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TRANSLOCASE INHIBITION IN ENDOTOXIC SHOCK: A LATE STEP IN THE SEQUENCE OF LETHAL EVENTS

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Abstract—Respiratory control ratios and translocase-mediated rates of 8-[^{14}C]ADP entry were studied in liver mitochondria isolated from mice in which shock had been induced by the intraperitoneal injection of a lethal amount of a lipopolysaccharide endotoxin preparation from *E. coli*. No changes in either parameter were observed in the first half hr. ADP entry was found to be already substantially inhibited in liver mitochondria isolated from saline-injected control animals, but greater levels of inhibition were consistently seen in organelles from mice which had received endotoxin 6 hr previously; the respiratory control ratio was not significantly affected as a result of the administration of endotoxin. The full extent of translocase inhibition could not be assessed precisely *in vivo*, partly because artefacts arising during the isolation procedure of the organelles tended to reverse the effect to some degree.

In 1970, Williamson, Refino and LaNoue [1] suggested that in endotoxin-induced shock the primary metabolic fault produced by the toxin might be an effect on the anionic permeability of the inner mitochrondrial membrane (IMM). Mela and her associates [2] referred briefly to a possibility that the translocase-mediated exchange of ATP for ADP across the IMM might become defective in the shocked condition. Three lines of evidence were later presented in support of the concept that shock appears to represent an attenuation of the energised movement of essential metabolites across the IMM [3]. Later, in a detailed analysis of the shock syndrome [4], the following sequence of events was advanced to account for the fundamental metabolic changes that occur in the syndrome: severe injury⟶lipolysis within adipose tissue, usually mediated by elevated, sustained catecholamine secretion → increase in levels of circulating non-esterified fatty acids (NEFAs) → uptake of NEFAs by target organs → esterification with coenzyme A to give long-chain fatty acyl thioesters (LCFACoAs) → competitive inhibition by LCFACoAs [5] of the adenine nucleotide translocase, which catalyses the exchange of cytosolic ADP for mitochondrial ATP [6, 7].

Since only the last of these changes lacked experimental verification, the purpose of the present study has been to examine the activity of the adenine nucleotide translocase in liver mitochondria from mice treated with a lethal dose of endotoxin. A preliminary communication has appeared [8].

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MATERIALS AND METHODS

Animals and the induction of shock. White female mice NMRI; Brönger, Halle-Westfalen, 8–16 weeks old and weighing 20–32 g, were injected i.p. with previously dried endotoxin (lipopolysaccharide W: *Escherichia coli* 055:B5, Difco, Detroit) in pyrogen-free 0.15 M saline at 60 mg/kg body weight, or with saline (8 ml/kg) as controls. Previous experiments had shown that this dose of endotoxin is of the order of twice the LD_{50}; fatalities generally occurred 8–10 hr after administration. Ambient temperatures ranged from 20.8° to 23.7°.

Preparation of mitochondria. The animals were killed by decapitation $\frac{1}{2}$ hr or 6 hr after endotoxin injection. In the latter experiments, rectal temperatures were measured just prior to injection and $5\frac{1}{2}$ hr thereafter. Livers were quickly removed, freed from the gall bladder, weighed, cut up and homogenised by hand with four passes of a loosely-fitting Teflon Potter–Elvehjem homogeniser (clearance, 0.3 mm) in 7 vol of chilled medium containing 0.25 M sucrose, 20 mM triethanolamine hydrochloride (TRAP), and 1 mM ethylene glycol-*bis*-(2-aminoethyl)-tetra-acetic acid (EGTA), pH 7.2. The method was essentially that of Klingenberg and Slenczka [9], but with one important modification of technique. After the initial centrifugation at 7800 *g* and the careful removal of floating fat, the mitochondrial pellet was not resuspended, but was washed gently with about 20 vol medium, causing minimal agitation, and respun at the same speed. Traces of fat were again carefully removed before re-homogenising the pellet to give a final volume approximately equivalent to that of the original liver. All steps following cutting the livers up into small pieces were conducted at 4°.

Measurement of respiratory control. Respiratory controls ratios (oxygen uptake in the presence of

inorganic phosphate, substrate and ADP, or State 3 ÷ oxygen uptake in the presence of inorganic phosphate and substrate, or State 4) were measured as soon as the mitochondrial preparations were available by means of an oxygen electrode at 25° linked to a chart recorder; the basic solution contained 0.14 M potassium chloride and 5 mM morpholinopropane sulphonic acid at pH 7.0. Successive additions of phosphate (final concentration 2.2 mM), succinate (10 mM) and ADP (135 μM) were made once the mitochondrial suspension (20 μl) had been added. The number of State 3–State 4 transitions recorded from any single sample of mitochondria was sometimes as many as six or seven, and never less than four

Activity of the adenine nucleotide translocase. Adenine nucleotide translocation was measured at 0° by following the entry of 25 μM 8-[^{14}C]-ADP (54,000–68,000 dpm; New England Nuclear) into liver mitochondria (about 0.8–1.4 mg protein per sample) in a final vol of 520 μl. All solutions were prepared in the sucrose/TRAP/EGTA homogenising medium. Duplicate vials were set up at the same time, in which the mitochondria (100 μl) were pretreated at 0° for 5–6 min with a preparation of bovine serum albumin (BSA; final concentration, 40 mg/ml) that was essentially free from fatty acids (Sigma). In some experiments amounts of mitochondria and reaction volumes were halved throughout. Translocation was initiated 145–175 min after the deaths of the animals. Entry was terminated after 30 sec by the addition of the specific inhibitor carboxyatractyloside [10] (Boehringer) at a final concentration of 50 μM; carboxyatractyloside was also present in control tubes from the start. Mitochondria were promptly spun down at 10,000 *g* for 4 min. The pellet surfaces were carefully washed once with about 700 μl chilled medium and respun for 2 min before dispersing in 250 μl 2% aqueous Lubrol WX (Imperial Chemical Industries Ltd.). Radioactivity was measured in 200 μl aliquots of both the mitochondrial and the supernatant fractions; rates of ADP entry were related to the protein contents of the preparations as measured by a biuret method.

Temperature falls in moribund and killed mice. Falls in rectal temperature were also measured at appropriate intervals in mice that had received endotoxin at 60 mg/kg, or which had been killed by cervical dislocation. In the latter cases the rectal thermometer, consisting of a small thermistor mounted in glass (2.4 × 9 mm), was left in position within the dead animal during the period of measurement.

RESULTS

Response to endotoxin. Animals treated with endotoxin soon became inactive, and appeared comatose; their fur became ruffled, and diarrhoea was usually apparent. Rectal temperatures fell by an average of 3.6 ± 1.6° in the first 5½ hr; the range of falls was 1.5–6.1°, while temperatures in saline-treated control animals fluctuated randomly to lesser extents. In other experiments, the rectal temperatures of mice given endotoxin began to fall within the first hr. The average rate of temperature decrease in the 6 hr animals was therefore almost −0.7° per hr. The rates of cooling of four mice killed by cervical dislocation were much more rapid; the temperature fell by about 4° in the first 20 min, at which time the rate of temperature loss had become −11.1° per hr.

Respiratory control and translocation. Mitochondrial integrity and function, as judged by measurements of respiratory control, were unaffected after ½ hr of endotoxin treatment (Table 1), while damage was negligible after 6 hr (Table 2). Rates of ADP entry into experimental and control mitochondria are also given in Tables 1 and 2. The intention of including BSA in the reaction medium was to reveal the uninhibited activity of the translocase [11]. No changes were observed during the first half hour of endotoxin treatment (Table 1), but comparisons of enzymic activities in the absence and presence of BSA indicate a substantial measure of endogenous inhibition in mitochondria from saline-injected animals (Tables 1 and 2). Although rates of ADP entry in the absence of BSA were consistently and signifi-

Table 1. Respiratory control ratios and rates of translocase-mediated ADP entry into liver mitochondria from mice 30 min after injection with endotoxin (60 mg/ml) or saline vehicle

Treatment	Respiratory control ratios	ADP entry, *p*-moles per mg mitochondrial protein per 30 sec		Percentage of translocase activity revealed by BSA
		No BSA	BSA, 40 mg/ml	
Endotoxin	3.1	1200	2190	45.2
Saline	3.4	1420	2420	41.2
Endotoxin	4.0	1420	2200	35.6
Saline	3.8	1280	2040	37.2
Endotoxin	3.9	1320	2240	41.1
Saline	3.6	1130	1960	42.3
Endotoxin	4.3	950	1750	45.7
Saline	4.3	950	1780	46.6
Averages Endotoxin	3.8 ± 0.5	1220 ± 200	2100 ± 230	41.7
Averages Saline	3.8 ± 0.4	1200 ± 200	2050 ± 270	41.6

*Means ± standard deviations are given in the last two lines of the first three columns of figures. For pairs of percentage values, $P < 0.475$ ($t = 0.057$).

Table 2. Respiratory control ratios and rates of translocase-mediated ADP entry into liver mitochondria from mice 6 hr after injection with endotoxin (60 mg/ml) or saline vehicle

Treatment	Respiratory control ratios	ADP entry, *p*-moles per mg mitochondrial protein per 30 sec		Percentage of translocase activity revealed by BSA
		No BSA	BSA, 40 mg/ml	
Endotoxin	3.1	1020	2360	56.8
Saline	3.9	1540	2740	43.9
Endotoxin	4.0	770	2150	64.3
Saline	4.5	1420	2730	47.8
Endotoxin	3.6	800	1800	55.5
Saline	3.4	1310	2170	39.6
Endotoxin	3.9	1090	1870	41.9
Saline	3.9	1550	2280	32.2
Endotoxin	3.0	500	1160	56.8
Saline	2.7	1440	2090	30.9
Endotoxin	3.6	610	1630	62.5
Saline	3.9	1010	1870	46.1
Endotoxin	3.2	880	2000	55.9
Saline	4.4	1220	1990	38.7
Endotoxin	4.3	680	1790	61.9
Saline	4.5	1350	2150	37.2
Averages Endotoxin	3.6 ± 0.5	790 ± 200	1840 ± 360	57.0
Averages Saline	3.9 ± 0.6	1360 ± 180	2250 ± 320	39.8

Means ± standard deviations are given in the last two lines of the first three columns of figures. For pairs of percentage values, $P < 0.0005$ ($t = 9.0$); pairs of test and control values without BSA, $P < 0.0005$ ($t = 8.3$); pairs of test and control values with BSA, $P < 0.0025$ ($t = 4.3$).

cantly lower after 6 hr of endotoxin-induced shock (Table 2), the largest differences lay in the extent of translocase inhibition. While inhibition ranged from 30.9 to 47.8 per cent in mitochondria from control animals, corresponding values after 6 hr of endotoxin varied from 41.9 to 64.3 per cent. The average figures were 39.8 per cent (control) and 57.0 per cent (experimental).

Artefacts in the isolation of mitochondria. The study of rates of translocation *in vivo* is beset by difficulties that appear insurmountable with techniques currently available. Evidence obtained in the course of this investigation suggests that the above data fail to indicate the full extent of the endogenous inhibition of adenine nucleotide movement *in vivo*. For example, repeated washing of mitochondrial preparations from untreated animals with chilled sucrose/TRAP/EGTA medium was found to increase the activity of the translocase at each stage by between 12 and 28 per cent. For this reason the final washing step [9] was omitted from the preparative procedure. Also, when the translocase was specifically inhibited [5] to an extent of 99 per cent by adding palmitoyl coenzyme A (Sigma; 86 per cent pure) to a suspension of liver mitochondria from untreated mice at 33–48 μg per mg mitochondrial protein, spinning down and resuspending in fresh medium, each of four subsequent washes in the same medium restored the original uninhibited activity by extents ranging from 7 to 78 per cent. Lastly, when similarly-inhibited preparations were treated with the fat-free supernatant fraction of liver homogenates which had been centrifuged at 26,000 *g* for 10 min, greater values for translocase activity were obtained than by washing with the sucrose/TRAP/EGTA medium. A similar reversal of inhibition was seen in the same preparations with supernatants from shocked livers, but the extent of restoration was usually somewhat less than with supernatants from control tissues, as Table 3 shows. The protein content of the supernatant fractions ranged from 9 to 11 mg/ml.

Most of these points are illustrated in the single experiment presented in Table 3. Average values from the total number of four experiments that were carried out are not presented, because the precise extent of partial inhibition of the translocase was difficult to reproduce with accuracy by treating mitochondria with palmitoyl coenzyme A. The effectiveness of the supernatants in reversing the inhibition produced by the thioester tended to be greater when the inhibition was more substantial, but appreciable activation of the translocase was also recorded when untreated mitochondrial preparations from saline-injected animals were washed with supernatants some two hours after the initial fractionation, spun down, and resuspended in fresh medium. Any estimate of the significance of the reversal in terms of additional inhibition *in vivo* is acutely difficult to make, but, as

Table 3. Rates of translocase-mediated ADP entry into mitochondria from control mice under various conditions

Tissue	Treatment	ADP entry, p-moles per mg. mitochondrial protein per 30 sec	Activity as percentage of (b)	Respiratory control ratio
Liver mitochondria from two control mice injected with saline	(a) None	1010	54%	3.9
	(b) BSA, 40 mg/ml	1870		
	(c) Inhibited with palmitoyl co-enzyme A (8 μg/mg protein).	700	37%	—
	(d) As (c), but washed once with buffered sucrose medium.	900	48%	—
	(e) As (c), but washed with supernatant of liver homogenate from 6 hr-shocked mouse.	1100	59%	—
	(f) As (c), but washed with supernatant of liver homogenate from control mouse.	1150	62%	—
	(g) As (c), with BSA at 40 mg/ml.	1480	79%	—
Liver mitochondria from mouse after 6 hr. endotoxin shock	(h) None	610	—	3.6
	(i) BSA, 40 mg/ml	1630		

Protein concentrations in the supernatant fractions of liver homogenates from shocked (e) and control (f) animals were 10.7 and 10.0 mg/ml respectively.

the results in Table 3 indicate, the effect does not appear to be negligible.

Effects of endotoxin on homogenate preparations. Endotoxin from *Salmonella typhosa* is taken up from the bloodstream by the reticulo-endothelial system, the chief sites of uptake being the spleen, the lungs, and the Kupffer cells [12]. The likelihood that the 6 hr findings set out in Table 2 were produced directly by the toxin itself was considered remote, especially in view of the totally negative findings at $\frac{1}{2}$ hr (Table 1). Nonetheless, the possibility of a direct action has been examined in the following way. Whole liver homogenate from untreated mice was divided into two; one half was mixed with endotoxin (5 mg/ml) in sucrose/TRAP/EGTA solution in an amount equivalent to a dose of 240 mg/kg in the whole animal, while the other half received the same volume of endotoxin-free solution. No differences in respiratory control were recorded in a series of four mice, but whereas the extent of inhibition of ADP entry was 40 per cent in untreated mitochondria, it tended to be greater in the endotoxin-treated organelles by a variable amount; the average inhibition was 44 per cent. No differences were seen in two similar experiments in which the equivalent dose of toxin was halved. In other words, the amounts of endotoxin needed to produce effects *in vitro* that were less than a quarter in magnitude than those seen *in vivo* were substantially greater than the amounts actually injected. Moreover, Sies and Mela were unable to show any effect of endotoxin on the respiration of isolated perfused rat liver [13]. This finding may be explained in terms both of the absence of a whole body response to endotoxin in experiments of this kind, and also of the small proportion of liver tissue accounted for by the Kupffer cells [14].

Further studies on the action of BSA. The effect on the translocase of increasing the concentration of BSA was found to be very similar in liver mitochondria from shocked or from saline-injected animals, regardless of whether the preparations had been treated with palmitoyl coenzyme A or not. Translocase activity rose as BSA levels were increased until a plateau was reached between 20 and 40 mg/ml. The plateau was virtually flat in the case of untreated mitochondria from saline-injected mice, but organelles either obtained from shocked animals or treated with palmitoyl coenzyme A demonstrated a slightly increasing slope. In addition, a single treatment with BSA at 40 mg/ml never permitted full recovery of translocase activity in preparations inhibited by palmitoyl coenzyme A (Table 3), although repeated washing with sucrose/TRAP/EGTA medium increased the activity each time.

DISCUSSION

The action and specificity of BSA in these experiments calls for comment. The assumption has been made that BSA acts as an adsorbent for LCFACoAs (compare [11]), which is consistent with the report that the presence of the protein in the reaction medium prevents inhibition of adenine nucleotide translocation in rat liver mitochondria by oleyl coenzyme A [15]. The translocase is localised on the IMM [6, 7], and is isolated from the extramitochondrial space by a region enclosed by the outer mitochondrial membrane [7]. In well-preserved organelles this membrane appears to be impermeable to macromolecules [7]; BSA may produce its effect by removing adsorbed LCFACoAs from the outside, thereby upsetting an equilibrium between thioesters adsorbed onto both inner and outer membranes. The mechanism of action is presumed to be similar to that of the supernatant fraction of liver homogenate (Table 3), but in neither case have other modes of action been

ruled out. High-speed supernatants of homogenates from various tissues [16] including liver [16, 17] contain proteins of low molecular weight (12000–14000) which can bind LCFACoAs with high affinity. While it is quite feasible that such proteins sequestrate other cellular components in homogenates, the amount and binding capacity of at least one of these proteins is sufficient to account for the entire LCFA-CoA content of rat liver [16]. Recent work [18] has shown that, in contrast with carboxyatractyloside [19] and bongkrekic acid [20], oleyl CoA can inhibit the adenine nucleotide translocase from either side of the membrane. It is therefore not inconceivable that the levels of inhibition recorded in the present experiments represent in substantial measure the inhibition exerted by LCFACoAs from within the IMM. On the other hand, the possibility that the stimulation of ADP entry by BSA is brought about by an increase in the proportion of exchangeable nucleotides within the IMM [6] has been shown not to be the case in mitochondria isolated from brown adipose tissue [11]. A similar finding has recently been made in mouse liver mitochondria (E. Pfaff and G. R. N. Jones, unpublished data).

The evidence presented in Table 2, together with the similarity of the responses to BSA at different concentrations of the translocase in mitochondria subjected to various procedures *in vivo* as well as *in vitro*, argues in favour of a powerful endogenous inhibition of enzymically-catalysed adenine nucleotide movement across the IMM, and that this inhibition is mediated by a substance or a group of substances whose action closely resembles that of palmitoyl coenzyme A. The existence of an endogenous inhibition of the translocase, and the capacity of that inhibition to vary substantially from one treated animal to another at different times (Table 2), though apparently not in different animals at the same time (Table 1), are all suggestive that excessive inhibition is likely to have pathological consequences *in vivo*.

The concept that the translocation of adenine nucleotides constitutes a rate-limiting step [7, 21, 22] in the overall synthesis and hydrolysis of ATP is by no means new. In Table 2 the recorded level of translocase inhibition in normal mitochondria was on average as high as 40 per cent; after 6 hr of endotoxin the inhibition increased to 57 per cent. This corresponds to a net decrease in enzymic activity of at least 28 per cent in going from the normal to the pathological state. Actual rates of cooling in shocked animals used in the study of translocation were not determined at the time of decapitation, but are not considered likely to exceed the average value of $-0.7°$ per hr over the first $5\frac{1}{2}$ hr. This figure, however, represents only 6 per cent of the rate of temperature decrease in freshly-killed mice ($-11.1°$ hr), in which energy production had been totally stopped. Since the rate of fall in temperature is directly proportional to the rate of loss of heat, the cutback in availability of translocase activity in this particular tissue is seen to be several times greater than the actual cutback in overall heat production.

This suggests that the system normally functions with some measure of reserve capacity, which might be partly accounted for in the following way. The basic purpose of the translocase is to exchange cytosolic ADP for mitochondrial ATP across the IMM; this might be termed functional exchange. On the other hand, the specificity of the translocase also permits exchange of ADP for ADP, ATP and ATP, and even cytosolic ATP for mitochondrial ADP [6, 7]; this might be regarded as futile exchange. Quantitative distinction between functional and futile exchange is difficult to make under physiological conditions, and will depend on the local concentrations of ADP and ATP in the two cellular compartments, the internal and external affinities of the translocase towards each substrate, and the competition of ADP and ATP, both with each other and with LCFACoAs, for the enzyme. If the system comes under pressure through accelerated conversion of ATP to ADP, then the resulting decrease in the cytosolic ADP concentration would be expected to bring about a readjustment by increasing the proportion of functional exchange at the expense of futile exchange. There must, however, be an ultimate limit to this kind of compensation. A more precise evaluation of the situation should become possible when the appropriate parameters are compared in non-pathological and in shocked tissue.

The capacity of LCFACoAs to inhibit powerfully an extensive range of enzymic reactions, sometimes in amounts well below those encountered in the cell overall, has been noted by a number of workers (see, for example, [22A, 23], and [24] for limited review). Certain specific enzymic steps other than translocation which are directly involved in oxidative pathways are especially sensitive to LCFACoAs. Existing evidence is consistent with the inhibition of such reactions at early stages in the development of shock, and also with the general depression of metabolism [25] and resulting hypothermia commonly seen in the shocked condition [25, 26]. For example, the excretion of $^{14}CO_2$ by rats injected with 1-[^{14}C]-palmitate at 60 and 90 min after a 4 hr period of bilateral hind-limb ischaemia was 40–50 per cent of that by control animals shortly after palmitate administration [27]. Significant depletion of ATP in the liver did not follow until several hr later [28], even though the body temperature falls promptly after tourniquet removal in this particular model [29]. The link with LCFACoAs may be traced as follows. The β-oxidation of long-chain fatty acids depends on the functioning of the carnitine cycle [30], in which reaction between carnitine and LCFACoAs is catalysed by acyl carnitine transferase to give free CoA and the corresponding acyl carnitine derivative. The transferase is subject to powerful substrate inhibition by LCFACoAs ($K_i = 3\ \mu$M for palmitoyl CoA, while the K_m is 10 μM [31]), and is hence uniquely placed for a rate-controlling function.

Furthermore, the depressed activity of the tricarboxylic acid cycle observed after injury from bilateral hind-limb ischaemia has been ascribed to a slowing of citrate synthase [32]; in this connection a strong inhibition of the enzyme by palmitoyl coenzyme A has been reported [33, 34], the K_i for pig heart synthase being only 4.6 μM [34]. Such an inhibition could similarly account for the depression of palmitate oxidation referred to above [27].

In addition to their direct actions, the indirect effects of LCFACoAs may render the situation more

richly complex than might appear from this tentative analysis. For example, various metabolic consequences of an increase in the inhibition of ATP ⇌ ADP exchange across the IMM have been predicted [35]. These include a decreased flux through the tricarboxylic acid cycle, and increases in both the mitochondrial [ATP]/[ADP][P_i] and the [NADH]/[NAD^+] ratios. The active form of pyruvate dehydrogenase is generated from an inactive phosphorylated state by phosphatase action [36]; experiments *in vitro* reveal that the proportion of the enzyme in the active form is decreased in response to increases in both the mitochondrial [ATP]/[ADP] [37, 38] and [NADH]/[NAD^+] ratios [38–40]. Also, a decrease in the proportion of the active form of pyruvate dehydrogenase and a concomitant increase in the [ATP]/[ADP] ratio have been reported in isolated fat cell mitochondria in response to palmitoyl coenzyme A addition [40A]. Recent experiments have shown that full activation of pyruvate dehydrogenase by ADP in rat liver mitochondria is hindered by palmitoyl coenzyme A in low concentration [41]. An impairment of pyruvate oxidation might therefore be expected to arise in conjunction with increases in these ratios as well as in the level of inhibition of the translocase. In fact such an impairment has been described in the intact rat as a result of bilateral hind-limb ischaemia [42], and at times when significant alterations to the overall concentrations of ADP in the liver also occurred [28]. The concept that shock arises as a result of an attenuation of the translocation of essential metabolites across the IMM [3, 4] does not necessarily present too restricted a view of the prerequisite alterations associated with the condition. On the other hand, differential inhibitory effects of LCFACoAs at other susceptible loci of intermediary metabolism may play important roles in the development of the syndrome in different organs and at different times.

Extrapolation of the current findings to the clinical condition would appear premature, but lowering plasma NEFA concentrations by β-adrenergic blockade confers benefit in the treatment of shock arising from burn trauma in human patients [43]. Also, β-blockade in conjunction with supportive treatment that included sodium bicarbonate, calcium chloride and glucose not only increased the survival rate among dogs in haemorrhagic shock, but also largely suppressed the degenerative pathological changes typically seen in control animals not treated with the drug [44]. Similar findings were obtained with β-blockade in dogs treated with endotoxin [45], while the beneficial results of β-blockade have also been described in human patients in the later stages of septic shock [46].

The administration to experimental animals in shock of certain substances for which specific translocatory mechanisms exist can have advantageous effects, probably once the substances have traversed the plasma membrane; the way in which such compounds might stimulate translocation generally has been set out [4]. The provision of a high-affinity acceptor for fatty acyl groups which is sufficiently non-polar to penetrate to sites of enzymic inhibition, or the stimulation of metabolic pathways in which LCFACoAs participate, form further theoretical lines of enquiry yet to be explored. The precise manner in which the inhibition of translocation is accentuated is not known, although various mechanisms, including the diverse inhibitory potentials of LCFACoAs [15], have been discussed [47]. A much fuller understanding of the fundamental biochemistry of the metabolic disorder must still be regarded as an indispensable preliminary to the enlightened and more successful therapy of the shock syndrome.

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