THE EFFECT OF INJURY BY TOXIC AGENTS UPON OSMOTIC PRESSURE MAINTAINED BY CELLS OF LIVER AND OF KIDNEY*

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In a preceding publication (1) the significance of osmotic changes in the particulate bodies, in large part mitochondria, which occupy a considerable portion of the cytoplasm of cells of liver and of kidney was discussed and experiments to determine the relation of these changes to hydropic swelling of the same bodies caused by injurious agents, namely, chloroform and butter yellow (dimethylaminoazobenzene), were described. In the same and a later publication (2) swelling of mitochondria and removal of reacting mitochondrial material by hypotonic and even by isotonic solutions, *e.g.* Ringer's solution, were described and experiments defining the relation of these histological changes to the specific gravity of the tissue were recorded.

The environment of tissue cells is the fluid in the tissue spaces and this in turn varies with changes in the circulating medium of the body; that is, the blood plasma. It has been established long ago that the osmotic pressure of the blood is maintained at levels which vary within very narrow limits among different mammalian species and within even smaller limits in the same species under changing conditions. The integrity of the red blood corpuscles is dependent upon the maintenance of osmotic homeostasis (3) and they have an osmotic pressure equivalent to that of the plasma, which approximates a 0.15 molar solution of sodium chloride. It has been tacitly assumed that the same holds true for the cells of all fixed tissues. But study of the movement of water in tissues removed from the body and immersed in various fluids has lately shown (4) that solutions of sodium chloride isotonic for parenchymatous cells of liver have twice the molar concentration of sodium chloride present in the blood serum and isotonic with red blood corpuscles. The metabolism of these cells presumably maintains within their cytoplasm a molar concentration which is considerably greater than that of blood serum and of the fluid surrounding them. The following experiments are described because they show that certain injurious agents administered to animals disturb only temporarily the osmotic equilibrium of liver and kidney cells and lower their level of isotonicity so that it approximates for a time that of blood serum and of erythrocytes.

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Methods

The initial change of weight of tissue slices immersed in solutions of sodium chloride of various concentrations has been used to determine the concentration that is in osmotic equilibrium with the tissue. Weight of the slices has been measured by a torsion balance and the procedure followed has been that described in an earlier publication (4). The movement of water to or from normal liver tissue during the first 10 minutes of immersion is proportional to the concentration of the immersion fluid and has a linear course when plotted in this relation. The point at which the line crosses the abscissa (Fig. 1) determines the concentration of sodium chloride which has osmotic pressure equal to that of the tissue under examination (Table I). The use of osmotically equivalent concentrations of sodium chloride as a measure of osmotic pressure has been discussed by Lipson and Visscher (3).

Injury by Chloroform

Chloroform selects for injury the hepatic cells about the central veins of the liver and when administered in sufficient quantity by inhalation, by introduction into the stomach, or by subcutaneous injection causes necrosis which with increasing severity may destroy all parenchymatous cells save those immediately about the portal spaces.

The necrotic cells lose their nuclei and their cytoplasm has a homogeneous appearance when stained with acid dyes. These changes first appear after an interval of 6 to 10 hours (5) and are usually assumed to be the result of autolysis. At the periphery of the nectrotic foci surrounding the central veins or immediately about these veins, when the poison in small quantity has failed to cause necrosis, liver cells are swollen, have sharply defined rounded outlines, and contain mitochondria of which a considerable part have lost their characteristic reactions to stains and have become enlarged and vesicular (1). These swollen bodies may give the cytoplasm a vacuolated or even foam-like appearance. Though stains for fat show that these spaces contain none, fat droplets in abundance are usually found in liver cells in a broad zone about the swollen cells.

The progress of repair which follows when chloroform poisoning does not cause death of the animal was described by Whipple and Sperry (5). Granulocytes and mononuclear phagocytes in large number enter the areas of necrosis and after 3 or 4 days the acidophile cytoplasm of necrotic cells has disappeared. Mitotic figures are seen in adjacent liver cells 24 to 36 hours after the administration of chloroform and are numerous after 48 hours. The rapidity with which recovery occurs evidently varies with the extent of necrosis about the central veins.

Chloroform mixed with twice its volume of paraffin oil has been injected into the subcutaneous tissue of the flank of white rats weighing with few exceptions from 150 to 250 gm. and of no selected strain. The quantity has been 0.25 cc. per hundred gm. of body weight. With favorable diet some of the animals that receive this quantity survive (6). Especially noteworthy in relation to the present study is the occurrence of nephrosis characterized by necrosis of some of the cells of the convoluted tubules and the presence of fat droplets elsewhere in the convoluted tubules and in the loops of Henle. When death occurs after 3 or 4 days there is pleural effusion with compression of the lungs and less advanced peritoneal effusion, occasionally with edema about the pancreas.

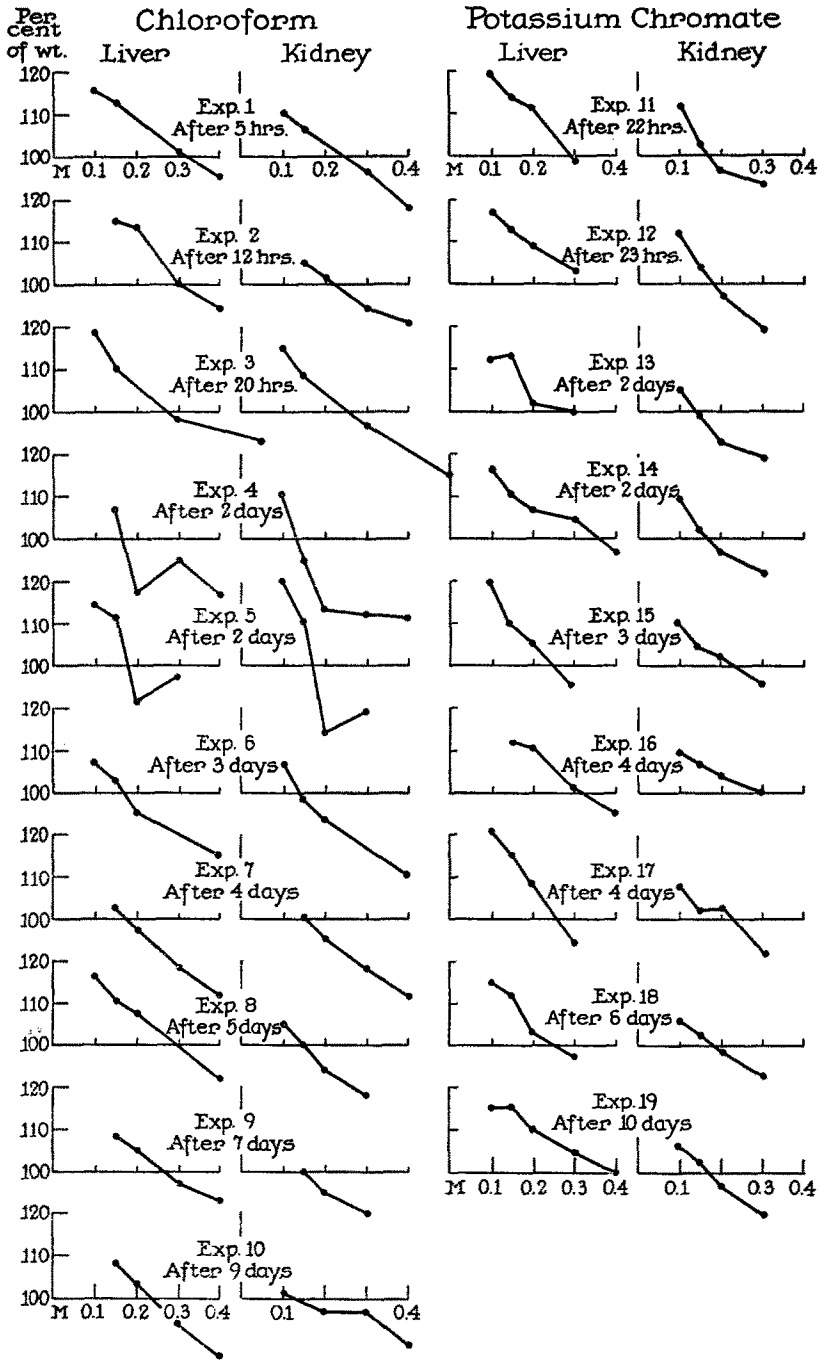

FIG. 1. Changes in the per cent of weight of slices of liver and of kidney tissue found 10 minutes after immersion in solutions of sodium chloride. The tissues were obtained from animals at different intervals after administration (*a*) of chloroform and (*b*) of potassium chromate.

During an interval of 6 to 12 hours immediately following the administration of chloroform, at a time when scant, if any, microscopic change was found, liver tissue maintained its usual isotonicity (Fig. 1, Table I) with solutions of sodium chloride having a concentration somewhat more than twice that in blood serum (4). Of tissues from two animals examined after 20 hours the liver of one had undergone in this relation no significant change but that of the other was isotonic with salt solution of much lower concentration (0.18 molar). After 2 days, at a time when the liver was the site of well defined necrosis, swelling of mitochondria, and deposition of fat about central and hepatic veins, the level

TABLE I

The Effect of Chloroform on the Level of Isotonicity Maintained by Liver Tissue and by Kidney Cortex

Interval after administration of chloroform	Liver		Kidney	
	Molar concentration of sodium chloride isotonic with liver	Maximum intake of water in per cent of weight of liver	Molar concentration of sodium chloride isotonic with kidney cortex	Maximum intake of water in per cent of weight of kidney cortex
Normal average	0.34	200.4	0.25	187.0
5 hrs.	0.32	176.2	0.24	178.0
12 "	0.3	171.5	0.22	169.0
20 "	0.28	178.9	0.27	165.0
20 "	0.18	174.3		
2 days	0.17	149.2	0.14	137.5
2 "	0.18	151.2	0.17	129.6
3 "	0.17	135.2	0.14	118.6
4 "	0.18	136.7	0.16	112.2
5 "	0.3	189.1	0.16	143.7
7 "	0.27	190.5	0.15	138.8
9 "	0.24	177.1	0.17	174.2

of isotonicity was that of 0.17 to 0.18 molar sodium chloride and little above the isotonicity of red blood corpuscles. The same level was maintained during 3 days but after 5 days the liver tissue had approximately regained its usual osmotic relation to solutions of sodium chloride. Concurrently with this change regeneration was in progress and liver cells had almost completely regained their normal appearance. After 9 days (see Table I) the level of isotonicity was below that found in normal animals and with associated nephrosis the liver cells were the site of fat deposition.

In association with severe injury to the cells of the convoluted tubules of the kidney caused by chloroform changes in the osmotic pressure of this tissue were similar to those of the liver. Slices of the cortex less than 1 mm. in thickness and cut parallel with surface of the organ were immersed in various solutions.

As with the liver little morphological change was found with kidney during the early period following chloroform administration but later advanced injury was evident. There was widespread necrosis of the cells of the convoluted tubules with loss of nuclei and disintegration of cytoplasm. Minute globules of fat were found in cells that were not destroyed and tubular casts were usually abundant. Evidence of injury persisted longer in the kidney than in the liver and after 9 days the organ was swollen and pale yellow and casts were numerous within the tubules of cortex and medulla.

During the 1st day following chloroform administration kidney cortex maintained its usual isotonicity with solutions of sodium chloride slightly less concentrated than those isotonic for liver but coincident with the decrease of the level of isotonicity of liver tissue evident on the 2nd day similar change was found in kidney cortex (Fig. 1, Table I) so that immersed slices were in water equilibrium with solutions of sodium chloride from 0.14 to 0.17 molar; that is, the approximate concentration of sodium chloride in blood serum. With the quantity of chloroform that was administered this low level of isotonicity was maintained throughout the experiments and corresponded with histological evidence of severe nephrosis in all the animals.

Immersion of tissues in distilled water has no resemblance to conditions present during life and promptly causes destruction of the tissue. Nevertheless it may give evidence that factors related to osmotic movement of water within the tissue have been altered. Coincident with the fall in the level of isotonicity of liver and kidney tissue caused by chloroform, water intake of the same tissue immersed in distilled water was greatly modified. The tissue took up less water as determined by increase of its weight than normal tissue under the same conditions and a maximum was reached sooner. Fig. 2 shows the effects of chloroform administration on changes in the weight of liver and kidney tissue during 2 hours of immersion in water. 5 hours after chloroform administration weight of the tissue slices, like that of the normal tissues (see Figs. 1 and 8 of an earlier publication (4) increased approximately 80 per cent and reached a maximum after 1 or 2 hours. 20 hours after chloroform administration water intake of liver was little changed but that of kidney was somewhat less than before. After 2 days each took up much less water, reached a maximum sooner, and then rapidly fell. After 2, 3, and 4 days these changes persisted and were more conspicuous with kidney than with liver. After a longer period parallel with histological evidence of repair of liver tissue the graph for liver assumed its original form but the kidney failed to take up water in quantity approximating that of normal tissue until 9 days after chloroform administration (Table I).

Injury by Carbon Tetrachloride

Carbon tetrachloride, like chloroform, injures liver cells and causes greatest change in those about the central veins but injury to the kidney is manifestly less than that with chloroform.

Cameron and Karunaratne (7) injected 0.1 to 0.25 cc. of undiluted carbon tetrachloride into the subcutaneous tissue of white rats. Little change was found after 5 hours but after 24 hours there was necrosis about the central veins and a surrounding zone in which cells had

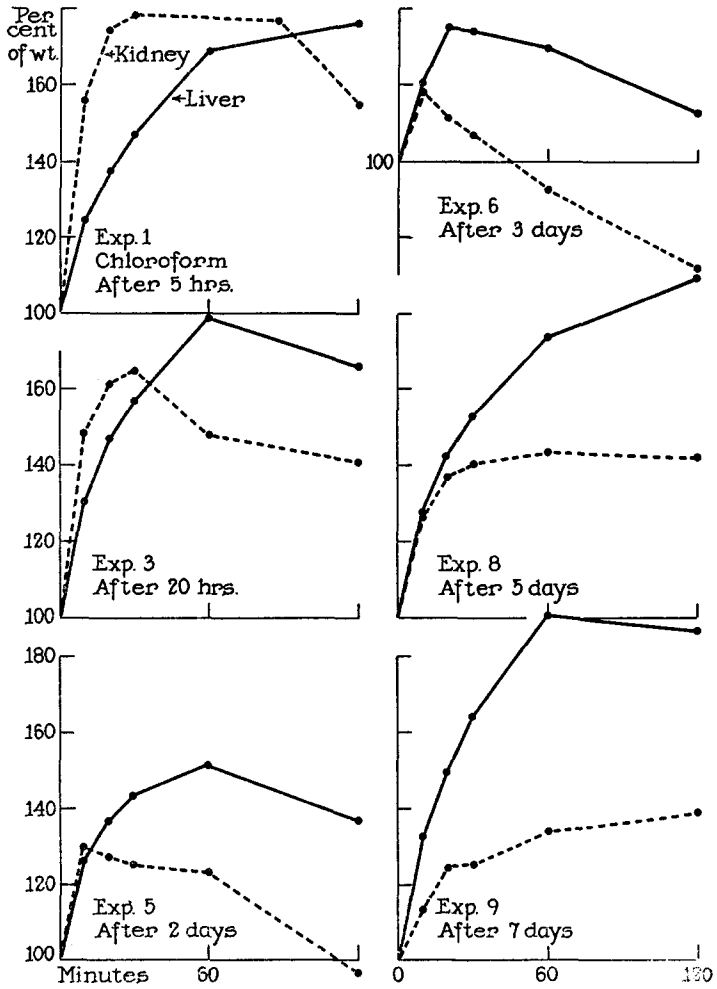

FIG. 2. Changes in per cent of weight of liver slices immersed in distilled water at different intervals after administration of chloroform.

undergone hydropic change. Lymphocytes and histiocytes appeared in the injured tissue. After 3 days necrotic liver cells had disappeared and regeneration characterized by mitotic figures in liver cells was in progress. After 7 days the greater part of the liver appeared to be normal but a few spaces were not filled by regenerated cells.

The movement of water in slices of liver tissue after intervals of 1 to 10 days following the administration of carbon tetrachloride is shown in Table II. The

level of isotonicity fell and after 2 days was that of 0.14 molar sodium chloride, approximating that of blood serum. Later it rose and after 10 days had reached the normal level. Histological changes found in the liver were those that have been cited. In the kidney on the contrary the level of isotonicity underwent

TABLE II

The Effect of Carbon Tetrachloride on the Level of Isotonicity Maintained by Liver Tissue and by Kidney Cortex

Interval after administration of carbon tetrachloride	Liver		Kidney	
	Molar concentration of sodium chloride isotonic with liver	Maximum intake of water in per cent of weight of liver	Molar concentration of sodium chloride isotonic with kidney cortex	Maximum intake of water in per cent of weight of kidney cortex
Normal average	0.34	200.4	0.25	187.0
23 hrs.	0.2	144.3	0.26	186.7+
2 days	0.14	123.1	0.22	173.2
3 "	0.2	160.3	0.26	164.3+
5 "	0.29	167.1	0.25	168.6
10 "	0.34	161.3	0.35	157.7

TABLE III

The Effect of Potassium Chromate on the Level of Isotonicity Maintained by Liver Tissue and by Kidney Cortex

Interval after administration of potassium chromate	Liver		Kidney	
	Molar concentration of sodium chloride isotonic with liver	Maximum intake of water in per cent of weight of liver	Molar concentration of sodium chloride isotonic with kidney cortex	Maximum intake of water in per cent of weight of kidney cortex
Normal average	0.34	200.4	0.25	187.0
22 hrs.	0.3	175.4	0.17	143.5
23 "	0.34	199.7	0.18	140.6
2 days	0.3	170.2	0.14	137.8
2 "	0.36	198.7	0.17	164.1
3 "	0.25	183.6	0.22	147.8
4 "	0.32	193.8	0.3	150.0
4 "	0.26	212.6	0.24	145.0
6 "	0.26	181.5	0.18	164.0
10 "	0.4	164.0	0.17	176.3

little change, falling slightly after 2 days, and no histological evidence of injury was found. The maximum intake of water after immersion of liver in distilled water (see Table III) diminished when the level of isotonicity fell.

Injury by Potassium Chromate

The effect of potassium chromate upon the level of isotonicity of liver or kidney as determined by immersion in solutions of sodium chloride was studied

because this substance causes necrosis of the cells of the convoluted tubules of the kidney cortex, whereas in contrast to chloroform and carbon tetrachloride it leaves the liver apparently uninjured.

The quantity of potassium chromate injected into the subcutaneous tissue was 0.1 cc. of a 2.5 per cent solution, that is 25 mg. per 100 gm. of body weight, save in one experiment (after 23 hours) in which 0.15 cc. was injected.

Within 24 hours slices prepared from the cortex of the kidney lost their normal isotonicity with solutions of sodium chloride approximately 0.26 molar (4) and were in osmotic equilibrium with solutions of sodium chloride 0.17 or 0.18 molar (Table III, Fig. 1). Though the isotonicity of the kidney cortex approached that of blood serum and of erythrocytes, that of liver tissue maintained its usual water equilibrium with solutions of sodium chloride with almost twice this concentration. The level of isotonicity of kidney tissue remained low during 2 days. At this time the proximal convoluted tubules of the cortex had in large part undergone necrosis; nuclei were lost, and the cytoplasm of the cells stained homogeneously with acid dyes. Nevertheless distal convoluted tubules and loops of Henle situated within cortical striae and in the subcortical zone were well preserved though deposition of fat droplets indicated that these structures had undergone minor injury. In two experiments 4 days after administration of potassium chromate isotonicity had risen to approximately normal levels represented by 0.3 and 0.24 molar sodium chloride.

In two experiments 6 and 10 days after administration of potassium chromate the level of isotonicity remained low, being that of 0.18 and 0.17 molar solutions of sodium chloride. In one instance there was persisting widespread necrosis of convoluted tubules and in the other nephrosis characterized by dilatation of tubules and deposition of fat within surviving cells of convoluted tubules and of the loops of Henle and some proliferation of interstitial tissue.

Following administration of potassium chromate the level of isotonicity of liver tissue in no instance diminished below that represented by 0.26 molar sodium chloride. It is noteworthy that the maximum intake of water by liver tissue (Table III) was little changed whereas that of kidney was almost uniformly diminished.

RECAPITULATION AND DISCUSSION

Metabolic changes within the cytoplasm of liver and kidney cells presumably maintain its molecular concentration at a level that establishes osmotic equilibrium with solutions of sodium chloride having a concentration approximately twice that of the blood serum. When excised liver or kidney tissue is immersed in blood serum it takes up water (4). The experiments here described show that

severe injury to liver or to kidney by chloroform causes the level of their isotonicity to fall so that it approximates that of blood serum and of erythrocytes. Like the histological changes that follow injury by chloroform this osmotic change is not immediate and becomes evident after an interval of variable length following administration of the toxic agent. The low level of isotonicity is maintained during several days but after 5 days the level returns to that of normal liver concurrently with regeneration of liver cells. Isotonicity of kidney tissue severely injured by chloroform in the quantity administered, undergoes changes similar to those of the liver but histological evidence of persisting injury and the osmotic pressure, show less tendency to return to normal.

Changes in the isotonicity of liver tissue and kidney cortex are evidently referable to the parenchymatous cells and not to the interstitial tissue of these organs. A preceding study has shown that interstitial tissue of the thymus and the connective tissue of the omentum are approximately isotonic with blood serum (4). It is noteworthy that hypotonic solutions cause great swelling of interstitial tissue of thymus or pancreas whereas the much less abundant fibrous tissue of liver and kidney is little changed; parenchymatous cells and the mitochondria of their cytoplasm are swollen (2).

Carbon tetrachloride which, like chloroform, causes necrosis of parenchymatous cells of liver about central veins likewise causes the level of isotonicity of liver tissue to fall to that of blood serum and it later returns to normal as repair is effected. Injury to the kidney is less with carbon tetrachloride than with chloroform and the level of isotonicity of kidney tissue is little changed.

Potassium chromate, which injures severely the cells of the proximal convoluted tubules of the cortex of the kidney reduces the level of isotonicity of kidney cortex approximately to that of blood serum but scarcely changes that of liver, which escapes structural injury. Recovery from this state of diminished isotonicity may occur promptly and after 3 or 4 days isotonicity may have returned to a normal level. In some instances, with persistence of severe injury recognizable by histological examination, its low level may persist.

Immersion of liver tissue in distilled water is a drastic procedure with little resemblance to processes within the living body. Nevertheless it may give evidence that structures upon which osmotic interchange depends have been profoundly modified. The experiments show that fall of the level of isotonicity of liver tissue or of kidney cortex exposed to water is accompanied by diminution of water intake by the tissue, when compared with that of the normal tissue immersed in water; water intake reaches a maximum sooner; and then falls rapidly.

Pancreas has a level of isotonicity somewhat higher than that of liver (4). The osmotic pressure maintained by cells of liver, kidney, pancreas, and perhaps other glandular organs has doubtless a significant relation to the functional

activity of these organs. It is noteworthy that hepatomas derived from liver cells, and cholangiomas, maintain an osmotic pressure (4), which is little greater than that of the fluid and red corpuscles of the blood.

CONCLUSIONS

As shown in a previous paper the cells of the liver and of the kidney maintain an osmotic pressure approximately twice that of blood and of erythrocytes, exceeding this slightly in the case of liver and being slightly less in that of kidney.

When liver cells are injured by chloroform or by carbon tetrachloride the osmotic pressure they maintain falls to the level of the medium that surrounds them but is promptly restored when recovery from the injury, with some regeneration of liver cells, occurs.

When nephrosis is caused by potassium chromate or by chloroform the osmotic pressure maintained by parenchymatous cells of the renal cortex falls to that of the medium about them but returns to its normal level with recovery from the injury.

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