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REVERSIBILITY OF CARBOHYDRATE AND OTHER CHANGES IN RATS SHOCKED BY A CLAMPING TECHNIQUE

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Secondary shock may be produced by a variety of methods. In the experiments to be described, a simple clamping technique has been used to induce shock in rats. Employing this procedure it is possible to demonstrate that certain changes in the shocked animals may be reversed and the fatal outcome prevented. Liver glycogen studies indicate that during the period of shock that follows the removal of the clamps, the rats exhibit a reduced ability to store glycogen when glucose is administered. The ability to store glycogen in the liver returns when the injured limbs are reclamped.

Methods

Female albino rats of the Wistar strain, weighing between 150 and 250 g., were used as the experimental animals. Each rat was suspended in a sling, the design of which was a slight modification of one previously used by Bunker & Solandt in this department. A clamp was applied to each hind leg of the rat. The clamps were made from rubber tubing clamps by the addition of curved metal plates (Fig. 1). The clamps were tightened sufficiently to cut off the circulation to the limbs. The nut on the clamp was tightened with a small, specially constructed, constant torque wrench. The clamps were retightened 15 min. after they were first applied. The clamps were left on overnight (12–15 hr.), though a shorter period may be used. When the clamps were removed, the limbs became swollen rather quickly and the animals died within a few hours. Throughout the test the rats were kept in a constant temperature box maintained at 27° C.

For all experiments in which glycogen estimations were made, food and water were removed 24 hr. prior to the estimated time of sacrifice. One c.c. 25 % glucose was given by stomach tube at the time the food was removed. The animals were anaesthetized with sodium amytal just before the liver samples were taken. The liver glycogen was determined by the method of

Good, Kramer & Somogyi [1933]. Blood sugar levels were obtained by the Somogyi method [1937]. The absorption of glucose from the gastro-intestinal tract was estimated by the method of Cori [1925]. The limb volume was estimated by measuring the volume of fluid displaced from graduated cylinders

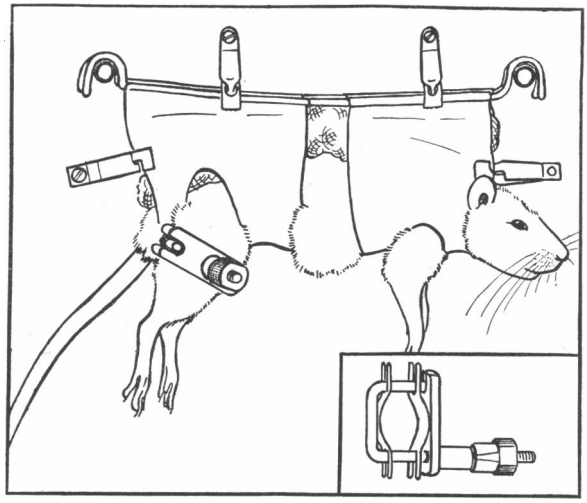

Fig. 1.

when the leg was immersed to a known level. The leg was first placed in water containing a wetting agent and each estimation was made in triplicate. The method, while relatively crude, gives values which are reproducible. This is shown by the results of three separate series of twelve successive single measurements:

Leg	Average volume c.c.	Standard deviation
1	12·3	±0·3
2	9·8	±0·4
3	8·3	±0·3

Results

When the clamps are removed from the rats, the limbs swell and the animals die within a few hours. The survival times for 84 rats are shown in the histogram (Fig. 2). The average survival time is 3 hr. 12 min. and the mode lies between 2 and 2½ hr.

Liver glycogen in shock. In the shocked rats (clamps removed) low values for liver glycogen were observed (average, 0·043 g. % for 20 rats). The values are also low in the control rats fasted for 24 hr., though usually not as low as those in the shocked animals (average, 0·142 g. % for 20 rats). Animals which have the clamps left in position show intermediate values but are nearer the control levels (average, 0·085 g. %). The glycogen levels are illustrated in Fig. 3.

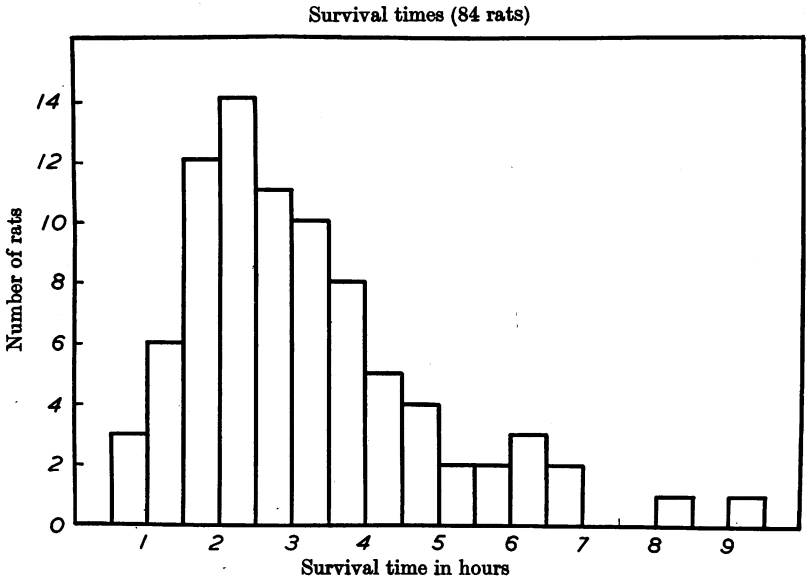
Survival times (84 rats)
Number of rats
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
Survival time in hours

Fig. 2.

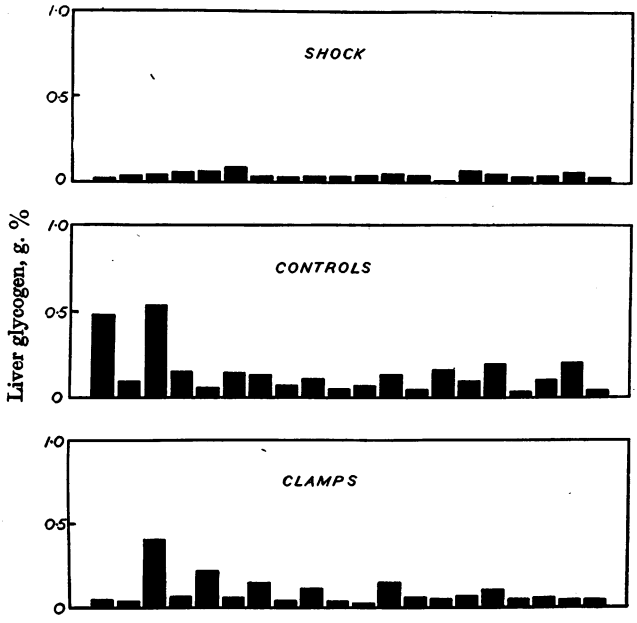
Liver glycogen, g. %
1·0
0·5
0
SHOCK
CONTROLS
CLAMPS

Fig. 3.

Glycogen storage following glucose administration. At the time the clamps were removed from the shocked rats, 2 c.c. 25 % glucose was given by stomach tube to each of the control and shocked animals. Liver glycogens were determined 4 hr. after the clamps were removed. Samples were obtained from control and shocked animals at the same time. Twenty control rats showed an

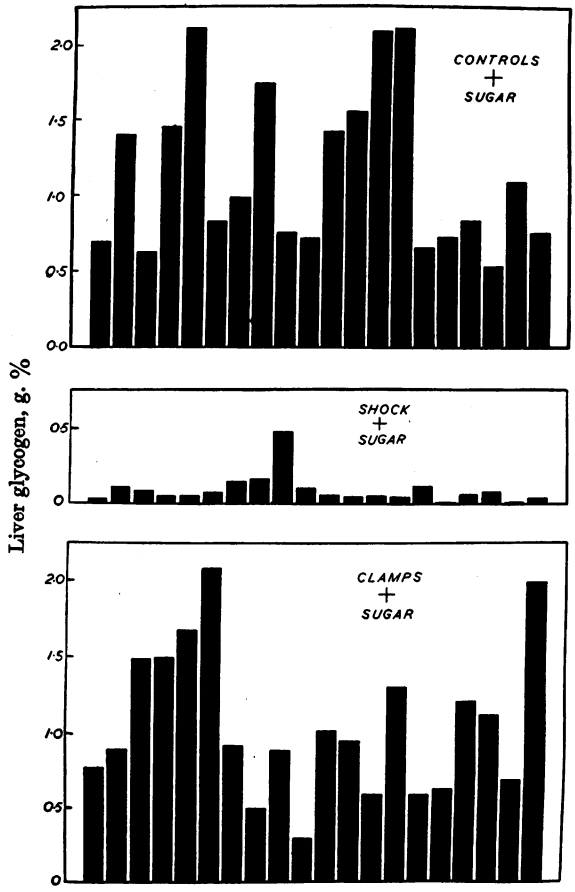

Fig. 4.

average liver glycogen value of 1·16 g. %, an increase of 1·0 g. %, or ten times the fasting value. Twenty shocked rats gave an average value of 0·093 g. %, an increase of 0·05 g. %. This increase is relatively slight, compared with that of the controls. The difference is not due to the clamping itself, since rats having the clamps left in place until after the liver was removed, responded like the controls when glucose was given. The average glycogen value for the clamped animals receiving glucose was 1·05 g. % or twelve times the level in

clamped rats receiving no glucose. The glycogen changes in the different groups following the administration of glucose are compared in Fig. 4. From this chart it will be evident that the shocked rats exhibit an impaired ability to store glycogen in the liver when sugar is fed. The change occurs only after the removal of the clamps, that is, after the restoration of circulation to the legs, since the clamped rats store glycogen as do the unclamped controls.

Absorption of glucose from the gastro-intestinal tract. It was necessary to determine whether or not the impaired storage of glycogen in the shocked rats was due to a poor absorption of glucose from the gastro-intestinal tract. First, intraperitoneal injections of glucose were tried. The results were the same as when the glucose was given by stomach tube. Next, the absorption of glucose was compared in 10 shocked rats and in 10 clamped controls by determining the residue of reducing substances in the gastro-intestinal tract 4 hr. after the glucose was given by stomach tube. In both the shocked and control animals practically all the glucose had been absorbed at that time (Table 1).

TABLE 1. The absorption of glucose from the gastro-intestinal tract. (2 c.c. 25 % glucose given by stomach tube. Rats killed in 4 hr.)

Clamped controls				Shocked rats			
Rat wt. g.	Blood sugar mg. %	Intestinal residue. Reducing substances mg.	Glycogen g. %	Rat wt. g.	Blood sugar mg. %	Intestinal residue. Reducing substances mg.	Glycogen g. %
211	186	12	—	159	340	44	—
200	170	109	—	203	234	18	—
170	168	92	—	213	325	9	—
197	120	10	—	240	680	12	0·148
188	174	11	1·64	221	363	32	—
234	185	19	1·37	202	363	31	0·436
229	235	22	0·88	204	420	124	0·055
217	181	29	0·71	189	402	127	0·073
210	246	11	0·79	189	396	70	0·077
198	260	23	1·17	223	613	71	0·222
Average	192	34	1·09		414	54	0·168

While the actual rate of absorption is not measured in this experiment, it seems evident from the table that the impaired storage of liver glycogen in shocked rats is not due to poor absorption of sugar from the intestinal tract. The reduced storage of liver glycogen occurs despite the fact that the blood sugar level is very high in the shocked rats given glucose. The average blood sugar values for several different groups of rats are presented in Table 2.

It is clear that in the shocked animals there is a greatly diminished ability to store glycogen in the liver at a time when the blood sugar is very high. This suggests the possibility that the changes might be associated in some way with an insulin deficiency. The blood sugar levels in the clamped control rats were higher than in the fasting animals which were not clamped. The liver

TABLE 2. Blood sugar changes

Sample	No. of rats	Average blood sugar mg. %
Control rats (no clamps)	5	90
Control rats, 4 hr. after sugar	5	144
Clamped control rats	5	148
Clamped, 2 hr. after sugar	5	248
Clamped, 4 hr. after sugar	10	192
Shocked rats (clamps off)	5	76
Shocked, 2 hr. after sugar	5	406
Shocked, 4 hr. after sugar	10	414

glycogen values seemed to be slightly lower in the clamped group. This would seem to indicate that the clamping procedure itself has some effect on the breakdown of liver glycogen. However, as far as the storage following glucose administration is concerned, the clamped animals responded like the control rats.

The effect of insulin administration. In order to test the possibility that an insulin deficiency might be involved, 2 units of insulin were injected subcutaneously into shocked rats. Some difficulty was encountered because of the early death of many of the rats. The effect of insulin administration on liver glycogen is shown in Table 3. The data in Table 3 indicate that insulin injection does not increase the ability of the shocked rats to store glycogen in the liver.

TABLE 3. The effect of insulin administration in shocked rats

Shocked rats, each given 2 units of insulin subcutaneously		Clamped controls given no insulin, sacrificed at corresponding times	
Rat wt. g.	Liver glycogen g. %	Rat wt. g.	Liver glycogen g. %
194	0·244	165	0·82
212	0·061	200	0·20
197	0·035	180	1·23
189	0·061	220	0·26
195	0·071	220	0·43
160	0·008	176	1·42
176	0·063	210	0·80
182	0·041	180	1·36
193	0·000	186	1·24
200	0·037	196	0·68
219	0·014		
209	0·011		
189	0·045		
220	0·088		
Average	0·056*		0·84

* Average liver glycogen for shocked rats given glucose: 0·093 g. %.

Table 4 shows the average blood sugar levels for the different groups at corresponding times after the glucose was given by stomach tube. From this it would appear that in the shocked rats the injection of insulin results in a very definite reduction in the blood sugar level, even though it does not enhance the storage of liver glycogen.

TABLE 4. Average blood sugar levels at corresponding times after sugar administration. (500 mg. glucose/rat)

Group	No. of rats	Average blood sugar mg. %
Clamped controls	5	248
Shock	5	406
Shock-insulin	13	134

The effect of oxygen lack on glycogen storage. Because of the prevalent opinion concerning the importance of fluid loss and the resultant development of tissue anoxia, it seemed desirable to determine whether or not oxygen lack, in itself, would alter the ability of the animal to store glycogen in a manner comparable to that observed in shock. Experiments were conducted at different atmospheric pressures in a special chamber. Each rat was given 2 c.c. 25 % glucose solution by stomach tube and the liver glycogen was determined at the end of 4 hr., as in the shock experiments. Immediately after the glucose was given, the animals were put in the chamber and the pressure was reduced. The low pressure was maintained throughout the 4 hr. period. The results are shown

TABLE 5. Glycogen formation in low oxygen atmospheres
Liver glycogens in g. % 4 hr. after glucose administration at approximate altitudes of 16,000 ft. (409 mm. Hg), 25,500 ft. (275 mm. Hg), and 30,500 ft. 220 mm. Hg)

	Liver glycogen, g. %		
	409 mm. Hg	275 mm. Hg	220 mm. Hg
	0·96	0·487	0·487
	0·98	0·161	0·107
	1·27	1·180	0·123
	0·65	0·113	0·091
	1·26	1·220	Died
	—	—	0·074
	—	—	0·027
	—	—	0·066
	—	—	0·109
	—	—	Died
Average	1·02	0·632	0·135

in Table 5. From this table it will be seen that at 409 mm. Hg (16,000 ft.) the animals stored glycogen in normal amounts. At 275 mm. Hg (25,500 ft.) some rats showed diminished storage but some did not. At 220 mm. Hg (30,500 ft.) two of the animals died and with one exception the others showed reduced ability to store glycogen. When the oxygen want is severe the diminished storage of liver glycogen approaches that observed in shock, but of course the conditions are not necessarily comparable, as will be considered later.

Reversibility of shock

The effect of reclamping on survival. In the experiments already outlined, shock was produced when the circulation was restored to limbs that had been clamped for 12–15 hr. After removal of the clamps the limb volume increased

rather quickly, reaching a maximum within 2 hr. from the time that the clamps were taken off. At 1 hr. after removal the major part of the swelling had occurred. These facts are evident in Fig. 5, which shows the increase in volume of the hind limbs with time.

It has been found that if the limbs are reclamped at a time when the swelling is complete, or nearly so, most of the animals will survive. The clamps used at this time are very narrow and are placed as high on the legs as possible. They

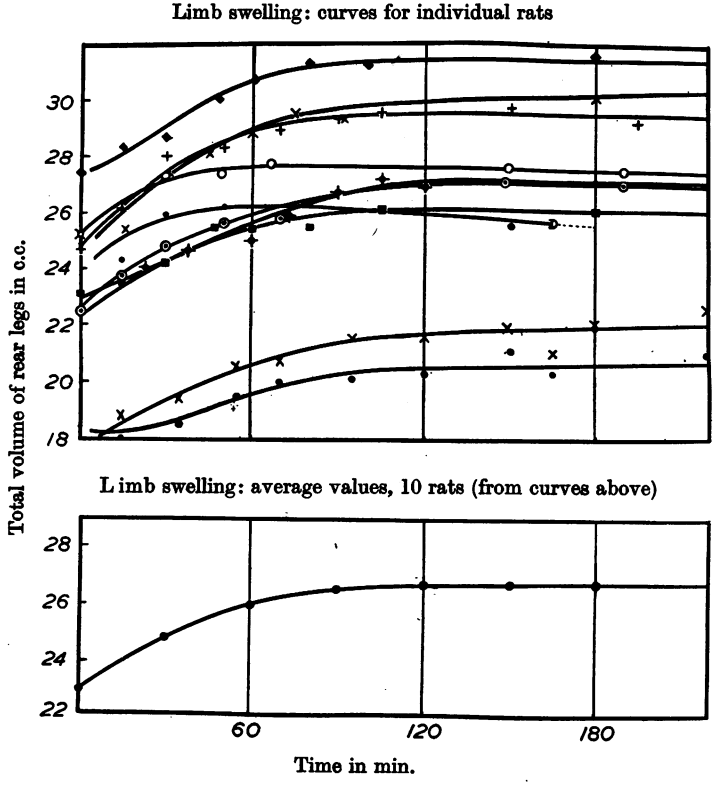

Fig. 5.

are tightened sufficiently to stop the circulation to the limbs. Limb volume measurements taken many hours after the reapplication of the clamps indicate that there is no significant reduction in limb volume after reclamping. The fluid trapped in the injured limbs is lost to the body as a whole.

In the shocked rats before death the respirations become laboured and finally gasping in type. Breathing stops several minutes before the heart. Reclamping after the respirations become laboured seems to be less effective. Animals reclamped at that time usually die within the first hour after the clamps are reapplied. Some of the reclamped animals used in glycogen and

sugar studies were killed 4 hr. after reclamping. They were in good condition at that time. All the others were killed, in good condition, more than 7 hr. following reclamping. Thirty-five were kept for more than 20 hr. before they were killed. Twenty-four shocked rats, reclamped at 2 hr., were kept for 48 hr. before they were killed. None had received food since 24 hr. prior to release of the clamps. Some of the data concerning the reclamped animals are given in Table 6. It is evident from the table that reclamping the injured limbs encourages survival despite the fact that the local fluid loss appears to be practically complete at the time that the clamps are reapplied, and the loss is maintained by the reclamping.

TABLE 6. Reclamping and survival

No. of rats shocked	Time after removal when clamps reapplied hr.	No. dying before reclamped	No. reclamped	No. dying in 30 min. after reclamping. Almost dead before	Survivals
61	1	13	48	5	41
78	2	18	60	7	40

The effect of reclamping on the storage of liver glycogen. It was important to determine whether there was a restoration of the ability to store liver glycogen when the clamps were reapplied. Experiments were performed on rats in which the clamps were reapplied 1 hr. and 2 hr. after removal. Each rat received 2 c.c. 25 % glucose solution by stomach tube immediately after the clamps were reapplied. Glycogen determinations were made 4 hr. after the glucose was given. The results are shown in Table 7.

TABLE 7. The effect of reclamping on glycogen storage in the livers of shocked rats. (Sugar was given at the time of reclamping. Glycogen was estimated 4 hr. later)

	Liver glycogen g. %		Blood sugar mg. %	
	Reclamped after 1 hr.	Reclamped after 2 hr.	Reclamped after 1 hr.	Reclamped after 2 hr.
	1·87	0·43	430	382
	1·09	0·37	256	336
	1·48	0·46	240	388
	0·95	0·59	343	421
	0·18	0·63	239	255
	0·38	0·37	204	180
	0·29	0·19	228	253
	1·18	0·94	—	—
	0·59	0·34	—	—
	0·12	0·30	—	—
	0·45	—	—	—
Average	0·78	0·46	277	316

Average liver glycogen for shocked rats given sugar: 0·093 g. %.

The ability of the rat to store liver glycogen returns towards normal when the injured legs are reclamped. It would seem that this restoration may be more rapid when the clamps are reapplied earlier in shock. Since the animals survive, it is likely that other abnormalities in the shocked rats also return

to normal. It is well to point out, however, that the return in the ability to store glycogen in the liver takes place very quickly.

A comparison of liver glycogen and blood sugar values for all groups. A comparison of the average values for liver glycogen and blood sugar in the different groups is given in Fig. 6.

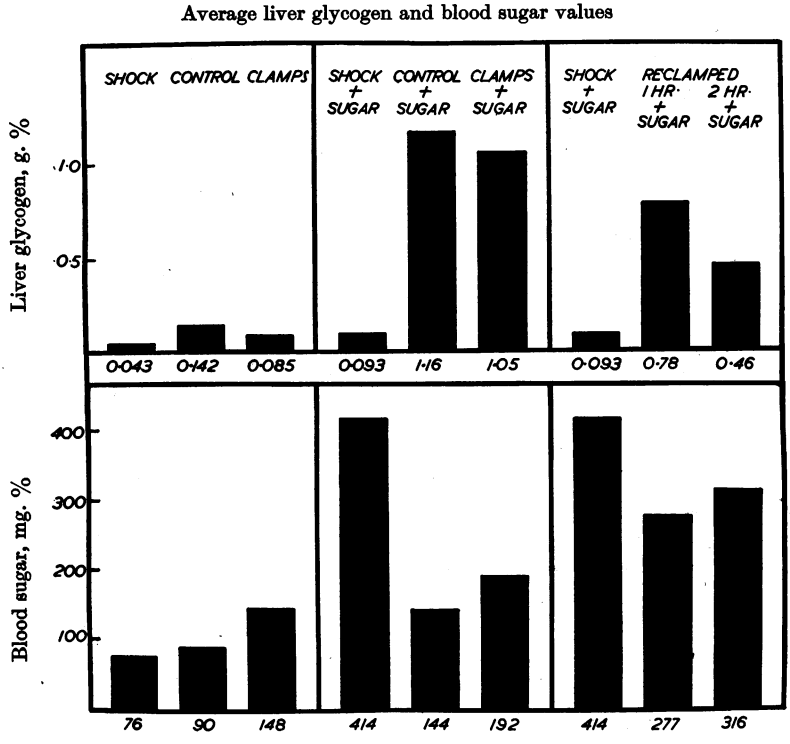

Fig. 6.

Discussion

A method of producing shock in rats has been described. Rats shocked by this procedure exhibit an increase in limb volume. Most of the increase appears in the part distal to the clamped region. The degree of swelling and the rapidity with which it occurs probably indicate the extent of the restoration of circulation to the limbs. If the swelling is rapid and extensive the animal is more apt to die quickly. If the swelling is slight the animal is likely to survive for a longer time. It is reasonable to suppose that the major factor in shock must be associated with the limb swelling in some way. At first thought this might suggest that the local fluid loss itself is the important factor in causing shock. Since the reapplication of clamps to the damaged limbs after they have swollen does not seem to diminish appreciably the local fluid loss, and since the

reclamped rats survive, it seems unlikely that local fluid loss itself is the fundamental factor in this type of shock. The possibility that fluid spreading above the clamped area or that loss of fluid from the surface of the legs may become important factors after the local swelling is complete, must be considered. Since the clamps are reapplied relatively close to death, the importance of these factors becomes questionable. The fact that the limb swelling is maintained after reclamping argues against any significant influence of fluid loss from the surface of the legs.

The information in the literature concerning glycogen changes in shock is not very extensive. Reduced values for liver glycogen have been reported following haemorrhage [Aggazzotti, 1927]. In our experiments, the fasting shocked rats (clamps removed) showed a reduction in liver glycogen below that of control animals with or without the clamps. Despite this, the blood sugar in the shocked animals was lower than in the control groups, indicating either (1) that there was an increased peripheral utilization of glucose, or (2) that there was a diminished production of glycogen from non-sugar sources in the shocked rats. Engel, Winton & Long [1943] mention some unpublished work of Russell & Engel on eviscerated rats which indicates that there may be an increased rate of glucose utilization by peripheral tissues in the shock following haemorrhage.

When the shocked rats are given sugar by stomach tube it is found that they exhibit a diminished ability to store glycogen in the liver. The clamped control animals store glycogen essentially as well as the unclamped controls. Hence, the clamping, in itself, is not responsible for the changed function. The absorption experiments show that the diminished ability to store liver glycogen is not due to poor absorption of glucose from the gastro-intestinal tract. Also the poor storage occurs despite the fact that the blood sugar level is very high. Insulin administration lowers the blood sugar level in the shocked animals but does not seem to help the animal to form glycogen from ingested sugar. The evidence seems to indicate that the poor storage of liver glycogen in shock is probably not due to a deficiency of insulin. However, since injected insulin will lower the blood sugar in the shocked rat and since the blood sugar in shocked animals receiving glucose is maintained at a high level for long periods, it follows that either insulin secretion is not regulated by the blood sugar level or that such a mechanism is not functioning adequately in shock.

The experiments concerning the effect of reduced atmospheric pressure show that when oxygen lack is extensive there is a diminished storage of liver glycogen after glucose administration. The diminished storage approaches that observed in shock. Evans [1934] reported that low atmospheric pressures lead to an increase in liver glycogen. However, the conditions for the experiments reported here are different in that the exposure was for a much shorter time and the atmospheric pressure was lower. It is realized that a condition of

generalized anoxia of this type probably is not comparable to the condition in the shocked animals since in shock the cardio-vascular compensations tend to maintain an adequate supply of oxygen to the brain, and the anoxia where it exists is due to a reduced blood flow through the tissues rather than to poor oxygenation of the blood.

It is desirable to know whether the changes in the shocked animals are due to a local oxygen deficiency or whether they are brought about by a secondary more generalized anoxia. When the clamps are reapplied to the legs and the damaged (and anoxic) tissue is cut off from the rest of the body, most animals survive and the ability to form liver glycogen is restored. The local loss of fluid is maintained by the reclamping, hence if a generalized anoxia following the local change is the important factor it is difficult to see why this factor ceases to operate in the reclamped animals. If there is a generalized anoxia that is not the result of a reduction in blood volume following the local fluid loss but the result of an altered circulation due to other causes, then reclamping might bring about its effect by restoring the circulation.

It is evident from these experiments that some change occurs in the damaged (and anoxic) tissue which affects the activity of tissues in other parts of the body remote from the site of injury. This influence can be removed by reclamping or blocking off the injured part from the rest of the body. The nature of the process by which the effect on more remote tissues is brought about has not been elucidated as yet.

One important point emerging from the reclamping experiments is that the profound changes in the shocked animals are reversible until late in shock, even at a time when transfusion is of little use. It is probable that the important irreversible changes, if such there be, occur just prior to death. The hope is raised that, by reversing some of the earlier changes, shock may be alleviated or prevented. Obviously, reclamping is not a practical means of achieving this and some more feasible procedure must be found.

Summary

1. A method of producing shock in rats by the application of clamps to the limbs is outlined.

2. The average survival time of 84 rats shocked by this procedure is 3 hr. 12 min.

3. The liver glycogen values are somewhat lower in the shocked rats than in clamped control animals or in unclamped controls.

4. Following the administration of sugar by stomach tube, the shocked rats are unable to store glycogen in a normal fashion. This occurs despite the fact that the blood sugar level is very high in the shocked rats and the absorption of sugar from the gastro-intestinal tract is almost as complete as in the clamped control animals.

5. Insulin administration does not improve the glycogen storage in the liver, but lowers the blood sugar level in the shocked rats.

6. With severe oxygen want there is a diminution in the storage of liver glycogen after glucose administration approaching that observed in shock.

7. When the shocked animals are reclamped before they are on the point of death and after the local fluid loss is practically complete, most of them survive. This happens despite the fact that the fluid trapped in the limbs is lost to the body.

8. When the shocked animals are reclamped, they rapidly recover the ability to store glycogen in the liver when glucose is administered.

9. It is concluded that local fluid loss is not the fundamental factor in shock in these animals.

10. Some change occurs in the damaged (anoxic) tissues which affects the activity of tissues in other parts of the body. How this effect is mediated has not been determined.

11. Extensive changes in the shocked animals are reversible until late in shock.

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