6. A nitrogen mustard altered the catalase distribution ratio in males and females to the same value, intermediate between their normal values.

7. 17-Methyltestosterone injected into females caused a change in the catalase distribution ratio to the male value. Testosterone behaved similarly, but the distribution ratio was altered to a point intermediate between the male and female values.

8. 17-Methyltestosterone injected into males did not affect the distribution ratio.

9. It is concluded that the catalase levels in the granules and extraparticulate cytoplasm are interdependent; that catalase probably migrates from the granules into the extraparticulate cytoplasm; and that androgenic hormones influence the rate of migration in a way which suggests that they increase the permeability of the granule membranes to catalase.

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The Behaviour of Liver Mitochondria Isolated from Rats with Different Body Temperatures after Limb Ischaemia or after Injection of 3:5-Dinitro-*o*-Cresol

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The liver is one of the main sites of heat production in the body (Federov & Shur, 1942; Birnie & Grayson, 1952) and the heat formed there mainly arises during the oxidations carried out by the mitochondria. It is therefore possible that changes in hepatic heat production might reflect alterations within the mitochondria or be due to variations in the amount of substrate being fed to mitochondria whose potentialities had remained unaltered.

The changes in liver temperature on simple exposure of the rat to different environmental temperatures are quite small (Stoner, 1958*a*), although the changes in overall metabolic rate may be large and rapid (Depocas, Hart & Héroux, 1957). Exposure to cold increases heat production but, according to Panagos, Beyer & Masoro (1958), mitochondrial changes occur only as the rat becomes acclimatized.

Much greater changes in liver temperature occur in rats which have been injured by limb ischaemia, particularly if the environmental temperature is

also altered (Stoner, 1958*a*). The possibility of mitochondrial changes in these rats is of special interest as several workers (Strawitz & Hift, 1956*a, b*; Packer, Michaelis & Martin, 1958; Hift & Strawitz, 1958) have sought to relate the metabolic decline and death of the injured rat to alterations in the mitochondria.

Fatal doses of 3:5-dinitro-*o*-cresol, which has the same biochemical action as 2:4-dinitrophenol, produce very high liver temperatures in the rat (Stoner, 1956) and the acute effects of this compound have also been investigated.

Experience in the production of a highly reproducible preparation of liver mitochondria (Aldridge, 1957) has allowed us to seek a possible association between acute changes in temperature (heat production) and the biochemical behaviour of the isolated mitochondria. It was found that except in rats treated with 3:5-dinitro-*o*-cresol large changes in liver temperature were unaccompanied by similar changes in the behaviour of the isolated mitochondria.

METHODS

Animal technique. Male albino rats (200–250 g. each) of the Porton strain fed on M.R.C. diet 41 b (Bruce & Parkes, 1956) were used, food being provided without restriction until the start of the experiment. Bilateral hind-limb tourniquets (Rosenthal, 1943) were applied under ether and the controls were given a similar short period of ether anaesthesia. The tourniquets were kept on for 4 hr. The changes in environmental temperature were made as before (Stoner, 1958*a*). Two rats, an experimental and a control, were used in each test so that both were subjected to the same environmental conditions. To avoid direct trauma to the liver the liver temperature itself was not measured but was inferred from the 'high' colon temperature determined with a mercury thermometer inserted through the anus for a distance of 8 cm. This temperature is only slightly below that in the liver (Stoner, 1958*a*).

In two experiments 3:5-dinitro-*o*-cresol was injected subcutaneously as a 1% (w/v) solution in 0.1 M- Na_2CO_3 .

Preparation of mitochondria. The rats were killed by decapitation and the liver mitochondria were isolated as previously described (Aldridge, 1958). The mitochondria from the control and experimental rats were prepared at the same time, the times of deaths of the two animals being separated by about 10 min.

Manometric technique. The reasons for the composition and the pH of the medium have been previously discussed (Aldridge, 1957). Each flask contained 3 ml. of a solution containing adenosine 5-phosphate (1.15 mM), adenosine triphosphate (ATP; 1.06 mM), KCl (0.1 M), MgCl_2 (14 mM), ethylenediaminetetra-acetic acid (1.0 mM), potassium phosphate (15 mM), sucrose (0.03 M), and substrates which were all 0.01 M except fumarate (1.0 mM). For studies of oxidative phosphorylation each flask contained, in addition, glucose (180 μmoles), glycylglycine (50 μmoles) and hexokinase (200–400 units). This medium was adjusted to pH 6.7–6.8 with KOH, a glass electrode being used, and all experiments were carried out at 37°. Carbon dioxide was absorbed by 0.15 ml. of 5*N*-KOH; the gas phase was air. The mitochondria were suspended in 0.3 M-sucrose. In all experiments, 0.30 ml. of mitochondrial suspension, equivalent to 0.15 g. wet wt. of original liver, was used.

Determination of adenosine triphosphatase activity. Each beaker contained 3 ml. of a solution containing ATP (3 mM), KCl (0.1 M), MgCl_2 (14 mM), ethylenediaminetetra-acetic acid (1.0 mM), glycylglycine (16.7 mM) and sucrose (0.03 M). This solution was adjusted to pH 6.7–6.8 with KOH. The beakers were shaken at 37°. After temperature equilibration mitochondria were added and the mixture was incubated for 10 min. The reaction was stopped by the addition of 0.5 ml. of 65% (w/w) HClO_4 and inorganic phosphate was determined.

Analytical methods. Inorganic phosphate was determined by the method of Fiske & Subbarow (1925). Mitochondrial protein was measured by the biuret method of Robinson & Hogden (1940), modified as described by Aldridge (1957), and has been expressed as mg. of albumin. 3:5-Dinitro-*o*-cresol was determined by the method of Parker (1949).

Special chemicals and reagents. The following chemicals were obtained from the sources indicated: glycylglycine, sodium pyruvate (Roche Products Ltd.); adenosine 5-phosphate, disodium salt of ATP (Sigma Chemical Co., St Louis, Mo., U.S.A.); sodium fumarate, 3:5-dinitro-*o*-

cresol and 2:4-dinitrophenol (British Drug Houses Ltd.). All free acids were neutralized to approx. pH 6.8 with KOH before being used. Potato apyrase was prepared by the method of Lee & Eiler (1951). This preparation liberated 1100 μg .atoms of inorganic phosphate/hr./ml. from the medium used for adenosine triphosphatase assay at 37°. Hexokinase was prepared from baker's yeast by a modification by V. H. Parker (unpublished work) of the method of Berger, Slein, Colowick & Cori (1946). The preparation was taken to the equivalent of their step 3*a* and when assayed by their procedure (but at 37° instead of 30°) had an activity of 3500 units/ml.

Units used. The oxidative activity of the mitochondria (q_{O_2}) is expressed as $\mu\text{l. of O}_2/\text{mg. of protein/hr.}$ Adenosine triphosphatase activity is given as μg .atoms of P liberated/mg. of protein/hr.

RESULTS

Controls

Since each control rat was exposed to the same environmental conditions as the experimental one the controls may be divided into three groups (Tables 1 and 2). First there is the group from Expts. 1, 4, 12 and 13, in which the rats were kept at an air temperature of 16–21°. The controls in Expts. 2 and 3 were exposed to an air temperature of 3°. This has previously been found to raise the liver temperature about 0.6° without affecting its blood flow (Stoner, 1958*a*). According to the work of Depocas *et al.* (1957) the overall metabolic rate of these rats would have been increased 1.7-fold. In the third group in Expts. 5–10 the environmental temperature was raised to the so-called critical level for the rat. Under these conditions the liver temperature falls slowly and there is a 31% decrease in the rat's total oxygen consumption compared with that at 20° (Stoner, 1958*a*). In one rat of this group (Expt. 11) the air temperature was raised to 36°, which is about the limit of tolerance, and at this air temperature the liver temperature rises.

Despite the different metabolic rates of these control rats no differences were found in the behaviour of the mitochondria isolated from them (Tables 1 and 2). In respect of their oxygen uptake and its stimulation by apyrase, their adenosine triphosphatase activity and its stimulation by 2:4-dinitrophenol, and their protein content, there was no consistent difference between liver mitochondria from rats at high and low environmental temperatures and those from rats at air temperatures of 16–21°. The results in the last group were within the range consistently found for such rats in this Laboratory over the past 2½ years.

Effect of limb ischaemia

More striking changes in liver temperature were produced by subjecting rats to limb ischaemia (Tables 1 and 2).

A 4 hr. period of bilateral ischaemia of the hind limbs at an air temperature of 18–22° gives an 85 % mortality rate with a survival time of about 13 hr. (Stoner & Threlfall, 1959). After removal of the tourniquets the liver temperature falls steadily in both fatal cases and survivors. After about 4 hr. its recovery begins in those which survive but the fall continues in those which die (Stoner, 1958a). In the rats which die, the liver blood flow does not fall significantly until about 4 hr. after removal of the tourniquets, i.e. it would be greatly below the normal level in Expt. 1, where the rat was moribund, but not in Expt. 4. Exposing the rats to an environmental temperature of 3° some time after removal of the tourniquets, as in Expts. 2 and 3, increases the mortality rate to 100 % and greatly accelerates the fall in liver temperature without markedly affecting its blood flow (Stoner, 1958a). In Expts. 2 and 3 the rats were at an air tempera-

ture of 3° for 55 and 90 min. respectively before they were killed.

Despite these gross falls in colon (liver) temperature there were no correspondingly large changes in the behaviour of the isolated mitochondria (Table 1 and 2).

After limb ischaemia the liver temperature can be made to rise if the rat is kept in an environmental temperature of 30° during and after a 4 hr. period of hind-limb ischaemia (Stoner, 1958a). When the tourniquets are removed the liver temperature rises until just before death, when it falls suddenly. The liver blood flow falls rapidly when the tourniquets are removed. This procedure increases the mortality rate to 100 % and shortens the survival time to about 2 hr. (Stoner & Threlfall, 1959). This was done in Expts. 5, 6, 8 and 10. In Expt. 7 the environmental temperature was not raised until after the period of limb ischaemia. The

Table 1. *Oxygen uptake of mitochondria, from control and experimental rats, with pyruvate as substrate*

Measurements were made as described under Methods. 'With apyrase' indicates addition of apyrase of activity corresponding to liberation of 110 $\mu\text{g. atoms}$ of inorganic P/hr. under assay conditions described in the Methods section. C, Control rats; E, experimental rats. In Expts. 1–11 the experimental rats were subjected to a 4 hr. period of bilateral hind-limb ischaemia. The tourniquets were applied under ether anaesthesia and the controls were given a similar short anaesthesia. Dose of 3:5-dinitro-*o*-cresol: 25 mg./kg. body wt. subcutaneously.

Expt. no.	Environmental temp.	Interval after removal of tourniquets	Final colon temp.	Oxygen uptake (q_{O_2})		Stimulation (ratio b/a)
				No apyrase (a)	With apyrase (b)	
Ischaemic shock						
1C	16°	—	38.0°	24.6	90	3.7
1E	16	18 hr.	22.0	29.5	109	3.7
2C	18/3*	—	38.6	29.0	111	3.8
2E	18/3	3 hr. 55 min.	25.3	35.0	121	3.5
3C	17/3	—	38.0	26.9	97	3.6
3E	17/3	4 hr. 15 min.	26.0	27.2	104	3.8
4C	20	—	37.2	25.1	107	4.3
4E	20	2 hr. 38 min.	32.7	27.8†	106†	3.8
5C	30	—	37.8	30.0	106	3.5
5E	30	1 hr.	38.3	34.8	112	3.2
6C	30	—	—	26.0	—	—
6E	30	38 min.	39.2	28.0	—	—
7C	20/30	—	37.8	28.2	87	3.1
7E	20/30	1 hr. 16 min.	39.2	31.0	117	3.8
8C	30	—	—	—	85	—
8E	30	50 min.	—	—	87	—
9C	30	—	—	30.0	89	3.0
9E	30	45 min.	—	20.0	87	4.3
10C	30	—	—	23.0	87	3.8
10E	30	30 min.	—	25.0	86	3.4
11C	36	—	39.0	23.8	88	3.7
11E	36	15 min.	40.5	29.8	102	3.4
3:5-Dinitro- <i>o</i> -cresol						
12C	18	—	38.0	18.5	89	4.8
12E	18	—	42.0	49.0	111	2.3

* 18/3 indicates a period at 18° followed by one at 3° (see text).

† Calculated from mean recovery of mitochondrial protein (cf. Table 3).

rise in colon temperature between removal of the tourniquets and killing the rats was 0.7°, 2.2° and 2.2° in Expts. 5-7 respectively. It was not measured in Expts. 8-10 but the average rise in liver tempera-

ture during this period in previous experiments was 0.6° (Stoner, 1958a).

Again these temperature changes were not reflected in the behaviour of the liver mitochondria *in vitro* (Tables 1 and 2). Their oxygen uptake with and without added apyrase, their adenosine triphosphatase activity with and without the addition of 2:4-dinitrophenol and their protein content was not strikingly different from those from the controls or from the injured rats with low liver temperatures. In Expts. 9 and 10 it was also found that the oxygen uptake with L-glutamate and succinate as substrates, both with and without apyrase, was likewise unchanged after limb ischaemia.

A higher liver temperature was produced by keeping the rat in an air temperature of 36° during and after the period of limb ischaemia (Expt. 11). This reduces the survival time after removal of the tourniquets to about 30 min. (Stoner & Threlfall, 1959). Under these conditions the liver temperature rises during the period of limb ischaemia with a further rise afterwards. Liver blood flow is depressed while the tourniquets are in place and falls precipitously when they are removed (Stoner, 1958a). Although in Expt. 11 conditions in the liver must have been grossly abnormal for about 3 hr. before it was removed, the subsequent behaviour of the mitochondria *in vitro* was not affected.

Inspection of the results in Tables 1 and 2 shows that despite the different environmental conditions there is a close similarity among those obtained in the controls and among those in the injured rats. In view of this the results in the two groups have been pooled, when certain overall differences become apparent (Table 3). The oxygen uptake by the mitochondria from the injured rats, both with and without apyrase, is significantly higher than in the control mitochondria but the degree of stimulation by apyrase is the same in the two groups. Similarly, the adenosine triphosphatase activity of the 'experimental' mitochondria is less than that of the 'controls', but in both cases the same degree of activity can be produced by the addition of an amount of 2:4-dinitrophenol which is known

Table 2. Adenosine triphosphatase activity and protein of mitochondria from control and experimental rats

Measurements were made as under Methods. C, Control rats; E, experimental rats. For treatment of rats see Table 1 and text. DNP, 2:4-Dinitrophenol (30 μ M).

Expt. no.	Final colon temp.	Protein (mg./g. wet wt. of liver)	Adenosine triphosphatase	
			No DNP (μ g.atoms of P/mg. of protein/hr.)	With DNP
Ischaemic shock				
1C	38.0°	30.8	0.49	11.7
1E	22.0	29.3	0.16	12.3
2C	38.6	28.5	0.26	—
2E	25.3	30.9	0.07	—
3C	38.0	27.8	0.14	13.0
3E	26.0	30.1	0.08	13.2
4C	37.2	28.2	0.16	—
4E	32.7	—	0.04*	—
5C	37.8	24.7	0.44	12.8
5E	38.3	23.9	0.35	13.0
6C	—	25.5	0.45	14.4
6E	39.2	28.9	0.11	13.8
7C	37.8	27.8	0.99	—
7E	39.2	30.5	0.35	—
8C	—	30.1	—	—
8E	—	39.0	—	—
9C	—	28.9	—	—
9E	—	34.0	—	—
10C	—	34.0	—	—
10E	—	38.2	—	—
11C	39.0	27.4	0.41	13.5
11E	40.5	24.7	0	14.8
3:5-Dinitro- <i>o</i> -cresol				
12C	38.0	30.5	0.17	13.1
12E	42.0	30.1	5.20	11.8
13C	38.3	29.5	0.14	12.6
13E	41.5	25.4	3.04	12.1

* Calculated from mean recovery of mitochondrial protein (cf. Table 3).

Table 3. Pooled results on mitochondria from control rats and rats in ischaemic shock (Expts. 1-11)

Values given are means $\pm$ s.d., with number of determinations in parentheses. Significance of differences were tested by Student's *t* test.

Assay	Control	Experimental	P
Protein (mg./g. wet wt. of liver)	28.6 $\pm$ 2.5 (12)	30.9 $\pm$ 4.7 (11)	0.08
Oxygen uptake, no apyrase (q_{O_2})	24.1 $\pm$ 3.5 (11)	29.1 $\pm$ 2.9 (10)	0.001
Oxygen uptake, with apyrase (q_{O_2})	94.2 $\pm$ 9.4 (11)	103.8 $\pm$ 12.2 (11)	0.03
Stimulation (ratio)	3.73 $\pm$ 0.52 (11)	3.66 $\pm$ 0.32 (10)	0.33
Adenosine triphosphatase, no DNP (μ g.atoms of P/mg. of protein/hr.)	0.39 $\pm$ 0.26 (9)	0.145 $\pm$ 0.13 (8)	0.02
Adenosine triphosphatase, with DNP (μ g.atoms of P/mg. of protein/hr.)	13.08 $\pm$ 0.88 (6)	13.15 $\pm$ 1.07 (6)	0.46

(Parker, 1958) to produce maximal stimulation. Varying the order in which the rats were killed eliminated the possibility that these differences were related to the intervals between death and the commencement of the assays *in vitro*.

Effect of 3:5-dinitro-o-cresol

Very high liver temperatures can be produced by fatal doses of 3:5-dinitro-*o*-cresol. The rise occurs during the period of hyperventilation and the liver blood flow shows variable changes, usually falling (Stoner, 1956). In Expts. 12 and 13 the rats were killed and the liver mitochondria isolated when the effects were judged to be maximal, 50 and 73 min. after injection of 25 mg. of 3:5-dinitro-*o*-cresol/kg. body wt., which had produced an increase of 4.3° in the colon temperature.

The oxygen uptake of these mitochondria in the presence of apyrase, the recovery of mitochondrial protein and their adenosine triphosphatase activity in the presence of 2:4-dinitrophenol were unaffected. However, the oxygen uptake without added apyrase and the adenosine triphosphatase activity without 2:4-dinitrophenol were much increased in the mitochondria from the treated rats (Tables 1 and 2).

In Expt. 13 the concentration of 3:5-dinitro-*o*-cresol in the isolated mitochondrial suspension was found to be $9\mu\text{M}$. The relationship between the concentration of 3:5-dinitro-*o*-cresol and mitochondrial adenosine triphosphatase activity *in vitro* has been determined; an activity of $3\mu\text{g.}$ atoms of P liberated/mg. of protein/hr. would require a concentration of $8\mu\text{M}$ -3:5-dinitro-*o*-cresol in the mitochondrial suspension.

DISCUSSION

No differences were found in the behaviour of the mitochondria from the control rats exposed for short periods to widely differing environmental temperatures. It might be that the differences in metabolic rate thus produced were too small to cause detectable differences in the isolated mitochondria. Much greater metabolic changes occurred in the rats subjected to limb ischaemia, but there was no correlation between any of the properties measured on the isolated mitochondria and the colon temperature. Gross changes in the colon (liver) temperature in the range $22-40^{\circ}$ have not produced comparable changes in the ability of the mitochondria to oxidize pyruvate, in the degree of stimulation on the addition of apyrase or in their adenosine triphosphatase activity both with and without 2:4-dinitrophenol.

The normal oxygen uptake of the mitochondria of rat liver *in vivo* cannot be assessed but it may be

assumed that, after a lethal dose of 3:5-dinitro-*o*-cresol, when the effects are maximal mitochondrial oxidation is proceeding *in vivo* at its maximal rate (q_{O_2} around 100). The colon temperature at this time is $41-42^{\circ}$. With this as a reference point a temperature of 22° (Expt. 1) must clearly indicate that the mitochondria are working at a much lower rate; assuming a Q_{10} of 2 their oxidative rate would be 25 % of the maximum. The results in this paper do not suggest that this lowered activity is due to any intrinsic inability of the mitochondria to oxidize substrates of the tricarboxylic acid cycle.

When the results in the controls and in the rats after limb ischaemia are collected into two groups, certain differences in the mitochondria appear (Table 3). The oxygen uptake of the mitochondria from the experimental rats both with and without apyrase is significantly different from the controls but the degree of stimulation is not. These slight differences, which for adenosine triphosphatase activity are near the limit of resolution of the method, may be associated with a change in the composition of the liver after limb ischaemia, e.g. loss of glycogen (cf. Stoner, 1958*b*), altering the physical state of the homogenate and producing slight differences in the sedimentation of the particles. The differences in oxygen uptake and in adenosine triphosphatase activity could well be explained if the experimental mitochondrial preparation was less contaminated by other material.

The biochemical properties of the mitochondria as measured by our assay procedures may bear little quantitative relation to their behaviour *in vivo*. The ability of the mitochondria to respond to demands for ATP would seem to be an essential physiological mechanism, and the results in Expts. 12 and 13 have shown that 3:5-dinitro-*o*-cresol has a similar effect on mitochondrial adenosine triphosphatase activity whether added *in vitro* or *in vivo*. These results are not in conflict with those of Parker (1956) since in his experiments with 2:4-dinitrophenol the mitochondria were isolated at a much later stage, when the rats had recovered from the immediate effects of the compound. If our previous assumption is correct that oxygen uptake (and therefore adenosine triphosphatase activity, cf. Parker, 1958) is maximal *in vivo* after a lethal dose of 3:5-dinitro-*o*-cresol, then the much lower values obtained after isolation might be due to loss of 3:5-dinitro-*o*-cresol during the isolation procedures or to dilution during the assay. However, assuming the relation between mitochondrial protein and water found by Werkheiser & Bartley (1957), an overall concentration of $9\mu\text{M}$ in the mitochondrial suspension indicates a concentration of approx. $200\mu\text{M}$ in the mitochondria. Full understanding of these findings must await

more information on the distribution of 3:5-dinitro-*o*-cresol between mitochondria and medium under a variety of conditions.

Two ways in which mitochondrial metabolism could be influenced to give the lowered metabolism of the injured rats in Expts. 1-4 are structural damage to the mitochondria and alterations in the intracellular medium.

The first possibility seems very unlikely, for it would be necessary for this damage to be repaired during the isolation. Damage of this type would probably result in the loss of essential coenzymes and replacement of these coenzymes is not possible in these experiments since they have been carried out without further additions to the mitochondria.

Alterations in the concentrations of the normal intracellular constituents such as electrolytes would have to be very large to produce mitochondrial changes of the magnitude required, but the reactions leading to the formation of the substrates for the mitochondria might be more sensitive to such changes. The presence of an inhibitor in the intracellular fluid is a further possibility. This could be a direct inhibitor of the enzymes of the mitochondria or it could inhibit any enzyme leading to the formation of the substrates necessary for the mitochondria. There is evidence that the liver temperature in normal rats is depressed when mitochondrial oxidation is inhibited, as by fluoroacetate (Stoner, 1956), or when glycolysis is inhibited, as by D-2-deoxyglucose (Wick, Drury, Nakada & Wolfe, 1957; Stoner & Threlfall, 1959). In favour of the idea that the temperature fall in the early part of the response to limb ischaemia at normal environmental temperatures is related to a failure to form substrate (pyruvate) for the mitochondria is the accumulation of hexose diphosphate in the liver under these conditions (Stoner & Threlfall, 1959).

The rise in liver temperature after limb ischaemia when the environmental temperature is raised to 30° appears to be a local phenomenon (Stoner, 1958*a*). It is not accompanied by a general rise in metabolic rate, for the total oxygen consumption of the rat falls in the same way as in rats injured at a lower environmental temperature (20°). At the higher environmental temperature (30°) there is a fall in the ATP content of the liver (Stoner & Threlfall, 1954). As pointed out above, the attempt to repair this loss may stimulate metabolism and lead to increased heat production.

Workers on the mitochondria of other tissues have claimed that the properties of the mitochondria are changed in shock after various injuries. Packer *et al.* (1958) claim that oxidative phosphorylation by rat-heart and -brain mitochondria is decreased after limb ischaemia. The statistical evaluation of their published data is not

possible. Others claim to have found decreased oxidative phosphorylation by heart mitochondria in haemorrhagic shock (Strawitz & Hift, 1956*a, b*; Hift & Strawitz, 1958). The significance of their results depends on one extremely low P/O ratio out of seven experiments. It has been the experience of this Laboratory that it is very difficult to obtain P/O ratios with a low standard error and it is our view that significant changes in the mitochondria isolated from animals subjected to physical injury have yet to be demonstrated. Much more work is necessary before the acute changes in tissue temperature in these pathological states are understood.

SUMMARY

1. Different liver temperatures have been produced in rats by varying the environmental temperature in ischaemic shock and by subcutaneous injection of 3:5-dinitro-*o*-cresol.

2. No correlation has been found between the properties of the liver mitochondria isolated from rats in ischaemic shock and their colon temperatures (range 22.0-40.5°). Possible reasons for these temperature changes are discussed.

3. The behaviour of the isolated mitochondria is explained by their 3:5-dinitro-*o*-cresol content. The relation between this and the rise in temperature *in vivo* is discussed.

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The Metabolic Fate of the Ester Sulphate Group of Potassium *p*-Nitrophenyl [³⁵S]Sulphate

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The fate of arylsulphates in the animal body has been investigated by a number of workers. Early studies by Rhode (1923), Sperber (1948) and Garton & Williams (1949) suggested that almost the whole of the administered dose of potassium phenyl sulphate was rapidly eliminated unchanged in the urine. Hawkins & Young (1954), using radioactive techniques, observed only slight hydrolysis (4–12%) of orally administered and intraperitoneally injected phenyl [³⁵S]sulphate and naphthyl [³⁵S]sulphate. In contrast, the results of Hanahan & Everett (1950) indicate that the bulk of the dose of oestrone [³⁵S]sulphate is desulphated by adult female rats, and there is indirect evidence which suggests that appreciable hydrolysis of stilboestrol disulphate (Bishop, Richards & Perry, 1951) and of sulphated insulin (containing sulphated tyrosine residues, see Glendening, Greenberg & Fraenkel-Conrat, 1947) occurs *in vivo*. Michel, Roche, Closon & Michel (1958) noted that ¹³¹I-labelled 3:5:3'-tri-iodo-L-thyronine sulphate is partially desulphated *in vivo* by the rat and eliminated as the corresponding glucosiduronic acid derivative. At first sight these collected results might suggest that only the sulphate esters of phenols which can be regarded as 'physiologically active' are extensively hydrolysed *in vivo*.

Mammalian tissues contain at least three distinct enzymes which are capable of hydrolysing arylsulphates. The specificity of these enzymes is not yet clearly defined, but there are indications that two of them, arylsulphatases A and B, show high affinity and activity towards arylsulphates such as dipotassium 2-hydroxy-5-nitrophenyl sulphate and monopotassium 4-chlorocatechol monosulphate (Dodgson & Spencer, 1957; Dodgson & Wynn, 1958), but comparatively low activity towards simpler arylsulphates such as potassium *p*-acetylphenyl sulphate and potassium *p*-nitrophenyl

sulphate (cf. Dodgson, Rose & Tudball, 1959). In contrast, the third enzyme, arylsulphatase C, is particularly active towards these simpler arylsulphates. In spite of these differing specificities, all three enzymes show only comparatively feeble activity and affinity towards phenyl sulphate (Dodgson *et al.* 1959), and this can probably be attributed to the absence of an *ortho* or *para* electrophilic substituent group in the substrate molecule (cf. Dodgson, Spencer & Williams, 1956). It is perhaps not surprising therefore that phenyl sulphate is not extensively hydrolysed *in vivo*. Similar considerations probably apply to naphthyl sulphate.

In attempts to elucidate the physiological role of the arylsulphatase enzymes, recent work in these Laboratories has been directed towards a study of the ability of the sulphate esters of 'physiologically active' phenols to act as substrates for the various arylsulphatases both *in vivo* and *in vitro*. In many cases, the chemical synthesis of radioactive (³⁵S) specimens of these particular sulphate esters presents formidable problems. It was therefore considered desirable, in order to gain experience with various techniques, to conduct preliminary investigations with potassium *p*-nitrophenyl [³⁵S]sulphate, an arylsulphate which is readily prepared on a small scale and which, from theoretical considerations, might be expected to undergo appreciable desulphation *in vivo*.

The results of this preliminary study are presented in this paper.

MATERIALS AND METHODS

Potassium p-nitrophenyl [³⁵S]sulphate. This was prepared by a modification of the method of Burkhardt & Lapworth (1926). Carrier-free chloro[³⁵S]sulphonic acid (0.261 g., specific activity 10.4 mc/m-mole) was mixed with 0.28 g. of