STUDIES ON THE MECHANISM OF SHOCK. THE QUANTITATIVE ASPECTS OF GLYCOGEN METABOLISM AFTER LIMB ISCHAEMIA IN THE RAT

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THE mechanism of the general response to injury (shock) can be studied through its associated metabolic changes. The obvious effect of injury on energy metabolism directs attention to the changes in carbohydrate metabolism. Although it is generally agreed that the glycogen concentration in the damaged tissue, liver and muscle outside the damaged area is reduced the changes in other tissues are less certain. Little attention has been paid to the causation of the changes, their relation to the other consequences of injury, the influence of nutritional status on these changes and the total amounts of glycogen involved. The present paper is mainly concerned with the quantitative aspects of this problem.

Previous work (Stoner, 1958) has shown that the survival period in ischaemic shock can be divided into two stages depending on the adequacy of tissue oxygenation. The present results show how this is reflected in the changes in carbohydrate metabolism after limb ischaemia and emphasize the importance of the adrenal medulla in bringing about these changes. They also show that glycogen formation from glucose continues in the tissues even when they are unable to store glycogen but they do not provide any evidence for an increase in the formation of glucose from non-carbohydrate sources in ischaemic shock in these conditions.

METHODS

Experiments were performed on albino rats of the Porton strain (body wt. 222 ± 17 g.) fed on MRC diet 41 or 41b (Bruce and Parkes, 1949, 1956). Both diets contain 13·6 per cent digestible protein, 48·4 per cent soluble carbohydrate and 4·5 per cent fat, the only difference being the replacement of the cod liver oil in diet 41 by a stabilized vitamin supplement. Food and water were given *ad lib* until the start of the experiment when food was removed. When the rats were fasted, food was removed 24 hr. before the experiment and coprophagy prevented. Rats were brought to the laboratory from the animal house about a week before the experiment to ensure that they were acclimatized to laboratory conditions and that their diurnal feeding rhythm was normal (Stoner, 1956).

Adrenal medullectomy was performed by the method of Evans (1936) as quoted by Ingle and Griffiths (1942). These rats were given 1 per cent NaCl to drink for 1 week postoperatively and used after 53–67 days. The absence of medulla was confirmed histologically. Adrenalectomy was carried out under ether anaesthesia through a dorsal approach and the rats were given 1 per cent NaCl to drink afterwards. They were used after 4 days.

Bilateral hind-limb ischaemia was produced with rubber tourniquets (Rosenthal, 1943) applied during a 3 min. period of ether anaesthesia. Controls had a similar anaesthetic. The environmental temperature was 18–22°.

The tissues were sampled under Na pentobarbitone anaesthesia (5 mg. per 100. g. body wt., intraperitoneally ; Veterinary Nembutal-Abbott). For the brain, as soon as the rat was

anaesthetized it was plunged head first into liquid N_2 and the cerebrum dissected out in the frozen state. For muscle and heart glycogen, samples of the right pectoralis major, both ventricles and right gastrocnemius were removed in that order and frozen with liquid N_2 as quickly as possible. For liver glycogen, samples from the pars centralis (Gershbein and Elias, 1954) were put into tared tubes containing 40 per cent KOH for "total glycogen" or weighed and ground up with 10 per cent trichloroacetic acid (redistilled, A.R.) in a Potter-Elvehjm all-glass homogenizer for "acid-soluble glycogen" (Bloom, Lewis, Shumpert and Shen, 1951). Blood samples were obtained from the thorax with a Pasteur pipette after division of the great vessels and heparin used as an anti-coagulant. Haematocrit values were obtained with Wintrobe tubes and corrected for trapped plasma (Chaplin and Mollison, 1952).

Blood sugar was estimated by the method of Haslewood and Strookman (King, 1946). Total glycogen was determined by the method of Good, Kramer and Somogyi (1933), the glucose liberated by hydrolysis being measured by Nelson's method (1944). For brain the cerebrosides were removed from the ethanol precipitate as described by Kerr (1936). Acid soluble liver glycogen was determined by the method of Bloom *et al.* (1951).

Thin slices of liver were fixed in Rossman's fluid for the histochemical demonstration of glycogen by the periodic acid-Schiff reaction with saliva control.

Uniformly ^{14}C-labelled glucose (Radiochemical Centre, Amersham) was injected into a tail vein in 0·9 per cent NaCl. After 2 hr. the rat was anaesthetized with Na pentobarbitone and the liver, hind-limb muscle and as much carcase muscle as possible rapidly excised and frozen in liquid N_2. Pure samples of the glycogen in these tissues were prepared according to the methods of Somogyi (1934) or Stetten, Katzen and Stetten (1954). The CO_2 obtained by combustion of the glycogen according to the method of Lindenbaum, Schubert and Armstrong (1948) was absorbed in NaOH. $BaCO_3$ was then precipitated with $BaCl_2$ and filtered on to sintered discs. The radioactivity of the $BaCO_3$ was determined at infinite thickness with an end-window counter (EHM 2S ; G.E.C.) attached to conventional scaling apparatus. A methylmethacrylate ^{14}C standard was used for comparison.

Results are expressed where possible as the mean $\pm$ standard deviation (S.D.), and statistical comparisons of the means made according to Student's t test (Fisher, 1934).

RESULTS

General Effects of Limb Ischaemia

The mortality rate and survival time after a 4 hr. period of bilateral hind-limb ischaemia at an air temperature of 18–22° are 85 per cent and 13·28 $\pm$ 0·90 hr. (Threlfall and Stoner, 1957). Fasting increases the mortality rate to 100 per cent and shortens the survival time (Threlfall and Stoner, 1954). The poorer condition of the fasted rats was evident within 3 hr. of removing the tourniquets. Adrenal medullectomy also raised the mortality rate to 100 per cent and shortened the survival time to 5·79 $\pm$ 4·72 hr. After total adrenalectomy the survival time was decreased still further to 1·86 $\pm$ 0·67 hr.

Fed normal rats

Blood sugar (Fig. 1).—Hyperglycaemia appeared during the ischaemic period and increased to a maximum about 3 hr. after the tourniquets had been removed. The level then fell progressively, reaching hypoglycaemic levels in fatal cases.

Liver glycogen (Fig. 2 and 3).—Part of the normal diurnal variation (Higgins, Berkson and Flock, 1933 ; Deuel, Butts, Hallman, Murray and Blunden, 1938) is shown by the control curve in Fig. 2. Normally the rat would begin to feed again about 6.0 p.m. but in the absence of food the fall continued until 4.0 a.m. The diurnal cycle is not completely abolished during the first 24 hr. of a fast (Higgins *et al.*, 1932) so that the 10.0 a.m. level in the 24 hr. fasted rats (Table I) was higher than that at 4.0 a.m.

At the end of the 4 hr. period of limb ischaemia the total liver glycogen was higher than would be expected from the controls. After removal of the tourniquets it was nearly always below that in the controls and within a few hours reached very low levels which persisted until death. Similar changes occurred in the acid-soluble glycogen level (Fig. 3). Histochemically the periportal cells were depleted of glycogen before the central cells in the same way as in the controls.

Muscle glycogen (Fig. 4).—The muscle glycogen level in the fed rat fell gradually to the level shown in Table I after a 24 hr. fast. With hind-limb ischaemia the

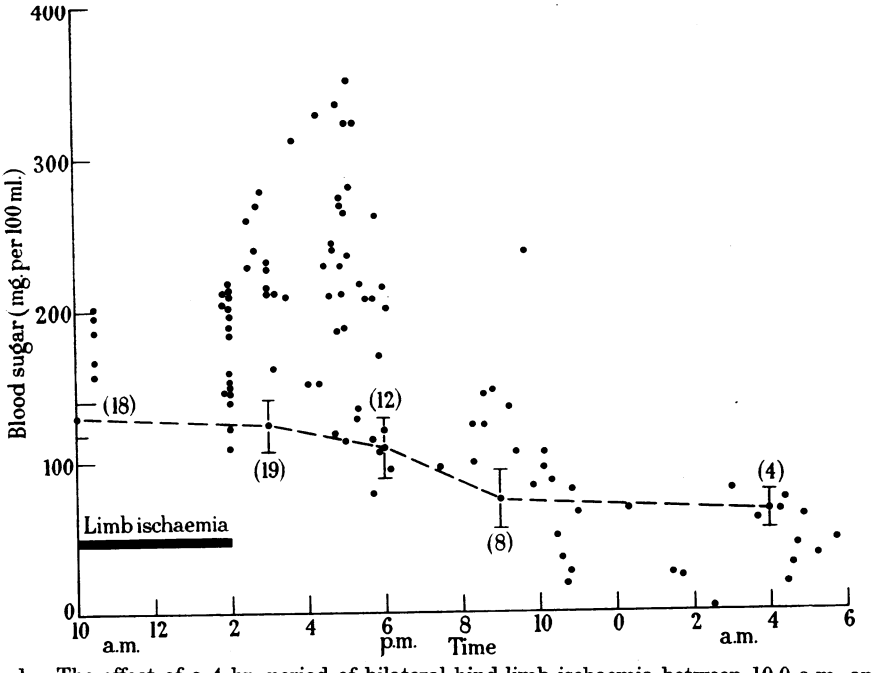

FIG. 1.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the blood sugar level of the fed rat, each point indicates the level in individual rats. The mean blood sugar level in fed control rats given a short ether anaesthesia at 10.0 a.m. is shown by the curve ●– – –● where each point indicates the mean of results in the number of rats shown in parentheses and the vertical line through the point represents the standard deviation of the mean.

glycogen contents of the pectoralis and gastrocnemius muscles fell similarly during the first 2 hr. of the experiment but during the next 2 hr. the fall was greater in the ischaemic muscle. If the tourniquets were kept on, this fall in the gastrocnemius continued so that after 6 hr. ischaemia its glycogen level was 108 $\pm$ 96 mg. glucose per 100 g. wet wt.

When the tourniquets were removed after 4 hr. the glycogen level in the pectoralis major continued to fall at about the same rate as before. The level in the gastrocnemius also continued to fall at first but after a few hours it rose and this higher level persisted. While the level in the gastrocnemius was below that in the pectoralis major when the tourniquets were removed, a few hours later it was consistently higher. This is shown in the later part of Fig. 4 where the pairs of points at any one time refer to the same rat. Examination of these extracts after

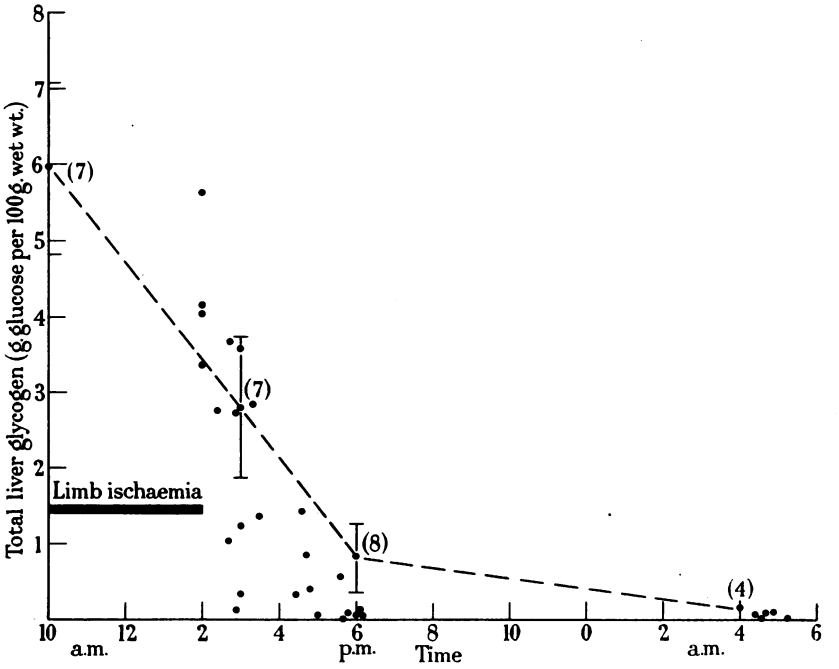

FIG. 2.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the total liver glycogen of the fed rat, each point indicates the level in individual rats. The mean level in fed control rats given a short ether anaesthesia at 10.0 a.m. is shown by the curve ●– – –● where each point indicates the mean results in the number of rats shown in parentheses and the vertical line through the point represents the standard deviation of the mean.

TABLE I.—*The Effect of a 4 hr. Period of Bilateral Hind-limb Ischaemia Between 10.0 a.m. and 2.0 p.m. on the Blood Sugar and Tissue Glycogen Levels (Mean ± S.D.) in Fasted Normal Rats*

(Number of observations shown in parentheses)

Group	Time	Blood sugar (mg. per 100 ml.)	Glycogen (mg. glucose per 100 g. wet wt.)			
			Ventricles	Liver	Pectoralis major	Gastrocnemius
Control	9.30 a.m.	76±21 (10)	504±55 (3)	251±114 (5)	337±90 (4)	311±110 (5)
,,	2.0 p.m.	69±14 (11)	499±24 (4)	84±65 (6)	249±90 (5)	389±95 (6)
,,	5.0 p.m.	55±14 (8)	447±55 (4)	84±62 (4)	250±123 (6)	365±69 (6)
Tourniquet	2.0 p.m.	86±25 (12)	507±83 (5)	273±146* (6)	120±52* (4)	36±37‡ (5)
,,	5.0 p.m.	90±29† (8)	450±44 (4)	20±13 (4)	65±36† (6)	132±113† (6)

* Significantly different from appropriate control mean at $P < 0{\cdot}05$.
† ,, ,, ,, ,, ,, ,, ,, $P < 0{\cdot}01$.
‡ ,, ,, ,, ,, ,, ,, ,, $P < 0{\cdot}001$.

acid hydrolysis and after neutralization by passing them down a column of Amberlite IR-4B(OH) showed that the reducing substance was yeast fermentable. Results obtained when glucose was added during the initial preparation of the muscle extracts excluded possible contamination by hyperglycaemic blood. This increase in the glycogen content of the post-ischaemic muscle was prevented by lengthening the period of ischaemia to 6 hr.

No attempt was made to divide the muscle glycogen into an acid-soluble and residual fraction since, in agreement with Carroll, Longley and Roe (1956), we

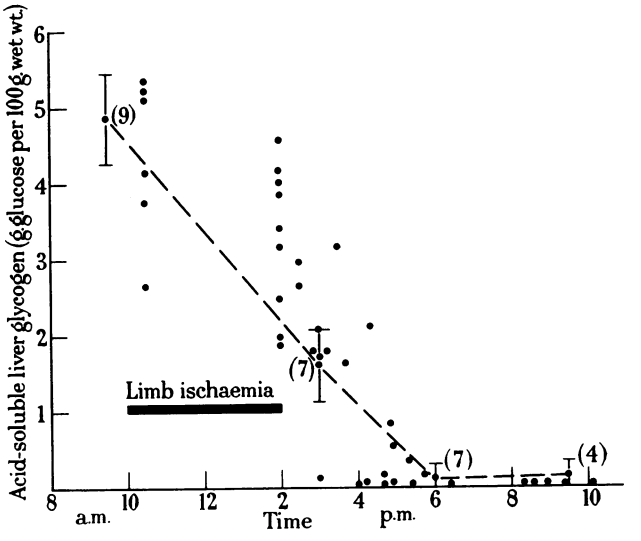

FIG. 3.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the acid soluble liver glycogen of the fed rat, each point indicates the level in individual rats. The mean level in fed control rats given a short ether anaesthesia at 10.0 a.m. is shown by the curve ●– – –● where each point indicates the mean of results in the number of rats shown in parentheses and the vertical line through the point represents the standard deviation of the mean.

have found that with efficient homogenization practically all the muscle glycogen is extractable with trichloroacetic acid.

Brain glycogen (Fig. 5).—The total glycogen content of the control brains showed little diurnal variation and the level in the experimental rats only fell when they were moribund.

Ventricular glycogen (Fig. 6).—The total ventricular glycogen level in the fed control rat rose steadily after removal of food at the beginning of the experiment. At the end of the limb ischaemia the level was considerably above that in the controls and this increase continued for about 2 hr. The level then returned to normal limits where it remained for most of the survival period. Terminally it was reduced.

Fasted normal rats

Fig. 1–6 show that an assessment of the major glycogen shifts could be made by comparing the levels at the removal of the tourniquets and 3 hr. later with the

control levels at 9.30 a.m., 2.0 and 5.0 p.m. The results in rats fasted for 24 hr. before the experiment are given in Table I.

Fasting greatly reduced the hyperglycaemic response to limb ischaemia and prevented the rise in the ventricular glycogen level. Liver glycogen again rose during limb ischaemia and then fell to a very low level, about the same as in the fed rats. The percentage reduction in the glycogen content of the pectoralis major was greater than in the fed rats. Glycogen deposition occurred in the gastrocnemius after removal of the tourniquets in all but 1 of the 6 rats.

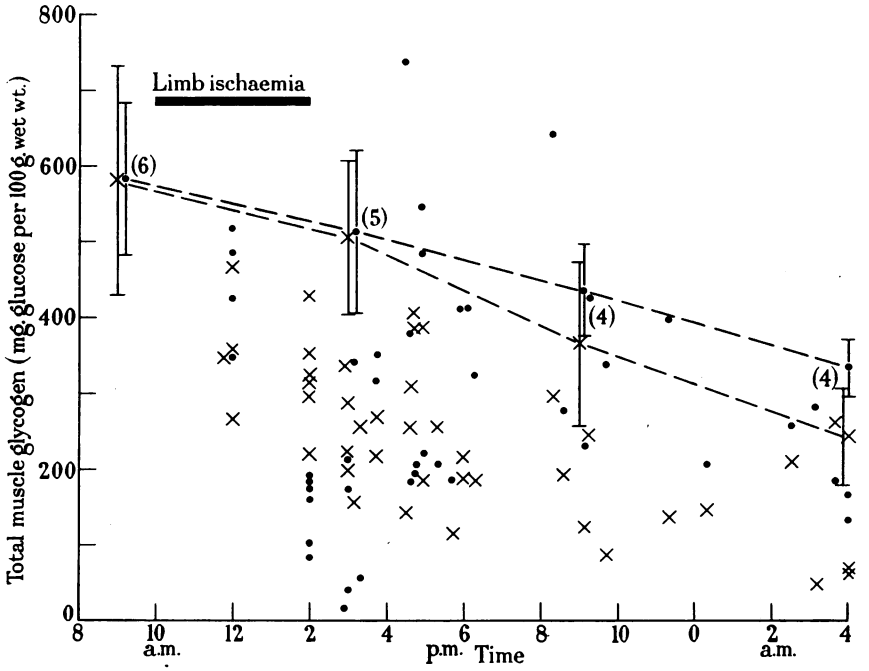

FIG. 4.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the total muscle glycogen in the pectoralis major (×) and gastrocnemius (●) of the fed rat, each point indicates the levels in individual rats. The mean levels in fed control rats given a short ether anaesthesia at 10.0 a.m. are shown by the curves, ×– – –× for the pectoralis major, ●– – –● for the gastrocnemius where each point indicates the mean of results in the number of rats shown in parentheses and the vertical lines through the points represent the standard deviations of the means.

Fed adrenal medullectomized rats

These results are shown in Table II. Medullectomy prevented the hyperglycaemia and 3 hr. after removal of the tourniquets the blood sugar level was significantly below that in the controls. The early onset of hypoglycaemia was in keeping with their poor clinical state. Medullectomy did not prevent the increase in ventricular glycogen. In some rats, not included in Table II, this rise continued for about 2 hr. after the period of limb ischaemia. The level then fell gradually, the final concentration being similar to that in injured normal rats at death. The liver glycogen level was unchanged during the limb ischaemia but fell afterwards at about the same rate as in the fed normal rats injured in this way. The glycogen

content of the undamaged muscle remained unaltered up to the time of death. The level in the ischaemic muscle fell as in the normal rats but there was no significant storage of glycogen in the post-ischaemic period and terminally the level fell again.

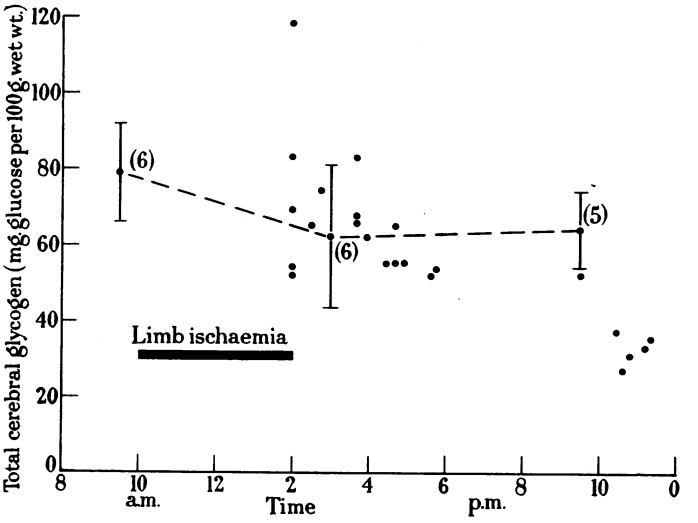

FIG. 5.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the brain glycogen of the fed rat, each point indicates the level in individual rats. The mean level in fed control rats given a short ether anaesthesia at 10.0 a.m. is shown by the curve ●– – –● where each point indicates the mean of results in the number of rats shown in parantheses and the vertical line through the point represents the standard deviation of the mean.

TABLE II.—*The Effect of a 4 hr. Period of Bilateral Hind-limb Ischaemia Between 10.0 a.m. and 2.0 p.m. on the Blood Sugar and Tissue Glycogen Levels (Mean ± S.D.) in Fed Medullectomized Rats*

(Number of observations shown in parentheses)

Group	Time	Blood sugar (mg. per 100 ml.)	Glycogen (mg. glucose per 100 g. wet wt.)			
			Ventricles	Liver	Pectoralis major	Gastrocnemius
Control	9.30 a.m.	114±8 (8)	384±72 (5)	6740±711 (3)	659±208 (5)	660±159 (5)
,,	2.0 p.m.	118±10 (7)	416±72 (3)	3895±1386 (4)	497±114 (5)	526±140 (5)
,,	5.0 p.m.	117±19 (5)	381±19 (3)	1540±544 (3)	491±130 (5)	414±157 (5)
Tourniquet	2.0 p.m.	130±30 (8)	589±21† (4)	4278±1096 (5)	520±76 (5)	159±36‡ (5)
,,	5.0 p.m.	61±41* (8)	486±102 (4)	275±294* (4)	659±174 (5)	196±143* (5)
,,	At death	33±9‡ (5)	271±60* (3)	79±23† (4)	529±121 (3)	62±43‡ (3)

* Significantly different from the control mean at $P < 0{\cdot}05$.
† ,, ,, ,, ,, ,, ,, ,, $P < 0{\cdot}01$.
‡ ,, ,, ,, ,, ,, ,, ,, $P < 0{\cdot}001$.

Fed adrenalectomized rats

In these rats (Table III) not only was hyperglycaemia prevented but when the tourniquets were removed the blood sugar level fell rapidly. The control glycogen levels were generally lower and more variable than in the other groups. In the injured rats the ventricular glycogen level was reduced by the end of the period of limb ischaemia and fell further when the tourniquets were removed. The liver glycogen level also fell more quickly than in the controls. There was no recovery in the glycogen content of the post-ischaemic muscle and that of the uninjured muscle showed a terminal fall.

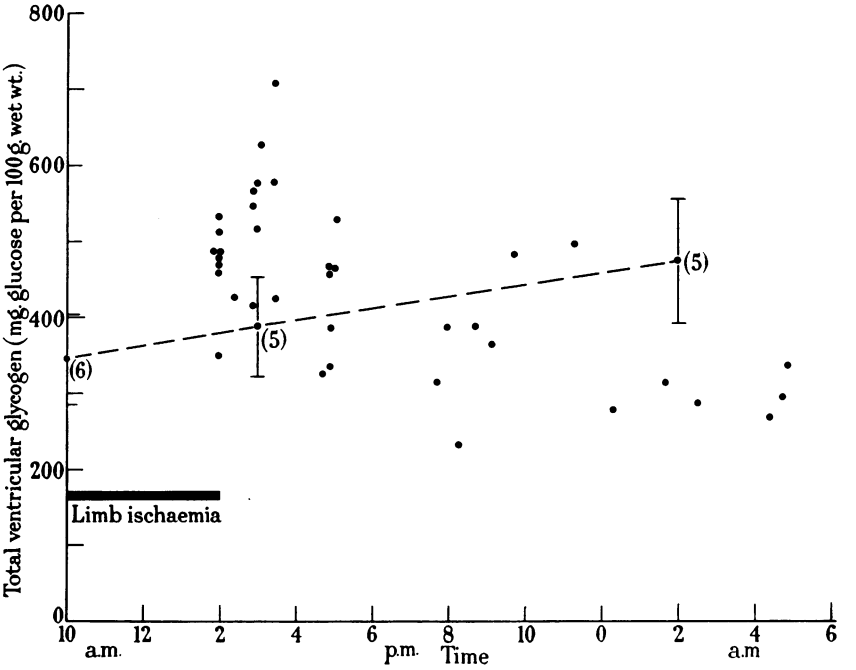

FIG. 6.—The effect of a 4 hr. period of bilateral hind-limb ischaemia between 10.0 a.m. and 2.0 p.m. on the total ventricular glycogen of the fed rat, each point indicates the level in individual rats. The mean level in fed control rats given a short ether anaesthesia at 10.0 a.m. is shown by the curve ●– – –● where each point indicates the mean of results in the number of rats shown in parentheses and the vertical line through the point represents the standard deviation of the mean.

Effect of Limb Ischaemia on Glycogen Storage and Turnover

The ability of the rat, under certain conditions, to store glycogen in the myocardium and in the muscles of the damaged limb after removal of the tourniquets has already been described.

When rats were given large amounts of glucose either by mouth (1·0 ml. 25 per cent aqueous soln. per 100 g. body wt.) or intravenously (0·5 ml. 6 per cent aqueous soln. per 100 g. body wt.) after a 4 hr. period of bilateral hind-limb ischaemia no effect was seen on the muscle glycogen level but the total liver glycogen concentration sometimes increased. This only occurred when the glucose was given shortly after the tourniquets had been removed. As the blood sugar

TABLE III.—*The Effect of a 4 hr. Period of Bilateral Hind-limb Ischaemia Between 10.0 a.m. and 2.0 p.m. on the Blood Sugar and Tissue Glycogen Levels (Mean ± S.D.) in Fed Adrenalectomized Rats*

(Number of observations shown in parentheses)

Group	Time	Blood sugar (mg. per 100 ml.)	Glycogen (mg. glucose per 100 g. wet wt.)			
			Ventricles	Liver	Pectoralis major	Gastrocnemius
Control	9.30 a.m.	119±11 (8)	308±50 (5)	2628±1400 (8)	346±128 (6)	374±112 (6)
,,	2.0 p.m.	101±21 (11)	252±54 (5)	537±271 (12)	312±87 (6)	357±55 (7)
,,	5.0 p.m.	63±18 (5)	344±49 (4)	114±88 (5)	363±37 (5)	359±40 (5)
Tourniquet	2.0 p.m.	86±36 (11)	179±18* (6)	170±136‡ (10)	338±68 (7)	45±24‡ (7)
,,	At death (approx. 4.0 pm.)	20±6‡ (8)	115±56‡ (5)	65±43 (7)	268±35† (5)	38±12‡ (5)

* Significantly different from the control mean at $P < 0.05$.
† ,, ,, ,, ,, ,, ,, ,, $P < 0.01$.
‡ ,, ,, ,, ,, ,, ,, ,, $P < 0.001$.

levels in the rats given oral doses of glucose were within the range for the untreated rats it is questionable how much of the glucose was absorbed.

The frequent inability of the injured rat to store glycogen in the skeletal muscle and liver does not mean that it was unable to synthesize it. The incorporation of ^{14}C-glucose into the glycogen of these tissues continued after limb ischaemia (Table IV) and the few available results suggest that this was decreased in the

TABLE IV.—*The Radioactivity (μc. per g. glucose) of the Glycogen Prepared from the Tissues of Rats 2 hr. After the Intravenous Injection of 20 μc. ^{14}C-glucose per 100 g. body wt.*

The rats were fed overnight and at 10.0 a.m. bilateral hind-limb tourniquets were applied under ether anaesthesia and the controls given ether. The tourniquets were removed at 2.0 p.m. and the ^{14}C-glucose injected in both groups at 2.15 p.m.

	Glycogen radioactivity (μc. per g. glucose)		
	Muscle (exc. hind-limb)	Hind-limb muscle	Liver
Control rat	0·932	0·475	0·146
,, ,,	3·010	1·655	0·440
,, ,, *	1·372†	—	0·188
Experimental rat	0·418	2·850	0·230
,, ,,	—	11·100	0·475
,, ,, *	0·381	14·800	0·460

* Glycogen prepared by method of Stetten *et al.* (1956), others prepared by method of Somogyi (1934).
† Including hind-limb muscle.

undamaged muscle and increased in the liver and injured muscle during the early part of the response. The last finding was in keeping with the increase in the glycogen content of the damaged muscle at that time.

Effect of Limb Ischaemia on " Total Body Glucose "

So far results have been expressed per unit tissue weight. They can also be expressed per 100 g. body wt. giving a value for the total amount of glucose in the body either free or combined as glycogen. This value will be called the " total body glucose ". The method of calculation is as follows :

Values of 4·15, 0·55 and 0·289 have been taken as the weights of the liver, brain and heart as percentages of the body wt. (Caster, Poncelet, Simon and Armstrong, 1956). A negligible error will be introduced by equating the glycogen level in the ventricles with that in the whole heart (Davies, Francis and Stoner, 1947). The damaged muscle in the hind-limbs was, by dissection, 8 per cent of the body wt., leaving 37·5 as the percentage for the undamaged muscle (Caster *et al.*, 1956). The justification for using the glycogen content of one muscle as a measure of that in the whole musculature has been discussed by Winternitz, Dintzis and Long (1957). In freshly drawn rat blood, the blood glucose is almost entirely confined to the plasma (unpublished observations) enabling the plasma concentration to be calculated from knowledge of the haematocrit (Table V). Steele, Wall, de Bodo and Altszuler (1956) have shown that glucose is distributed through the extracellular space of the body at its concentration in the plasma. The total extracellular space has been taken as 20 per cent of the body wt. (Manery, 1954) and it is considered to be unaltered by the injury (Green and Stoner, 1950 ; Rosenthal and Millican, 1954).

TABLE V.—*The Effect of a 4 hr. Period of Bilateral Hind-limb Ischaemia on the Haematocrit of the Rat*

Group	Average haematocrit (corrected)			
	Before ischaemia	At end of ischaemia	Time (hr.) after ischaemia	
			1	3	14
Intact	43	43	54	60	57
Medullectomized	42	41	—	70	—
Adrenalectomized	41	48	57*	—	—

* At death, approx. 2 hr. after ischaemia.

The results of these calculations are shown in Tables VI–IX. These total values are of the same order as those obtained for the " total body carbohydrate " (Threlfall and Stoner, 1954) indicating that no major source of glycogen has been omitted. The possible variability of these estimates of " total body glucose " is difficult to assess. The figures in parentheses below the total values in the table show the percentage increase which would have occurred if the mean glucose levels in the compartments had all been raised one S.D.

In intact rats subjected to limb ischaemia the loss of " total body glucose " during the ischaemic period was greater than in the controls during this period of the day. This was followed by a phase of normal consumption which was succeeded by a terminal phase of accelerated consumption. Medullectomy and adrenalectomy prevented the initial loss but the terminal phase commenced sooner.

TABLE VI.—*The Glucose Content of the Extracellular Space and the Glucose Present in the Organs as Glycogen Expressed as mg. glucose per* 100 *g. body wt. in Control Rats Fed up to the Time of the First Observations and in Similar Rats Subjected to Bilateral Hind-limb Ischaemia Between* 10.0 *a.m. and* 2.0 *p.m.*

Method of calculation and significance of figures in parentheses given in the text. The values used to calculate the amounts in the controls at 2.0 and 5.0 p.m. obtained by interpolation from the curves in Fig. 1, 2, 4, 5 and 6.

Time	9.30 a.m.	2.0 p.m.	3.0 p.m.	5.0 p.m.	6.0 p.m.	9.0 p.m.	4.0 a.m.
Controls :							
Extracellular space	45·2	44·0	43·6	40·6	38·2	26·0	23·2
Liver	246.5	143·0	116·2	62·3	33·6	24·9	5·6
Brain	0·4	0·4	0·3	0·3	0·4	0·4	0·4
Heart	1·0	1·1	1·1	1·2	1·2	1·2	1·4
Muscle (exc. hind-limb)	218·3	194·0	189·4	172·2	163·1	136·5	91·1
Hind-limb muscle	46·6	42·1	41·0	39·2	38·0	34·9	26·7
Total	558·0	424·6	391·6	315·8	274·5	183·9	148·4
	(20·9)	—	(21·9)	—	—	(48·2)	—
Experimental :							
Extracellular space	—	60·8	—	117·0	—	55·8	20·6
Liver	—	178.0	—	16·1	—	2·0	0·2
Brain	—	0·4	—	0·3	—	0·2	0·2
Heart	—	1·4	—	1·2	—	1·1	0·9
Muscle (exc. hind-limb)	—	120·5	—	98·8	—	70·5	48·8
Hind-limb muscle	—	12·0	—	26·7	—	30·5	16·4
Total	—	373·1	—	260·1	—	160·1	87·1

TABLE VII.—*The Glucose Content of the Extracellular Space and the Glucose Present in the Organs as Glycogen Expressed as mg. glucose per* 100 *g. body wt. in Control Rats After* 24 *hr. Fast and in Similar Rats Subjected to Bilateral Hind-limb Ischaemia, Between* 10.0 *a.m. and* 2.0 *p.m.*

Method of calculation and significance of figures in parentheses given in the text.

Time	9.30 a.m.	2.0 p.m.	5.0 p.m.
Controls :			
Extracellular space	26·6	24·2	19·3
Liver	10·4	3·5	3·5
Heart	1·5	1·4	1·3
Muscle (exc. hind-limb)	126·4	93·4	93·8
Hind-limb muscle	24·9	31·1	29·2
Total	189·8	153·6	147·1
	(28·8)	(31·9)	(40·3)
Experimental :			
Extracellular space	—	30·4	45·0
Liver	—	11·3	1·0
Heart	—	1·5	1·3
Muscle (exc. hind-limb)	—	45·0	24·4
Hind-limb muscle	—	5·2	10·6
Total	—	93·4	82·3
	—	(37·4)	(45·5)

TABLE VIII.—*The Glucose Content of the Extracellular Space and the Glucose Present in the Organs as Glycogen Expressed as mg. glucose per* 100 *g. body wt. in Control Adrenal Medullectomized Rats Fed up to the Time of the First Observations and in Similar Rats Subjected to Bilateral Hind-limb Ischaemia Between* 10.0 *a.m. and* 2.0 *p.m.*

Method of calculation and significance of figures in parentheses given in the text.

Time	9.30 a.m.	2.0 p.m.	5.0 p.m.	
Controls :				
Extracellular space	39·3	40·7	40·3	—
Liver	279·7	161·6	63·9	—
Heart	1·1	1·2	1·1	—
Muscle (exc. hind-limb)	247·1	186·4	184·1	—
Hind-limb muscle	52·8	42·1	33·1	—
Total	620·0	432·0	322·5	—
	(19·9)	(21·7)	(27·3)	—
Experimental :				
Extracellular space	—	44·1	40·7	22·0*
Liver	—	177·5	11·4	3·3
Heart	—	1·7	1·4	0·8
Muscle (exc. hind-limb)	—	195·0	247.1	198·4
Hind-limb muscle	—	12·7	15·7	5·0
Total	—	431·0	306·3	229·5
	—	(15·9)	(41·2)	(24·3)

* At death.

TABLE IX.—*The Glucose Content of the Extracellular Space and the Glucose Present in the Organs as Glycogen Expressed as mg. glucose per* 100 *g. body wt. in Control Adrenalectomized Rats Fed up to the Time of the First Observations and in Similar Rats Subjected to Bilateral Hind-limb Ischaemia Between* 10.0 *a.m. and* 2.0 *p.m.*

Method of calculation and significance of figures in parentheses given in the text.

Time	9.30 a.m.	2.0 p.m.	5.0 p.m.
Controls :			
Extracellular space	40·3	34·2	21·4
Liver	109·1	22·3	4·7
Heart	0·9	0·7	1:0
Muscle (exc. hind-limb)	129·8	117·0	136·1
Hind-limb muscle	29·9	28·6	28·7
Total	310·0	202·8	191·5
	(37·9)	(27·4)	(33·9)
Experimental :			
Extracellular space	—	33·1	9·3*
Liver	—	7·1	2·7
Heart	—	0·5	0·3
Muscle (exc. hind-limb)	—	126·8	100·5
Hind-limb muscle	—	3·6	3·0
Total	—	171·1	115·8
		(27·4)	(16·3)

* At death, approx. 4.0 p.m.

DISCUSSION

The effects of limb ischaemia on carbohydrate metabolism can be divided into three stages.

Changes during limb ischaemia

This period is characterized by a fall in the muscle glycogen level, a rise in the levels in the liver and heart and an increase in the amount of extracellular glucose. The release of glycogen from the uninjured muscle as lactate and pyruvate would be more than sufficient to account for the increase in the glucose content of the other compartments in both fed and fasted rats (Tables VI and VII) even allowing for the cost of the conversion of the lactate and pyruvate (Meyerhof, 1920). These changes are typical of the Cori cycle in the rat (Cori and Cori, 1928) and their suppression, except for those in the ventricles, by adrenal medullectomy shows that they are due to the release of adrenaline in response to the injury. The increased loss of " total body glucose " during this period could be explained by the metabolic cost of the continued movement of lactate and pyruvate through this cycle.

The rise in ventricular glycogen, first described by Cordier and Dessaux (1954), was not prevented by medullectomy in fed rats but did not occur in intact fasted rats or in fed adrenalectomized rats. Hormonal mechanisms are probably not directly involved in this. Very little glycogen is actually formed (Table VI). The effect does not depend on hyperglycaemia and the increase may be derived from the circulating lactate and pyruvate both of which can be utilized by the heart (see Evans, 1949) although they do not appear to form glycogen in the perfused heart (Bogue, Evans and Gregory, 1937 ; Braun-Menendez, Chute and Gregory, 1939). After medullectomy these compounds will still be released from damaged muscle just above the tourniquets. In the fasted rat the initial glycogen level is already maximal (Adronny and Russell, 1956) and after adrenalectomy the terminal accelerated destruction has already begun when the tourniquets are removed.

After limb ischaemia I

This stage lasts about 3–6 hr. and the changes during it are the most difficult to interpret. During this period total O_2 consumption and tissue temperature are falling rapidly although the liver, brain and muscle appear to be adequately oxygenated (Stoner, 1958).

In the fed rat the blood sugar rises to its maximum and there is a continuing fall in the glycogen content of the uninjured muscle. The level in the liver fell steeply but that in the ventricles continued to rise and there was some recovery in the damaged muscle. Brain glycogen was unaltered. Previous fasting reduced the size of most of these changes but the amount of glycogen lost from the uninjured muscle remained the same (Tables VI, VII). The mechanism of some of these changes is clear.

When the tourniquets are removed the circulation is flooded with lactate and pyruvate and this could account for the further rise in ventricular glycogen (see above). The latter did not occur in the experiments of Cordier and Dessaux

(1954) because the high environmental temperature (30°) they used would precipitate the onset of the final stage (Stoner, 1958).

Medullectomy prevented the hyperglycaemia and fall in the glycogen level in the uninjured muscle so that these changes can be attributed to the further release of adrenaline. Adrenaline does not affect the cerebral glycogen level (Kerr and Ghantus, 1936) so that this level remains unchanged. The constancy of this level supports a previous conclusion (Stoner, 1958) that the brain is adequately oxygenated for most of the survival period.

The glycogen stored in the damaged muscle appears to come from the circulating glucose (Table IV) and to reflect the height of hyperglycaemia. Like nucleotide regeneration (Threlfall and Stoner, 1957) it did not occur if ischaemia was prolonged for 6 hr. The glycogen is probably confined to those fibres capable of recovery (Moore, Ruska and Copenhaver, 1956). Although the very low intracellular glycogen level in the muscle fibres after ischaemia and the high extracellular glucose level would favour glycogen synthesis it is difficult to see why it should differ from the rest of the skeletal glycogen in its sensitivity to adrenaline unless the mechanism whereby adrenaline activates phosphorylase has been damaged during the ischaemia. According to Kovách, Takács, Menyhárt, Iranyi and Kalmár (1952) the phosphorylation of glycogen *in vitro* by minced muscle taken from the post-ischaemic limb is inhibited.

The increased loss of liver glycogen immediately after removal of the tourniquets is not due to the action of adrenaline nor, in the rat, would one expect it to be (Sherlock, 1949). It is also unlikely to be due to hepatic anoxia (Stoner, 1958). The mechanism of this is obscure and the possibility that it reflects changes in the electrolyte composition of the liver (Hastings, Ashmore and Cahill, 1956) remains to be explored.

Haist and Hamilton (1944) considered that there was a generalized failure in the ability to store glycogen after fatal limb ischaemia. Their clear-cut results were probably due to the severe injury they used, 12–15 hr. bilateral hind-limb ischaemia. With a shorter, but usually fatal, period of ischaemia this was not an invariable feature of ischaemic injury particularly during the early part of the response when glycogen is still being formed from glucose in the muscle and liver (Table IV).

A possible increase in the formation of glucose from non-carbohydrate sources cannot be excluded. However, the glycogen lost by the liver and uninjured muscle in addition to the lactate and pyruvate released from the damaged limbs when the tourniquets are removed is sufficient to account for the increase in extracellular glucose and glycogen content of the damaged muscle during the first 3 hr. after the ischaemic period even allowing for the cost of converting the lactate and pyruvate to glucose and for the consumption of glucose at the same rate as in the normal rat (Table VI). After the first 3 hr. none of the compartments shows any increase in glucose content so that after removal of the tourniquets in the fed rat it is never necessary to postulate increased gluconeogenesis. The picture is the same in the fasted rat (Table VII). While this almost certainly takes too static a view of carbohydrate metabolism it shows that more stringent tests must be applied if increased gluconeogenesis after this injury is to be established.

Increased gluconeogenesis has usually been postulated because of the effect of injury on the adrenal cortex. The results in the fed medullectomized rats (Table VIII) are of interest here since such rats have adequate amounts of cortical

tissue and can maintain their blood sugar level on fasting (Dexter and Stoner, 1952). They should correspond to the adrenalectomized rats maintained on cortical hormone in the experiments of Selye and Dosne (1941) and Engel and Fredericks (1957) and, in accordance with the " permissive " theory of adrenal cortical action (Ingle, 1951), increased gluconeogenesis should be seen after injury. The absence of any increase in extracellular glucose in the medullectomized rat after injury shows that the normal hyperglycaemia does not depend on a " permissive atmosphere " of cortical hormone or simply on an adequate level of liver glycogen (Engel and Fredericks, 1957) and emphasizes the causative role of adrenaline.

The arguments of the last two paragraphs depend on glucose consumption during this period not exceeding that in the controls. This seems to be the case (Tables VI, VII) but the same picture would be seen if consumption was greatly increased after injury and there was a compensatory increase in gluconeogenesis. This seems unlikely. Oxygen consumption is reduced (Stoner, 1958) so that if glucose catabolism was increased the accumulation of metabolites such as lactate and pyruvate would be seen but is not (Stoner *et al.*, 1952). Although the rate of disappearance of " total body glucose " at this stage is the same as in the controls even this may be too fast, for their body temperature may be as much as 5° below that of the controls and the Q_{10} for glycolysis is 1·47–1·63 (Bendall, 1951 ; Long, 1951).

After limb ischaemia II

The terminal phase in the rat sets in 3–6 hr. after the injury and is characterized by an increasingly rapid disappearance of the " total body glucose ". Its onset corresponds to the failure of O_2 transport and the changes can be largely attributed to the decreasing supply of O_2 to the tissues.

Adrenal medullectomy and especially adrenalectomy hasten the onset of this terminal phase. This can be interpreted in two ways.

In keeping with Cannon's (1929) concept of the adrenal medulla as a gland concerned in conditioning the animal to withstand assault it might be thought that the secretion of adrenaline will mobilize the carbohydrate stores into the extracellular space where the action of the adrenal cortical hormones, by inhibiting their utilization, will preserve them for the recovery period. Adrenal medullectomy and adrenalectomy prevent these defensive manoeuvres and shorten the survival period. However, if the absence of the adrenal, wholly or in part, simply interfered with the compensatory response of the cardiovascular system to injury so that the rat was unable to maintain the O_2 supply to the tissues the same changes in carbohydrate metabolism and shortening of the survival time would occur and there would be no need to postulate effects on the enzymes of carbohydrate metabolism. The teleologically attractive scheme outlined above must be examined more critically if it is to be established.

The relation of these results to other features of the general response to injury will be discussed elsewhere (Stoner and Threlfall, unpublished). Although further problems are created, by defining the changes in glycogen distribution after injury the results go some way to answering the questions posed in the introduction and explaining the mechanism of the changes. The most important features of the results are the emphasis they put on the role of the adrenal medulla in the

causation of the changes and the questions they raise about the role of the adrenal cortex.

SUMMARY

The effect of a 4 hr. period of bilateral hind-limb ischaemia on the blood sugar level and glycogen contents of the muscle, liver, ventricles and brain was determined in fed and fasted intact rats and in fed adrenal medullectomized and adrenalectomized rats at an environmental temperature of 18–22°.

In the intact rat, during limb ischaemia there is a fall in the glycogen level in the ischaemic and uninjured muscle with a rise in the blood sugar and liver glycogen levels. These changes depended on the presence of the adrenal medulla.

During the period of adequate oxygenation after limb ischaemia, the glycogen levels in the liver and uninjured muscle of the intact rat continued to fall with a rise in the blood sugar level and some recovery in the glycogen content of the muscle in the post-ischaemic limbs. The fall in muscle glycogen level was again dependent on the presence of the adrenal medulla. The ventricular glycogen level, if submaximal, rose during limb ischaemia and for a time afterwards. Glycogen formation from glucose continued in the uninjured muscle and liver although it could not usually be stored by these tissues.

When the O_2 supply to the tissues became inadequate there was a general decline in the blood sugar and tissue glycogen levels which continued until death. This was shared by the brain where the glycogen level had remained unaltered.

No evidence for the formation of glucose from non-carbohydrate sources was found in these experiments.

The increased loss of " total body glucose " during and after limb ischaemia was attibuted, in the early stages, to the metabolic cost of transformations in the Cori cycle and in the terminal stage to anoxia.

These results emphasize the importance of the adrenal medulla in the changes in carbohydrate metabolism during and after limb ischaemia.

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