which the first 4 have contained Krebs-Ringer solution for the immersion of control liver slices. The fifth flask in the series has contained 25 cc. of Krebs-Ringer solution with 0.5 cc. of chloroform. In the last 4 flasks in the direction of oxygen flow slices of liver have been immersed in the Krebs-Ringer solution which had been shaken with chloroform. An excess of chloroform has remained in the fifth flask throughout the experiment, and this chloroform presumably supplies to the distal flasks chloroform lost by evaporation. It is noteworthy that the chloroform used as a chemical reagent contains 0.75 per cent of ethyl

alcohol added to prevent oxidation. The dilution of this alcohol in the flasks containing chloroform is less than 1/25,000.

Water intake in the presence of chloroform has been greater than that of control liver slices throughout the experiment (Fig. 14). Water intake by kidney has exceeded that of the control after 2 hours and has reached a very high level after 3 hours.

TABLE III
Water Exchange of Liver Slices in Oxygenated Solution at 38° Containing Ethyl Alcohol

Experiment	Dilution of ethyl alcohol	Per cent of weight after 1 hr.	Per cent of weight after 2 hrs.	Per cent of weight after 3 hrs.
In Krebs-Ringer solution:—				
1	1/100	120.8	126.8	
	1/200	115.2	128.8	
	1/1000	123.2	126.3	
	None	116.3	115.9	
2	1/1000	122.9	134.2	130.6
	None	123.6	132.1	119.7
3	1/5000	120.2	126.6	126.8
	1/20,000	120.9	123.6	117.7
	None	120.9	120.3	117.8
In Krebs-Ringer Solution with two-fold molar concentration:—				
4	1/200	109.2	113.8	
	None	102.1	101.1	
5	1/100	114.8	120.1	
	None	108.9	113.3	

Living Colon Bacilli.—Increased permeability of tissues in the presence of simple chemical compounds along with well known pathological lesions have suggested experiments directed to determine whether bacteria and bacterial products cause similar change.

To test the effect of colon bacillus on permeability of liver slices to water under the conditions that have been described a bouillon culture of *Escherichia coli* 5 days old has been added to Krebs-Ringer solution in proportions of 1/100 to 1/100,000 and water exchange in its presence compared with that in Krebs-Ringer solution with corresponding quantities of the culture medium added to it (Table IV and Fig. 15).

A culture of *E. coli* diluted 1/1000 and 1/10,000 has increased the permeability of liver tissue (Table IV). Water intake with dilution of the culture 1/1000 has been less than that with the higher dilution. Inhibition of water intake by

FIG. 13. Water exchange of liver in Krebs-Ringer solution with ethyl-alcohol in dilutions of 1/100, 1/200 and 1/1000 and in control solution; in Krebs-Ringer solution with double molar concentration.

FIG. 14. Water exchange of liver in Krebs-Ringer solution with chloroform and in control solution; water exchange of kidney tissue under the same conditions.

still stronger solution, 1/100, is indicated. No increase has occurred when the culture is diluted 1/100,000.

Increase of the permeability of liver slices caused by *E. coli* has followed their immersion in Krebs-Ringer solution made approximately isotonic with the

tissue by doubling the usual sodium chloride content of the solution (Table IV). Two experiments of limited scope give somewhat uncertain evidence that filtrate of a 5 day old culture of *E. coli* has increased the permeability of liver tissue (Table V). In Krebs-Ringer solution the water intake has been greater

TABLE IV
Water Exchange of Liver Slices in Solutions Containing Culture of E. coli Compared with That in Control Solutions with Corresponding Quantities of the Culture Medium

Experiment	Dilution of culture	After 60 min.	After 120 min.	After 180 min.
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
In Krebs-Ringer solution:—				
1. <i>E. coli</i> culture	1/10,000	121.0	117.7	129.8
Control (with bouillon 1/1000)	None	121.2	118.8	113.9
2. <i>E. coli</i> culture	1/10,000		129.1	129.3
“ “ “	1/1000	123.1	122.6	124.7
Control (with bouillon 1/1000)	None	114.4	119.8	115.6
3. <i>E. coli</i> culture	1/100,000	120.0	114.5	100.
“ “ “	1/100	114.05	119.4	106
Control (with bouillon 1/100)	None	122.7	119.9	122.3
In Krebs-Ringer solution with molar concentration $\times 2$:—				
4. <i>E. coli</i> culture	1/10,000	115.1		
Control (with bouillon 1/10,000)	None	103.1		

TABLE V
Water Exchange of Liver Slices in Solutions Containing Filtrate of a Culture of E. coli

Experiment	Dilution of filtrate	After 30 min.	After 60 min.	After 120 min.
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
In Krebs-Ringer solution:—				
1. <i>E. coli</i> filtrate	1/10,000	105.0	110.1	
Control (with bouillon 1/10,000)	None	104.0	104.0	
In Krebs-Ringer solution with twofold molar concentration:—				
2. <i>E. coli</i> filtrate	1/10,000	98.7	98.0	102.4
Control (with bouillon 1/10,000)	None	99.0	97.8	100.7

after 1 hour in the presence of the filtrate but longer observations have not been made. In Krebs-Ringer solution approximately isotonic with the tissue the difference between filtrate and control has been slight.

Somatic Antigen of the Flexner Group of Dysentery Bacilli.—Through the kindness of Dr. Walther Goebel I have obtained the somatic antigen which he has

prepared from dysentery bacilli (*Shigella paradysenteriae*, Flexner) and have tested its effect upon the permeability of liver and kidney tissue to water. The antigen, extracted from the bodies of the microorganism by pyridine, is highly toxic for mice and rabbits (Goebel, Binkley, and Perlman, 11).

FIG. 15. Water exchange of liver in Krebs-Ringer solution containing a bouillon culture of *E. coli* in dilutions of 1/1000 and 1/10,000 and in the same solution with bouillon in dilution of 1/1000.

TABLE VI
Water Exchange of Liver Slices Immersed in Krebs-Ringer Solution Containing Somatic Antigen of *Paradysentery Bacilli*

Experiment	Dilution of somatic antigen	After 60 min.	After 120 min.	After 180 min.
1.	1/20,000	130.3	133.1	135.2
	None	128.7	132.7	128.8
2.	1/100,000	139.7	129.6	135.7
	None	125.3	131.4	125.8
3.	1/100,000	124.4	132.0	132.7
	None	123.9	131.1	122.5
4.	1/100,000		121.6	121.9
	1/1,000,000	114.1	118.1	120.8
	None	114.6	120.3	118.0
5.	1/1,000	125.0	132.3	134.2
	1/100,000	125.1	129.3	127.7
	None	126.3	126.0	120.7

Somatic antigen of *Sh. paradysenteriae* has been dissolved in Krebs-Ringer solution in the proportion of 1 mg. in 1 cc., that is, 1/1000 and further diluted as indicated in Table VI. In each experiment water intake of liver slices in the presence of the antigen has been compared with that of liver slices from the same animal with no antigen (Table VI and Fig. 16).

Somatic antigen of *Sh. paradysenteriae* in dilutions of 1/10,000, 1/2000, and 1/100,000 has increased the water intake of liver tissue. In dilution of 1/1,000,000 it has not been greater than that of control liver slices. Increased permeability is in most instances not evident or is inconspicuous until the toxic material has been in contact with the liver tissue during 2 hours, and is considerably greater after 3 hours.

FIG. 16. Water exchange of liver in Krebs-Ringer solution containing somatic antigen of *Shigella paradysenteriae* diluted 1/1000 and 1/100,000 compared with that of the control. Water exchange in the same solution containing the antigen diluted 1/20,000.

Killed Typhoid Bacilli.—

Typhoid bacilli have been removed from the surface of agar slants, suspended as an opaque mixture in physiological salt solution and killed by heat. The suspension has been diluted with Krebs-Ringer solution with concentration adjusted by addition of sodium chloride to twice the usual molar strength. Liver slices have been immersed in dilutions of 1/1000, 1/10,000 and 1/100,000 and their water exchange compared with that of liver tissue in the same Krebs-Ringer solution alone (Table VII).

Water intake in the presence of the suspension in dilutions of 1/1000, 1/10,000, and 1/100,000 has been greater than that of controls with none. In the first experiment (Table VII) it has exceeded that of the control after 60

and after 120 minutes and in the second there has been a consistent difference only after 120 minutes.

TABLE VII
*Water Exchange of Liver Slices Immersed in Oxygenated Krebs-Ringer Solution with
Molar Concentration $\times 2$ Containing Killed Typhoid Bacilli*

Experiment	Suspension of killed typhoid bacilli	After 60 min.	After 120 min.
		<i>Per cent</i>	<i>Per cent</i>
1.	1/1,000	113.7	123.4
	1/10,000	121.8	122.5
	None	105.8	120.2
2.	1/1,000	117.0	123.7
	1/10,000	114.5	120.0
	1/100,000	112.9	123.4
	None	115.6	116.9

TABLE VIII
*Water Exchange of Liver Slices in Oxygenated Krebs-Ringer Solution Containing
Diphtheria Toxin*

Experiment	Diphtheria toxin	After 30 min.	After 60 min.	After 120 min.
1.	1/1000	104.1	104.1	
	Control	101.7	102.65	
2.	1/1000	110.25	111.5	119.8
	Control	109.3	111.35	118.4
3.	1/1000	101.5	101.5	108.95
	Control	101.25	102.35	105.75
4.	1/200	105.2	111.35	124.95
	Control	107.1	110.45	120.4

Diphtheria Toxin.—

Diphtheria toxin has been tested by the procedure that has been described (Table VIII). In order to avoid deterioration of the toxin one per cent of gelatin has been added to the Krebs-Ringer solution with which it has been diluted and the same quantity has been added to the solution used for immersion of control liver slices.

Water intake recorded in Table VIII has been little greater in the presence of diphtheria toxin than in controls but after 2 hours has been consistently somewhat greater.

Urea and Certain Other Agents.—Liver tissue has shown slightly increased

permeability to water when urea has been added to Krebs-Ringer solution under the conditions of these experiments (Table IX). In Krebs-Ringer solution with the usual molar concentration addition of urea in concentrations 0.01, 0.05, and 0.1 molar has in general increased the water intake of liver slices but in Experiment 4 of Table IX increase of water intake has been evident only after 120

TABLE IX
Water Exchange of Liver Slices in Solutions Containing Urea

Experiment	Molar concentration of urea	After 60 min.	After 90 min.	After 120 min.	After 135 min.	After 180 min.
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>
In Krebs-Ringer solution:—						
1.	0.05	119.3		117.8		
	None	117.1		115.1		
2.	0.05	124.0		130.8		
	None	121.1		129.7		
3.	0.1		122.1		122.8	
	0.05		122.7		127.4	
	0.01		120.7		123.6	
	None		118.3		117.3	
4.	0.4	125.8		132.0		138.3
	0.05	130.8		145.5		126.0
	None	132.2		137.6		136.8
In Krebs-Ringer solution with molar concentration $\times 2$:—						
5.	0.05	106.5		117.9		
	None	110.0		114.7		
6.	0.05	107.4		115.9		123.1
	None	107.3		115.1		122.6
7.	0.1	106.9		118.2		
	None	114.4		120.7		

minutes. With urea in high concentration, 0.4 molar, water increase above the control has been evident only after 180 minutes. In solutions of Krebs-Ringer solution with molar concentration increased twofold by addition of sodium chloride (Table IX) and thus approximately isotonic with liver tissue water intake has been slightly increased when urea in the proportion of 0.05 molar has been added. No increase has occurred when the concentration of the added urea has been 0.1 molar, the molar concentration of the solution being then equal to or exceeding that isotonic with liver. When white rats have received urea

subcutaneously in excess of their ability to eliminate it by excretion isotonicity of liver tissue may reach 0.4 to 0.65 molar (12). The experiments indicate that under some conditions urea increases the permeability of liver tissue.

Histamine dihydrochloride has had no demonstrable effect upon the water exchange of immersed slices of liver. This result has been obtained in two experiments with dilution of 1/1000; in two, of 1/10,000; in one, of 1/100,000 and in one, of 1/1,000,000.

In the few tests that have been made glucose has not changed the permeability of liver tissues.

RECAPITULATION AND DISCUSSION

Experiments have been undertaken to determine what conditions are favorable for the measurement and maintenance of the osmotic activity of liver and of kidney tissue after their removal from the body. Changes in the permeability of these tissues caused by various injurious agents have been studied. Increase of osmotic pressure of liver or of kidney tissue above that of the blood plasma and extracellular fluid is referable to the cells of the part. Tissues of liver or of kidney immersed at 38°C. in Ringer's solution, as modified by Krebs's, with appropriate buffer and supplied with oxygen, undergo during at least 3 hours an orderly series of changes. But even under these conditions slowly progressive injury disturbs water movement. This inference is supported by study of the changes in the permeability of the tissue induced by a wide variety of agents.

Fluctuations in the weight of immersed tissue during the first few minutes of immersion (Fig. 2, 4, 7, 12) indicate that there is a rapid interchange of fluid between the extracellular fluid of the tissue and the immersing medium. This suggestion is supported by the slight diminution of the dry weight of the tissue which occurs during the 1st hour of immersion. Fibrinogen evidently escapes, in part, at least, from blood vessels, and after 2 hours may form a small coagulum free in the fluid. A greater diminution of dry weight may occur in the period after 3 hours of immersion (Fig. 5, 6) and is presumably referable to actual disintegration of tissue. The total loss of weight of the slice at this time is in considerable part referable to loss of tissue substance.

When water exchange of liver slices immersed in physiological salt solution is compared with that in Krebs-Ringer solution under the conditions of these experiments (Fig. 8) it is evident that greatly increased water intake has followed contact with an unfavorable environment and is doubtless referable to ensuing injury. Greater change in permeability to water has followed exposure of the tissue to temperatures above 38°. Maximum increase of permeability occurs at 50 to 52° and the experiments show that water may enter the tissue with great rapidity during the short period, 5 or 10 minutes, in which the

elevated temperature is acquired. Exposure to a temperature of 55° causes heat coagulation, and the tissue then ceases to take up water.

With anoxia caused by replacing oxygen with nitrogen liver tissue has become more permeable to water. Water intake of brain cortex, kidney cortex, liver, lung, and spleen was found by Stern, Eggleston, and Krebs (13) considerably greater under anaerobic than under aerobic conditions and they regarded respiration as an important factor in controlling fluid intake of tissues. Ethyl alcohol added to the immersion fluid in dilutions from 1/100 to 1/5000 has increased the permeability of liver tissue but no increase has occurred in a dilution of 1/20000. It is noteworthy that the quantity of alcohol in the blood of intoxicated persons may be 0.5 per cent (Jetter 14). Chloroform with ethyl alcohol added to prevent oxidation has increased the permeability of liver tissue when the proportion of alcohol in the immersion medium has been less than 1/25,000.

Cloudy swelling associated with many infectious processes suggest the possibility that infectious agents may cause swelling of cells *in vitro*. Bacterial cultures and products of bacteria in high dilutions have increased the permeability of liver and of kidney tissue. With cultures of colon bacilli in dilutions of 1/1000 and 1/10,000 water intake of liver slices has been greater than that in control solutions containing an equal quantity of the culture medium. Killed typhoid bacilli grown on agar and suspended in Krebs-Ringer solution has had similar effect. Diphtheria toxin seems to have increased slightly the permeability of liver tissue. Somatic antigen with high toxicity, extracted from the bodies of dysentery bacillus of Flexner type has increased the permeability of liver tissue in dilutions of 1/100,000. Increased permeability has not been evident until the agent has been in contact with the immersed tissue during 1 hour.

Most of the experiments that have been cited have been made by immersing tissues in Krebs-Ringer solution with concentration adjusted to 0.154 molar which is hypotonic for liver and for kidney tissue. When the concentration of Krebs-Ringer fluid has been approximately doubled by addition of sodium chloride it is approximately isotonic with liver tissue (4) and the experiments show that anoxia (Fig. 12) and various agents increase the permeability of liver tissue (Fig. 13, Table III, IV, V) but the increased water intake is then much less than that in the Krebs-Ringer solution of the usual concentration. In the solution of twofold concentration notwithstanding its approximate isotonicity with the tissue some of the injurious agent evidently enters the tissue.

In the present experiments a few of the vast number of agents that injure mammalian tissues have been tested in order to determine whether they alter the permeability of cells. It is obviously desirable that this list be much extended. All the agents that cause this change may not be concerned with

pathological processes. Substances ingested with food or formed as products of metabolism, notably urea, may have this effect.

SUMMARY AND CONCLUSIONS

Immersion of tissue slices of liver or of kidney in buffered Krebs-Ringer solution at 38°, with oxygenation, gives opportunity for the study of water exchange during 3 hours following removal of the tissue from the body.

Under these conditions a wide variety of physical and chemical agents cause changes in the permeability of the tissue to water, which are referable to the cells of the part; these agents include increased temperature, anoxia, ethyl alcohol, chloroform, bacteria, and bacterial products.

With heating of liver tissue increased permeability reaches a maximum at 50 to 52°C. and is lost at 55° when heat coagulation has occurred.

Permeability of liver cells has increased in the presence of cultures of colon bacilli, of culture filtrate, of killed typhoid bacilli and apparently of diphtheria toxin. Somatic antigen from paradysentery bacilli has caused increased permeability of liver cells.

The experiments indicate that a substance formed by normal metabolism, namely urea, may, under some conditions, increase the permeability of liver cells.

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