

STUDIES ON THE MECHANISM OF SHOCK
NON-ESTERIFIED FATTY ACID METABOLISM IN NORMAL AND
INJURED RATS

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A STRIKING feature of the acute metabolic response to severe physical injury is the fall in the rate of heat production which occurs very shortly after the trauma (Stoner and Pullar, 1963; Stoner, 1968; Caldwell and Miksche, 1968). In attempting to explain this phenomenon we have studied the early effects of an injury, hind-limb ischaemia, on carbohydrate metabolism in the rat. It was found that shortly after this injury the oxidation of injected pyruvate was markedly reduced, in fact, to a much greater extent than would be expected from the lower body temperature of the rat at that time (Ashby, Heath and Stoner, 1965). A natural complement to this is a study of fatty acid oxidation under similar conditions, since fatty acids are almost entirely oxidized *via* acetyl-CoA (Fritz, 1961) while only part of the pyruvate enters the tricarboxylic acid cycle *via* this path.

Comparatively little is known about the effect of injury on fat metabolism (Levenson, Einheber and Malm, 1961). The fat stores of the body, like those of carbohydrate, are mobilized by limb ischaemia (Stoner, 1962; Matthews, 1965; Stoner and Matthews, 1967) and this is usually reflected by an increase in the concentration of non-esterified fatty acid (NEFA) in the plasma. However, under certain conditions, such as after limb ischaemia in fasting rats, this increase is not seen, probably because of poor perfusion of the fat depots, since fasted rats are more severely affected by this injury.

In the present experiments $1\text{-}^{14}\text{C}$ -palmitate was injected *i.v.* as a tracer for the oxidation of plasma NEFA. It was found that $^{14}\text{CO}_2$ excretion was decreased after limb ischaemia in both fed and fasted rats. The availability of the $1\text{-}^{14}\text{C}$ -palmitate for oxidation was assessed by studying its distribution and preliminary calculations (Stoner, 1966) suggested that an idea of the size of the change in NEFA oxidation induced by injury could be obtained from a comparison of the kinetics of $^{14}\text{CO}_2$ excretion in control and injured rats.

MATERIALS AND METHODS

Radioactive chemicals.— $1\text{-}^{14}\text{C}$ -palmitic acid (specific activity 120–145 $\mu\text{c}/\text{mg.}$) was obtained from the Radiochemical Centre, Amersham. This was converted to the Na salt and added to fresh rat serum (Bragdon and Gordon, 1958). The radioactive serum was filtered before injection.

Animal techniques.—Male albino Wistar rats (mean body wt. 226 ± 21 g. S.D.) of the Porton strain fed on M.R.C. diet 41b (Bruce and Parkes, 1956) were used. The diet was available to the fed rats until 9 a.m. on the day of the experiment; in the case of the fasted rats it was removed 24 hr. before the experiment. A 4 hr. period (9 a.m.–1 p.m.) of bilateral

hind-limb ischaemia was produced with tourniquets applied under ether anaesthesia lasting about 3 min. (Rosenthal, 1943) and the controls were also given a 3 min. period of ether anaesthesia at 9 a.m. The environmental temperature was 18–22°. The radioactive serum, 0.1 or 0.2 ml ($< 0.2 \mu\text{Eq. palmitate}$) per rat, was injected into a tail vein about 1.5 hr. after removal of the tourniquets in the fed rats, about 1 hr. after in the fasted rats and at about the same time of day in the controls. The time for the fed rats was chosen so that they would be in a fairly steady state (Stoner, Heath and Collins, 1960). A shorter interval was taken for the fasted rats so that they would be more comparable with the fed ones, since fasted rats are more severely affected by this injury (Threlfall and Stoner, 1954).

The excretion of $^{14}\text{CO}_2$ was studied in a tubular chamber (Heath, 1962). Five control or 5 injured rats were put in the chamber for each run so as to have sufficient CO_2 for accurate estimation in the early stages. In each experiment groups of control and injured rats in the same nutritional state were tested at the same time. Immediately before beginning a run the air-flow through the chamber was started at 4.0 l./min. and maintained constant at this rate. The dead time from the centre of the chamber was about 0.6 min. at this flow rate.

For results to be strictly comparable the time of injection of each rat had to be accurately known. As each rat was injected the time of injection was taken (± 0.1 min.) and it was immediately placed in a wire mesh compartment which was slid into the chamber. The run was timed from the injection of the third rat. Table I shows the average time taken to inject 5 rats in each group of experiments and the fractions of these times taken to inject the first 3 rats. Except for fed control rats the injections were evenly spaced, *i.e.* fraction = 0.5 when the third rat was injected in the middle of the injection period; and the timing was sufficiently reproducible from run to run for accurate comparisons to be made.

TABLE I.—*The Time \pm S.D. Taken to Inject 5 Rats, and the Fraction of this Time taken to Inject the First 3 Rats*

	Fed		Fasted		All
	Control	Injured	Control	Injured	
No. of expts.	4	4	4	3	
Time for 5 rats (min.)	3.5 ± 0.5	3.6 ± 0.2	3.0 ± 0.3	3.9 ± 0.4	3.5
Fraction for 3 rats	0.65 ± 0.04	0.48 ± 0.06	0.55 ± 0.08	0.55 ± 0.08	0.56 ± 0.09

The $^{14}\text{CO}_2$ was collected in N.NaOH in 3 traps in series. Samples for analysis were taken from the first trap at 4, 8, 12 and 16 min. (± 0.05 min.) after the start of the run. The first trap was changed at set intervals thereafter. At the end of each run the contents of all 3 traps were analysed for $^{14}\text{CO}_2$. To calculate total $^{14}\text{CO}_2$ excretion (Heath, 1962) the efficiency of each trap was assumed to remain constant throughout the run. Except for the first sample, for which analyses could not be duplicated, the $^{14}\text{CO}_2$ estimations were precise to 2 per cent.

The plasma NEFA concentrations at the time of these injections were determined in separate experiments. Control and injured rats were given 0.1–0.2 ml. unlabelled rat serum intravenously with the same amount of handling as in the $^{14}\text{CO}_2$ excretion experiments and guillotined 3–5 min. later. Blood was collected from the severed neck vessels, and heparinized and the plasma separated.

The rate of disappearance of 1- ^{14}C -palmitate from the blood was studied on cannulated rats in the same way as for ^{14}C -glucose (Ashby *et al.*, 1965) except that the blood in the dead space of the cannula was removed before each sampling (0.1 ml.). At the end of the experiment a larger sample was withdrawn to determine the haematocrit value and plasma NEFA concentration.

Analytical methods.—The $^{14}\text{CO}_2$ from excretion experiments was estimated as $\text{Ba}^{14}\text{CO}_3$ (Heath, 1962), weighed specimens of which were counted as a suspension in Cab-O-Sil scintillator fluid [1 : 3 : 3 toluene, dioxan, ethyl cellosolve mixture containing 1 per cent PPO, 0.05 per cent di-methyl POPOP, 8 per cent naphthalene (Nuclear Enterprises Ltd., Edinburgh) and 4 per cent Cab-O-Sil (Packard Instrument Co., La Grange, Ill.)] in a Packard

Tri-Carb scintillation counter, standardized with $\text{Ba}^{14}\text{CO}_3$ prepared from $\text{Na}_2^{14}\text{CO}_3$ of known specific activity (Radiochemical Centre, Amersham).

^{14}C -NEFA in blood was extracted by the procedure of Dole and Meinertz (1960). Three heptane extractions were carried out. The extracts were bulked and evaporated to dryness in a counting vial and dissolved in 2.0 ml. toluene. Ten ml. scintillation fluid (0.5 per cent PPO, 0.03 per cent di-methyl POPOP in toluene) were added and the ^{14}C determined using ^{14}C -toluene (Packard Instrument Co., La Grange, Ill.) as an internal standard. The terminal blood volume was calculated from the haematocrit value, corrected for trapped plasma (Constable, 1963), assuming an erythrocyte volume of 2.8 ml./100 g. body wt. As these experiments only lasted about 10 min. corrections were made for the amount of blood removed. The ^{14}C in the blood was expressed as a fraction of the injected dose.

The doses of 1- ^{14}C -palmitate were determined by treating aliquots of the sera for injection in the same way as the blood samples. The efficiency of this procedure was tested by digesting 0.1 ml. labelled serum in a mixture of 2.5 ml. M. hyamine in methanol and 2.5 ml. ethyl-cello-solve, diluting it with 50 ml. scintillation fluid and then determining the label in 0.1 ml. of this solution as above. Results were 3 per cent higher than by the standard method.

To determine the distribution of radioactivity in the body, rats were guillotined 10 min. after the i.v. injection of 1- ^{14}C -palmitate and bled out. The organs and tissues were frozen in liquid N_2 , weighed and homogenized in 20 vol. chloroform : methanol mixture (2 : 1) using an Ultra-Turrax homogenizer (Janke and Kunkel K. G., Staufen i. Br.). The precipitate was filtered off next day and re-extracted with acidified chloroform : methanol mixture (1.0 ml. conc. HCl /300 ml. mixture). The extracts were then treated as described by Olivecrona (1962). The carcass (with any remaining organs) was either minced or, more usually, frozen and pulverized in a metal mortar before being extracted in the same way in a top-drive blender. The final extracts were evaporated to dryness in a stream of N_2 and their lipid contents determined gravimetrically before being dissolved in toluene. Specimens of these toluene solutions were analysed for ^{14}C as above. Recoveries of 1- ^{14}C -palmitate added to blood, liver and minced carcass are shown in Table II.

The plasma NEFA concentration was determined by the method of Dole and Meinertz (1960) using Nile Blue as an indicator. The results are expressed in terms of palmitic acid.

TABLE II.—*Recovery of 1 ^{14}C -Palmitate Added to Samples of Blood, Liver and Minced Rat Carcass*

Tissue	Percentage recovery	
	After first extraction	After second extraction
Blood . . .	106.3	—
Blood . . .	91.8	—
Liver . . .	96.8	108.6
Liver . . .	88.8	101.9
Carcass . . .	94.7	102.6
Carcass . . .	91.6	96.9

RESULTS

The clinical appearance of the injured rats was as in earlier experiments (Ashby *et al.*, 1965; Stoner, 1961*a, b*). None died during these experiments but, from previous work, most would have done so if they had not been killed first.

CO₂ excretion.—In each run the rate of CO_2 excretion fell 20–30 per cent in the first hour to a constant value (Table III). Probably the handling associated with injection elevated the initial rate. The average rates in the injured were 55 per cent of those in the controls.

Plasma NEFA concentrations.—The plasma NEFA concentration in rats in the same condition as for the $^{14}\text{CO}_2$ excretion experiments was determined in separate experiments (Table IV). The concentrations in the fed rats were

TABLE III.—Average Rates of CO_2 Excretion in mg./min./kg.

		Min. after injection	4-8	8-12	0-60	60-120
Fed	Controls	.	79	70	62	57
	Injured	.	42	40	35	30
Fasted	Controls	.	69	59	53	51
	Injured	.	38	30	30	26

TABLE IV.—NEFA Concentration and Pool size in the Plasma of Control Rats and Injured Rats 1.5 hr. (Fed) and 1 hr. (Fasted) after 4 hr. Bilateral Hind-limb Ischaemia, Guillotined 3-5 min. after i.v. Injection of Rat Serum (see text). The Haematocrit Values Used to Calculate the Pool Sizes are also shown.

Group and nutritional status	Plasma NEFA concentration μ Eq./ml.		Haematocrit	Plasma pool size μ Eq./100 g. body wt. (S.E. range)
	(Geom. mean; S.E. range)			
Fed, control (12)	0.592 (0.544-0.644)	.	43	2.0-2.4
Fed, injured (14)	0.685 (0.640-0.733)	.	54	1.5-1.8
Fasted, control (12)	1.079 (1.011-1.152)*	.	42	3.9-4.5
Fasted, injured (23)	0.733 (0.694-0.773)†	.	57	1.5-1.6

Number of rats given in parentheses.

* Greater than in fasted, injured rats ($P < 0.001$).

† Greater than in fed, control rats ($P < 0.05$).

higher than those found previously (Stoner, 1962), presumably due to the handling associated with the injection. As before, there was no significant difference between control and injured rats sampled at this time of day and at this interval after limb ischaemia. Higher concentrations were found in the fasted rats but those in the injured were lower than those in the controls. The possible significance of this has been discussed elsewhere (Stoner and Matthews, 1967).

Tissue lipids and their radioactivity (Table V).—In fed rats the concentrations of lipid extracted by chloroform : methanol per unit wet wt. of tissue were not

TABLE V.—The Total Lipid Concentrations and Distribution of Radioactivity in the Carcass and Certain Organs of Control Fed Rats and Fed Rats Killed 1.5 hr. after 4 hr. Bilateral Hind-limb Ischaemia and in the Liver of Control Fasted Rats and Fasted Rats Killed 1 hr. after the Injury. $1-^{14}C$ -Palmitate Injected i.v. 10 min. before Death.

Tissue	Total lipid (g/100 g. wet wt.)		Radioactivity	
	Mean \pm S.D.		Percentage of injected dose (range)	
	Control	Injured	Control	Injured
Carcass	9.3 \pm 0.7 (5)	9.8 \pm 1.6 (5)	35.8-53.8	35.3-53.5
Liver	4.18 \pm 0.61 (5)	4.09 \pm 0.31 (6)	19.3-37.1	15.2-39.5
Liver*	5.20 \pm 0.39 (5)	4.06 \pm 0.10 (5)†	10.0-12.2	8.7-11.8
Kidney	4.16 \pm 0.89 (5)	3.95 \pm 0.90 (6)	0.6-1.2	0.8-1.4
Heart	2.71 \pm 0.62 (5)	2.60 \pm 0.46 (6)	0.2-0.7	0.7-2.0
Gut	4.69 \pm 0.71 (5)	4.94 \pm 1.13 (5)	2.0-3.8	1.5-4.5
Blood	—	—	0.3-0.7	0.3-1.2
Total	—	—	74.7-81.2	72.3-86.4

Number of rats shown in parentheses. Carcass includes all the tissues not otherwise mentioned.

* Fasted rats.

† Less than fasted controls, $P < 0.01$.

affected by the injury nor was there any difference between the liver values when expressed as g./100 g. body wt. Variable amounts of material, usually < 1.0 g./100 g. wet wt. of tissue, were recovered by further extraction with acidified chloroform : methanol but there was no striking difference between the two groups in this respect.

In fasted rats only the livers were studied. In the controls they contained more lipid than in the fed controls ($P < 0.05$). An hour after removal of the tourniquets the concentration of liver lipid was significantly less than in the appropriate controls. This difference was also seen when the results were expressed as g. lipid/100 g. body wt.: controls 0.180 ± 0.011 (S.D.), injured 0.135 ± 0.010 , $P < 0.001$.

In both fed and fasted rats, studied 10 min. after the injection of $1\text{-}^{14}\text{C}$ -palmitate, injury had little effect on the distribution of the label outside the injured area. Fasting itself significantly lowered the fraction of the label remaining in the liver at 10 min. (Table V). The uptake of $1\text{-}^{14}\text{C}$ -palmitate by the damaged muscle of the hind-limbs was low. In 4 fed controls the ratio of the percentage of the dose/g. wet wt. in the hind-limb muscles to that in the pectoral muscles was 0.79 ± 0.34 . In 4 injured fed rats this ratio was 0.20 ± 0.02 . This would have little effect on the overall picture.

Disappearance of $1\text{-}^{14}\text{C}$ -palmitate from the blood stream.—The disappearance of $1\text{-}^{14}\text{C}$ -palmitate from the blood during the first 11 min. after i.v. injection is shown in Fig. 1. There were no significant differences between any groups. The average plasma NEFA concentrations at the ends of the runs are given in the legend to Fig. 1 and were higher than those usually found in uncannulated control or injured rats, due to heparinisation and handling.

$^{14}\text{CO}_2$ excretion.—The results for each run are shown in Table VI with the mean values for each class. Despite day-to-day variation in the percentage of the injected label excreted as $^{14}\text{CO}_2$ the fasted controls excreted more than the fed controls and the injured rats always excreted less than their controls. Comparing the $^{14}\text{CO}_2$ excretion in experiments done on the same day the average ratio, injured/control, at the end of 2 hr. was 0.646 ± 0.050 (S.D.) in the fed rats and 0.663 ± 0.084 (S.D.) in the fasted. The inhibition was much greater during the first few min. of the experiment. After 8 min. the same ratios were 0.439 ± 0.052 (S.D.) and 0.436 ± 0.044 (S.D.) respectively.

For a particular class of rat there was little variation in the shape of the $^{14}\text{CO}_2$ excretion curve. All the curves had at least 2 phases, an early rapid excretion of $^{14}\text{CO}_2$ followed by a much slower one. By plotting the curves for the data in Table VI it can be shown that all the curves extrapolate back to nearly zero time (< 1 min.) for zero excretion. This shows that there was very little delay between the uptake of $1\text{-}^{14}\text{C}$ -palmitate and the commencement of $^{14}\text{CO}_2$ excretion. This means that only very small pools of intermediates can be interposed between plasma palmitate and the oxidation of a fraction of it to CO_2 .

DISCUSSION

Before the depression of fatty acid oxidation after limb ischaemia, shown by the results in Table VI, can be attributed to metabolic alterations at a cellular level some other possibilities must be considered.

TABLE VI.—*Excretion of $^{14}\text{CO}_2$ After the i.v. Injection of 1- ^{14}C -palmitate in Injured and Control Rats*

Treatment	Exp. No.	Time (min.) after injection								
		4	8	12	16	30	60	90	120	180
		Percent of dose as $^{14}\text{CO}_2$								
Fed controls	1	3.77	8.54	12.72	14.96	20.90	29.95	34.84	38.24	43.04
	2	4.41	8.78	12.90	16.32	24.17	34.20	39.53	42.97	47.36
	3	5.26	10.15	14.23	17.55	23.72	31.41	36.01	—	42.53
	4	6.49	12.57	17.10*	19.50	24.72	32.76	37.78	41.10	45.08
	5	5.30*	11.42	14.45	17.01	22.72	31.69	36.38	40.02	45.08
	Mean	5.05	10.29	14.28	17.07	23.25	32.00	36.91	40.38†	44.58
Fed injured	1T	1.57	3.31	4.90	6.82	10.70	16.96	21.17	24.12	
	2T	1.75	3.85	6.22	7.72	12.71	19.07	22.99	25.66	
	3T	1.97	3.92	5.82	6.95	10.71	16.72	20.59	23.42	
	4T	2.63	6.20	8.34	10.40	15.49	22.29	26.09	28.87	
	5T	2.65	5.60*	7.38	8.75	13.34	20.07	24.35	27.77	
	Mean	2.11	4.58	6.53	8.13	12.59	19.02	23.04	25.97	
Fasted controls	6	6.28	12.86	—	21.91	31.78	40.78	46.23	49.74	54.01
	7	8.97	17.78	23.32	26.69	36.08	46.65	51.68	54.65	59.06
	8	9.52	17.87	23.06	28.14	36.31	45.64	50.73	53.98	57.58
	9	4.60	9.88	13.56	16.12	22.91	32.83	39.16	43.64	48.36
	Mean	7.34	14.60	19.56†	23.22	31.77	41.47	46.95	50.50	54.65
Fasted injured	6T	2.50*	4.94	6.90	8.89	13.84	20.17	24.08	26.70	
	7T	3.21	8.26	11.78	15.29	22.79	31.10	35.88	38.81	
	8T	3.88	7.44	11.37	13.05	20.42	29.70	34.50	37.67	
	9T	1.92	4.73	6.89	8.76	14.22	21.89	26.98	30.91	
	Mean	2.88	6.34	9.24	11.50	17.82	25.72	30.36	33.52	

For experimental details see text.

* Values were extrapolated from neighbouring points, never more than 1 min. away.

† indicates that when one value was missing the means were obtained by interpolating the missing value.

Experiments with same arabic number, e.g. 2 and 2T, were performed on the same day.

The first question concerns the distribution of the tracer dose—is this comparable in the injured rats and their controls? The very rapid turnover of the plasma NEFA makes it technically difficult to obtain accurate disappearance curves for the first few min. after the injection of 1- ^{14}C -palmitate. However, no significant differences were found between the curves which were obtained (Fig. 1) and so much of the dose had disappeared from the circulation by 2 min. that any differences which did exist must be relatively unimportant. There was clearly no hindrance to the uptake of 1- ^{14}C -palmitate by the tissues and its early distribution was very similar in the control and injured rats.

Is sufficient O_2 available to the tissues of the injured rats for substrate oxidation? From previous physiological and biochemical experiments (Stoner, 1958; 1961a, b, 1963; Stoner and Threlfall, 1954, 1960; Threlfall and Stoner, 1961) it was concluded that, in the fed, injured rats, O_2 transport to the tissues would be adequate at the time when they were studied. Less is known about fasted, injured rats but the shorter interval between removal of the tourniquets and injection should compensate for the earlier deterioration in their condition.

Mitochondrial changes in the injured animals are possible but Aldridge and Stoner (1960) found that liver mitochondria behaved normally *in vitro* even when they were isolated from injured rats on the point of death.

For rapidly oxidized substrates the rate of $^{14}\text{CO}_2$ excretion is controlled in the early stages by the hold-up of $^{14}\text{CO}_2$ in the bicarbonate pools of the body. An increase in the size of these pools would delay excretion but, in fact, their size is somewhat reduced after the injury (Stoner *et al.*, 1960; Ashby, *et al.*, 1965).

We have concluded that the differences in $^{14}\text{CO}_2$ excretion from the injured and controls reflected differences in their cellular metabolism and have attempted to estimate them semi-quantitatively.

The metabolism of NEFA can be summarized as in Fig. 2. Some of the NEFA is stored as triglyceride and is not oxidized in the course of these short experiments. This accounts for the incomplete recovery of the label as $^{14}\text{CO}_2$.

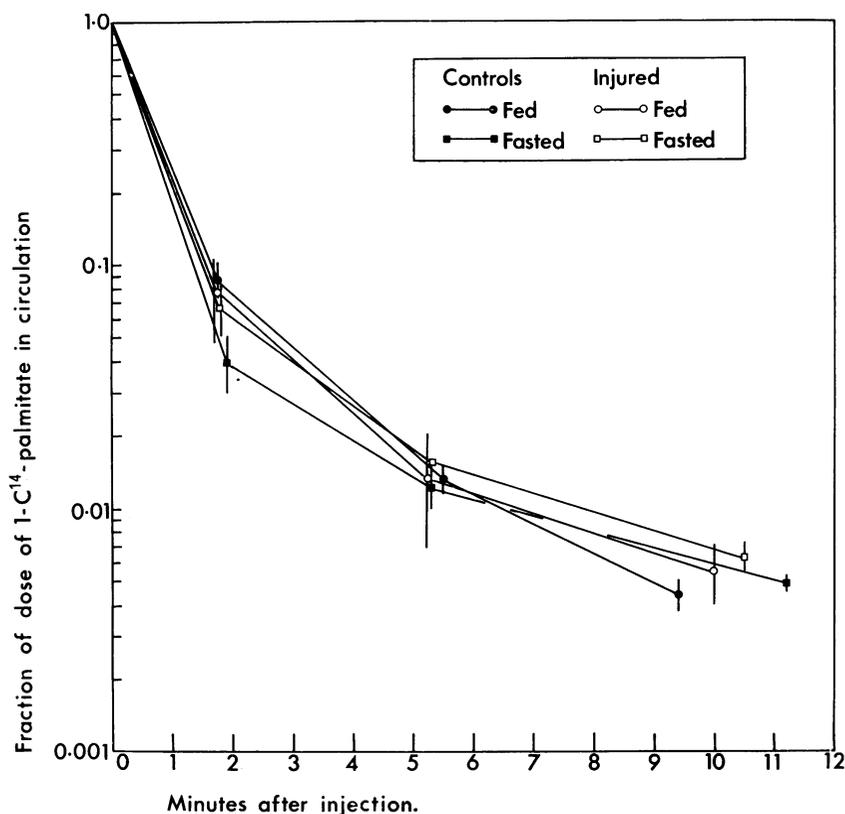


FIG. 1.—Disappearance of 1- ^{14}C -palmitate from the blood after i.v. injection. Fed injured injected 1.5 hr. and fasted injured 1 hr. after 4 hr. period of bilateral hind-limb ischaemia. Symbols indicate means and the vertical lines S.Ds.

Plasma NEFA concentration ($\mu\text{Eq./ml.}$) at end of run:

Type	No of rats	Mean	Range
Fed, control	4	0.78	0.44-1.24
Fed, injured	6	1.14	0.55-1.67
Fasted, control	2	1.27	1.24-1.30
Fasted, injured	3	1.33	0.61-1.72

Of the NEFA oxidized, some can be oxidized directly while some only indirectly, after it has passed through pools of intermediate compounds mainly of triglyceride and ketone bodies. Stein and Stein (1962) have described the existence of 2 triglyceride pools in the tissues with different rates of turnover. Consequently, after a single i.v. injection of 1- ^{14}C -palmitate some $^{14}\text{CO}_2$ will be produced rapidly while the rest will appear more slowly, as was observed (Table VI). The first part (direct oxidation) was more affected by injury than the second (indirect oxidation).

The distinction between direct and indirect oxidation is a practical one which has been demonstrated in man and fully discussed by Havel, Ekelund and Holmgren (1967). Direct oxidation involves several intermediates but present only in very small quantities as the delay between the disappearance of 1- ^{14}C -

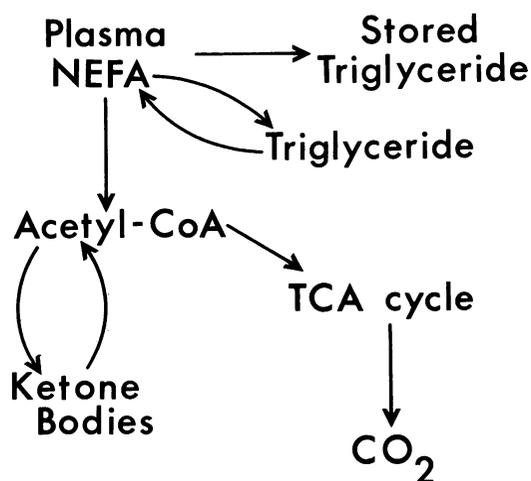


FIG. 2.—Pathways of plasma NEFA metabolism.

palmitate from the plasma and the appearance of $^{14}\text{CO}_2$ in the expired air is very short. From the marked change in the rate of excretion between 16 and 30 min. (Table VI) it would appear that excretion from direct oxidation was substantially completed by 30 min. The much slower excretion of $^{14}\text{CO}_2$ after the first 30 min. was due to indirect oxidation in which label is passed through relatively large intermediate pools. By 2 hr. after the injection $^{14}\text{CO}_2$ excretion had nearly ceased and the total excretion to 2 hr. is a measure of direct + indirect oxidation. This was 35 per cent less in the injured rats than in the paired controls (Results). Indirect oxidation makes some contribution to $^{14}\text{CO}_2$ excretion at all times but direct oxidation only in the first 16 min. At earlier times than this the reduction caused by injury was greater, *e.g.* 56 per cent at 8 min. This simple calculation shows that while indirect oxidation was inhibited less than 35 per cent, direct oxidation was inhibited by more than 56 per cent. The latter is comparable with the observed inhibition of 2- ^{14}C -pyruvate oxidation, *i.e.* about 50 per cent (Ashby *et al.*, 1965, their Fig. 5). It is difficult to convert these figures to absolute rates in a satisfactory fashion, taking account of the differences in plasma NEFA concentration, etc. However, such adjustments

would not affect the overall conclusion. It is clear that these results, with those of Ashby *et al.* (1965) imply considerable inhibition of the final common path of fat and carbohydrate oxidation at this early stage after ischaemic limb injury. It is of interest that Koltun and Gray (1957), in their study of acetate metabolism in rats after tumbling trauma, postulated reduced formation of citrate in liver, kidney and to a lesser extent muscle from either a decreased rate of formation of acetyl CoA or inhibition of citrate synthase.

Since the final steps in the direct and indirect oxidation of NEFA are the same, it appears anomalous that the indirect pathway can be less inhibited than the direct. The work of Coxon and Robinson (1959) on regional arterio-venous differences in the specific activity of blood CO₂ after the infusion of ¹⁴C-labelled substrates showed that organs differed in their rates of elimination of metabolic CO₂. The rates for liver and kidney were much faster than for skeletal muscle. If the severe inhibition of direct oxidation seen in our experiments occurred in those organs with rapid rates of ¹⁴CO₂ elimination the anomaly would disappear.

SUMMARY

1-¹⁴C-palmitate has been injected intravenously into fed and fasted rats 1.5 and 1 hr. after a 4 hr. period of bilateral hind-limb ischaemia. Its disappearance from the plasma, its initial distribution in the tissues and the production of ¹⁴CO₂ from it were studied and the results compared with those in similar control animals. Although the injury did not significantly alter the rate of removal of 1-¹⁴C-palmitate from the plasma or its distribution in the tissues, it depressed the rate of ¹⁴CO₂ excretion particularly during the first few minutes after the injection. These findings are attributed to inhibition of plasma non-esterified fatty acid oxidation by the tricarboxylic acid cycle.

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REFERENCES

- ALDRIDGE, W. N. AND STONER, H. B.—(1960) *Biochem. J.*, **74**, 148.
ASHBY, M. M., HEATH, D. F. AND STONER, H. B.—(1965) *J. Physiol.*, **179**, 193.
BRAGDON, J. H. AND GORDON, R. S.—(1958) *J. clin. Invest.*, **37**, 574.
BRUCE, H. M. AND PARKES, A. S.—(1956) *J. Anim. Techs. Ass.*, **7**, 54.
CALDWELL, F. T. AND MIKSCH, L.—(1968) *Ann. N.Y. Acad. Sci.*, in Press.
CONSTABLE, B. J.—(1963) *J. Physiol.*, **167**, 229.
COXON, R. V. AND ROBINSON, R. J.—(1959) *J. Physiol.*, **147**, 487.
DOLE, V. P. AND MEINERTZ, H.—(1960) *J. biol. Chem.*, **235**, 2595.
FRITZ, I. B.—(1961) *Physiol. Rev.*, **41**, 52.
HAVEL, R. J., EKELUND, L.-G., AND HOLMGREN, A.—(1967) *J. Lipid. Res.*, **8**, 366.
HEATH, D. F.—(1962) *Biochem. J.*, **85**, 72.
KOLTUN, W. L. AND GRAY, I.—(1957) *Am. J. Physiol.*, **190**, 183.
LEVENSON, S. M., EINHEBER, A. AND MALM, O. J.—(1961) *Fed. Proc.*, **20**, 99.
MATTHEWS, J.—(1965) *Experientia*, **21**, 611.
OLIVECRONA, T.—(1962) *Acta physiol. scand.*, **54**, 295.
ROSENTHAL, S. M.—(1943) *Publ. Hlth Rep. Wash.*, **58**, 1429.

- STEIN, Y. AND STEIN, O.—(1962) *Biochim. biophys. Acta*, **60**, 58.
- STONER, H. B.—(1958) *Br. J. exp. Path.*, **39**, 251.—(1961a) *Scientific Basis of Medicine Ann. Rev.*, p. 172.—(1961b) *Fed. Proc.*, **20**, 38.—(1962) *Br. J. exp. Path.*, **43**, 556.—(1966) Conference on Energy Metabolism and Body Fuel Utilization. Boston (Harvard University Printing Office) p. 104.—(1968) *Ann. N.Y. Acad. Sci.*, in Press.
- STONER, H. B., HEATH, D. F. AND COLLINS, O. M.—(1960) *Biochem. J.*, **76**, 135.
- STONER, H. B. AND MATTHEWS, J.—(1967) *Br. J. exp. Path.*, **48**, 58.
- STONER, H. B. AND PULLAR, J. D.—(1963) *Br. J. exp. Path.*, **44**, 586.
- STONER, H. B. AND THRELFALL, C. J.—(1954) *Biochem. J.*, **58**, 115.—(1960) In 'Biochemical Response to Injury'. Oxford (Blackwell), p. 105.
- THRELFALL, C. J. AND STONER, H. B.—(1954) *Q. J. exp. Physiol.*, **39**, 1.—(1961) *Biochem. J.*, **79**, 553.
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