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MITOCHONDRIAL BIOCHEMICAL LESION AND PYROGENIC EFFECT OF PENTACHLOROPHENOL

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Abstract—The biochemical activity of mitochondria isolated from liver of pentachlorophenol (PCP)-injected rats were studied before, during, and after the appearance of the hyperthermia produced by the compound. PCP was found to provoke in liver mitochondria definite biochemical lesions, already apparent 15 sec after PCP administration. The damage was revealed by a lowering of the P:O ratios, the loss of the respiratory control, the increase of the oxidation rate towards some substrates, and the increase of the latent ATPase activity. The return of liver mitochondria to the conditions of controls occurred gradually and lagged largely behind the return of the body temperature to the normal values. The results are discussed in relation to the genesis of the hyperthermia produced by PCP.

PENTACHLOROPHENOL (PCP) is a toxic compound for a number of animal and plant species,[1, 2] and it provokes marked body temperature increase in laboratory homeothermic animals.[2, 3] *In vitro* PCP uncouples phosphorylation in rat liver mitochondria[4–6] and submitochondrial particles,[7] and this effect, in rat liver mitochondria, cannot be reversed by washing.[8]

In a previous study[8] it was found that liver mitochondria, isolated from rats that had been injected with a lethal dose of PCP, showed an increased oxidation rate with pyruvate and L-glutamate as substrates, and a diminished phosphorylative capacity; concurrently the energy-rich phosphate (~P) concentration was markedly lowered in liver tissue. Thus PCP appeared to be a convenient tool to investigate possible correlation between the effects of an uncoupler of oxidative phosphorylation on body temperature and on some biochemical activities of liver mitochondria related to energy metabolism.

In the present investigation rats were injected with a dose of PCP capable of producing a marked pyrexia followed by a complete recovery of the animals, and liver tissue and mitochondria were studied at various stages of intoxication. The results obtained show that the pyrexia produced by PCP in rats is preceded and accompanied by biochemical lesions in liver mitochondria, and that these changes are reversible. A preliminary report on part of this work has been published.[9]

MATERIALS AND METHODS

Animal technique

The experiments were performed on 185 Wistar albino rats and on a few others from different breeds. Animals of either sex weighing 150–200 g were used. The rats were fed on a standard commercial diet, and received water *ad libitum*; from 16 to 18 hr before

the experiment the food, but not the water, was withdrawn. The animals during this time, and also that following the injection, were kept in a thermostatically controlled cabinet at 31–34°. Experimental and control rats were kept together and treated in the same way and under the same conditions. The temperature was determined with a mercury thermometer inserted through the anus for a distance of 3·5 cm. The rats were intraperitoneally injected, the experimental ones with the compound tested, and the control ones with equivalent volumes of 0·9% NaCl solution.

Preparation of mitochondria

The rats were killed by decapitation or by cervical fracture, and the liver removed and placed in 0·25 M sucrose at 0°. During all subsequent manipulations the material was maintained at 0–2°. Mitochondria were isolated from liver essentially according to the procedure described by Schneider;[10] a 10 per cent homogenate in 0·25 M sucrose + 0·0025 M K-EDTA was obtained by using an all-glass homogenizer. The mitochondrial pellet was washed twice and resuspended in an adequate volume of the same sucrose–EDTA solution used for the homogenization.

Preparation of liver slices

The rats were decapitated; the liver was removed, placed in glucose–Krebs Medium II (seebelow) at 0–2°, and kept at this temperature until the beginning of the experiment. It was then sliced with a razor blade.

Manometric technique

The conventional direct Warburg method was used. Liver mitochondria: each 14–16 ml flask contained in a final volume of 3·0 ml, 13·3 mM Na_2HPO_4–KH_2PO_4 buffer, pH 7·4; 10 mM Tris–HCl buffer, pH 7·4; 16·6 mM KCl; 13·3 mM NaF; 92 mM sucrose; 0·25 mM K-EDTA; 6·6 mM $MgSO_4$; and the substrates tested. Substrate concentrations were: 11 mM Na-pyruvate (plus 1·3 mM Na-DL-malate as a primer); 6·6 mM Na-α-oxoglutarate; 10 mM Na-L-glutamate; 6·6 mM Na-succinate; and 13·3 mM sodium citrate. When required, each flask contained in the side arm, 45 μmole glucose; 240 K.M. units hexokinase; and 0·4 μmole Na-AMP. The reagents were brought to pH 7·4 either with NaOH or HCl, a glass electrode being used. Mitochondria corresponding to 5·4–7·5 mg protein were added to each flask. CO_2 was absorbed by 0·1 ml 20% KOH and a slip pleated filter paper. The gas phase was air; the temperature 25°; the rate of shaking, 120–130 complete oscillations per min. The phosphate trapping system was tipped in after 6–7 min equilibration. Readings were taken every 5 min for 15–20 min, and the reaction was stopped by adding 1·0 ml ice-cold 32% (w/v) trichloroacetic acid (TCA). The mixture was filtered, and the filtrate used immediately for inorganic phosphate determination. In flasks for determination of respiratory control index the side arm contained 45 μmoles sucrose.

Liver slices. The following media were used, (a) complete-Krebs Medium II (the phosphate saline without Ca, and low in bicarbonate and CO_2 Medium II of Krebs[11]); (b) glucose–Krebs Medium II (same as (a), but with glucose only as substrate). The latter medium was used only for the preservation of the tissue. Each flask contained 2·0 ml medium; 5–15 mg dry wt. tissue, and 0·2 ml 30% KOH and a slip of pleated filter paper in the central well. The flasks were kept at 0° until used. The gas phase was O_2; the incubation temperature 40°; the rate of shaking 74 complete oscillations per

min; the equilibration time was 10–12 min. The oxygen uptake was measured at 10 min intervals for 40 min. At the end of the experiment the tissues were blotted and dried at 100° for 4–6 hr.

Determination of adenosine triphosphatase activity

Each test tube contained in a final volume of 1·0 ml, 40 mM Tris–acetate buffer, pH 7·4; 72 mM KCl; 1·2 mM Na-EDTA; 2·5 mM Na-ATP, and 1·2–1·5 mg mitochondrial protein. The reaction was started by addition of the enzyme. The tubes were incubated at 25° for 15 min without shaking. The reaction was stopped by adding 1·0 ml ice-cold 16% (w/v) TCA, and inorganic phosphate was determined quickly.

Preparation of tissue for analysis of phosphates

The rats were anaesthetized with ether, and a piece of about 0·5 g of liver was collected very rapidly. The tissue was immediately frozen by squeezing it between two blocks of solid CO_2. The liver fragments were maintained in CO_2 powder, weighed when still frozen, and ground in a previously cooled porcelain mortar with acid washed quartz sand together with solid CO_2 and 15 ml 5% $HClO_4$. Thus the tissue was ground practically in a dry state,[12] and progressively impregnated by the acid. The extracts were filtered and kept at 0°. The energy-rich phosphate was determined as inorganic phosphate liberated by hydrolysis in N HCl at 100° for 7 min, and it was assumed that it included the labile phosphate from adenosine triphosphate (ATP) and adenosine diphosphate (ADP).

Analytical methods

Inorganic phosphate was determined either by the Fiske and Subbarow method[13] (in the experiments of oxidative phosphorylation and adenosine triphosphatase activity) or by the method of Martin and Doty[14] as modified by Ernster, Zetterstrom and Lindberg[15] (for determination of acid soluble phosphate of the tissue). Mitochondrial nitrogen was estimated by the Kjeldahl–Markham method.[16]

Chemicals

Chemicals of analytical grade were used. Adenosine-5-phosphate, adenosine-5-trisphosphate disodium salt, hexokinase type III, nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), and yeast concentrate were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.; D-glucose, sodium pyruvate, α-oxoglutaric acid, sodium succinate from Hoffmann-LaRoche, Basel, Switzerland; sodium L-glutamate, sodium citrate from British Drug House Ltd., London; sodium DL-malate from Merck, Darmstadt, Germany; and pentachlorophenol from Fluka A.G., Buchs, Switzerland.

Assessment of results

The oxidative activity of mitochondria (Q_{O_2}) is expressed as μl of O_2/mg protein per hr. It indicates the rate of oxygen uptake in the presence of the phosphate trapping system. It was calculated from the oxygen actually consumed in 15–20 min, and no extrapolation was made to account for the initial short period of increment of the oxidation rate. The respiratory control of the mitochondria (R.C.) is represented by the ratio O_2 uptake with AMP–hexokinase–glucose/O_2 uptake without AMP–hexokinase–glucose. The phosphorylative activity of mitochondria (P:O) is expressed by

the ratio μmole inorganic phosphate disappeared/μatoms oxygen consumed. The oxidative activity (Q_{O_2}) is expressed, in the case of tissue slices, as μl O_2/mg dry wt per hr. It was calculated on the oxygen consumed in 40 min.

RESULTS

In the conditions selected, a dose of 20 mg PCP/kg body wt, given by intraperitoneal injection, invariably induced a body temperature increase of 1·5–2·5° in the rats. The rectal temperature began to rise 7–10 min after the injection of the toxic compound, and it reached its highest levels within 50–70 min, remaining constant or nearly so for 3–5 hr. Then it rapidly returned to the normal values (Fig. 1). This treatment did not seem to seriously affect the rats, and they recovered completely after a few hours of the injection.

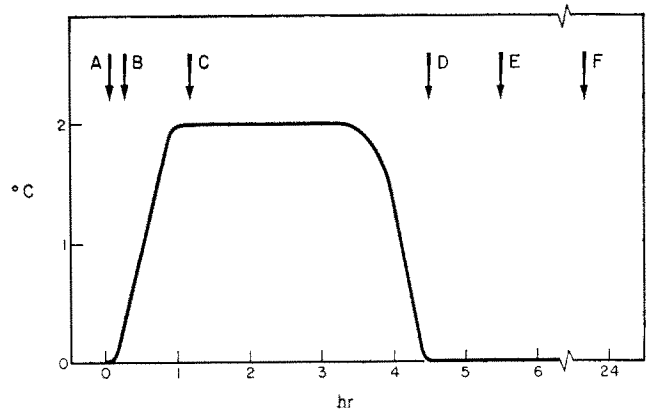

FIG. 1. Body temperature increase caused by pentachlorophenol in the rat. Arrows indicate the points at which measurements were made on liver mitochondria and tissue (see Table 1, 4 and 5): A, latent period between intraperitoneal injection of PCP and the rise of rectal temperature (15 sec to 7 min); B, period of rise of temperature (10 min); C, period of sustained high temperature (60–90 min); D, end of period of falling temperature (2·5–5 hr);E, early defervescence period (3–6 hr); and F, late defervescence period (24 hr). Mean curve given by 20 mg PCP/kg body weight.

The effect on oxygen uptake

As shown in Table 1, the rate of oxygen uptake was significantly increased for pyruvate and L-glutamate already 15–30 sec after the PCP injection, and it reached its highest level in about 7 min, before the beginning of pyrexia. During the entire pyretic period the oxidation rate remained approximately constant at the maximum levels, and at the end of the effect on the body temperature, the Q_{O_2} was still about 30 per cent above normal values. The oxidation rates towards other substrates were tested at 3 min of intoxication (Table 2), when the biochemical lesion of the mitochondria is most severe. The Q_{O_2} of fumarate was increased by 58 per cent, that of α-oxoglutarate was unchanged, and the oxidation rates relative to citrate and succinate were 76 and 47 per cent respectively reduced. The oxidation velocity of citrate could be increased by the addition of cofactors (Table 3).

The opposite effects on the respiration of isolated mitochondria observed with different metabolic intermediates stressed the need for a study of the effect of PCP

upon the respiration of organized tissue. Six rats were injected with 20 mg/kg per 3 min, and the respiration of liver slices measured in Krebs-complete Medium II. The mean Q_{O_2} for the experimental animals was 20·3, while that of the controls was 17·6. Thus PCP caused a significant increase of the oxidation rate of the liver tissue.

TABLE 1. OXIDATION RATE, P:O RATIO, AND RESPIRATORY CONTROL OF LIVER MITOCHONDRIA ISOLATED FROM PCP-INJECTED RATS

Time of intoxication	Δ Rectal temperature (°C)	Pyruvate			L-glutamate		
		Q_{O_2}	P:O	R.C.	Q_{O_2}	P:O	R.C.
Controls	—	16·7 (22)	2·6 (22)	2·6 (22)	26·9 (15)	2·45 (15)	3·8 (5)
15 sec	0	20·4 (7)	1·7 (7)	1·4 (6)	26·5 (7)	1·79 (7)	1·9 (6)
30 sec	0	20·1 (5)	0·7 (5)	1·0 (4)	29·2 (5)	1·35 (5)	1·1 (4)
1 min	0	21·1 (8)	0·1 (8)	1·0 (4)	30·3 (8)	0·78 (8)	1·1 (4)
3 min	0	21·2 (12)	0·4 (12)	1·0 (10)	32·1 (4)	0·72 (4)	1·1 (4)
7 min	0	25·7 (4)	0·9 (4)	1·2 (2)	31·9 (4)	1·33 (4)	1·4 (2)
10 min	+0·5	25·2 (4)	0·8 (4)	—	34·2 (4)	1·17 (4)	—
60–90 min	+2·0	25·9 (6)	0·9 (6)	1·0 (2)	34·8 (6)	1·33 (6)	1·2 (2)
2·5–5 hr	0	21·8 (6)	1·4 (6)	1·4 (2)	32·4 (6)	2·02 (6)	1·1 (2)
3·0–6 hr	0	17·8 (8)	1·4 (8)	1·5 (2)	30·1 (8)	1·84 (8)	2·1 (2)
18–24 hr	0	17·6 (5)	2·6 (5)	3·2 (4)	24·8 (5)	2·48 (5)	4·0 (4)

Figures in parentheses indicate the number of determinations. 20 mg PCP per kg body weight were injected.

TABLE 2. OXIDATION RATE, P:O RATIO, AND RESPIRATORY CONTROL OF LIVER MITOCHONDRIA FROM PCP-INJECTED RATS, WITH VARIOUS SUBSTRATES

Substrate	Q_{O_2}		P:O		R.C.	
	Controls	PCP-injected	Controls	PCP-injected	Controls	PCP-injected
Pyruvate	16·7 (22)	21·2 (12)	2·6 (22)	0·4 (12)	2·6 (22)	1·0 (10)
Citrate	27 8 (4)	6·8 (11)	2·6 (4)	0·0 (11)	Not determined	
α-oxoglutarate	18·9 (4)	19·5 (3)	3·2 (3)	0·7 (3)	3·1 (3)	1·1 (3)
Succinate	54·2 (3)	28·6 (3)	1·5 (3)	0·2 (3)	2·3 (3)	1·0 (3)
Fumarate	10·0 (3)	15·8 (3)	2·7 (3)	0·1 (3)	2·0 (3)	1·1 (3)
L-glutamate	27·0 (10)	32·1 (4)	1·4 (10)	0·7 (4)	3·8 (5)	1·1 (2)

Substrate concentrations were as indicated in the Methods. Liver mitochondria were isolated from normal and PCP-injected animals (20 mg/kg body weight per 3 min). In parentheses, the number of determinations.

TABLE 3. EFFECT OF VARIOUS COFACTORS ON OXIDATION RATE OF CITRATE BY MITOCHONDRIA ISOLATED FROM PCP-INJECTED RATS

Addition	Q_{O_2}
None	5·94
NAD	9·30
NADP	16·15
Yeast concentrate	23·60

Cofactors concentrations were, 0·8 mM NAD; 0·8 mM NADP; 0·8 mg/ml yeast concentrate. Liver mitochondria were isolated from rats injected with 20 mg PCP/kg body weight per 3 min.

The effect on the P:O ratio

The P:O ratio for pyruvate was rapidly affected, and already 1 min after the PCP injection it was reduced to zero (Table 1). It is noticeable that shortly after, before the onset of pyrexia, the P:O ratio had recovered somewhat. However, the restoration to the normal values tended to lag behind the recovery of the temperature. The same effect could be observed with L-glutamate as substrate. The fact that the P:O ratio for this substrate, and also for α-oxoglutarate, was reduced to near 1, and not to zero, can be accounted for by the substrate-linked phosphorylation of α-oxoglutarate, which is not affected by 2:4-dinitrophenol, and presumably also by PCP, that appears to have a somewhat similar biochemical mode of action.[6]

The effect on the respiratory control

The respiratory control was the index of mitochondrial activity most severely affected by PCP. In fact it was completely annulled before the P:O ratio had reached its lowest level, and began to recover much later than this variable.

The effect on the adenosine triphosphatase activity

The mitochondria showed a progressive increase of ATPase activity in absence of added activators, parallel with the loss of the phosphorylative efficiency (Table 4).

TABLE 4. ATPASE ACTIVITY OF LIVER MITOCHONDRIA ISOLATED FROM NORMAL AND PCP-INJECTED RATS

Time of intoxication	Δ Rectal temperature (°C)	ATPase Activity (μmole P_i/mg protein per hr)				
		No addition	1·5 mM Mg^{2+}	Δ	0·01 mM PCP	Δ
Controls	—	0·64 (21)	1·26 (12)	+0·62	5·37 (19)	+4·73
15 sec	0	1·11 (5)	2·32 (5)	+1·21	4·49 (5)	+3·38
30 sec	0	2·42 (3)	3·16 (3)	+0·74	2·26 (3)	−0·16
1 min	0	3·35 (9)	4·01 (5)	+0·66	2·05 (9)	−1·30
3 min	0	3·00 (2)	3·88 (2)	+0·88	2·37 (2)	−0·63
7 min	0	1·80 (2)	2·90 (2)	+1·10	1·66 (2)	−0·14
60–90 min	+0·5	2·67 (2)	3·71 (2)	+1·04	4·18 (2)	+1·51
2·5–5 hr	+2·0	1·02 (2)	2·25 (2)	+1·23	4·84 (2)	+3·82
3·0–6 hr	0	1·50 (2)	2·19 (2)	+0·69	4 33 (2)	+2·83
18–24 hr	0	0·47 (4)	0·87 (4)	+0·40	4·08 (4)	+3·61

The rats were injected with 20 mg PCP/kg body weight. In parentheses, the number of determinations.

The effect of the addition of PCP *in vitro* was also tested in order to gain further information upon the mitochondria isolated from the poisoned rats. In fact Weinbach showed that low concentrations of PCP stimulated latent ATPase activity from normal rat liver mitochondria, and inhibited that of mitochondria variously damaged *in vitro*.[5] The same worker observed also that concentrations of PCP above a certain level caused inverse activating effects on normal mitochondria and even an inhibition of their ATPase activity. As shown in Table 4, the activation lessened progressively, until at the maximum of the biochemical damage, the addition of PCP resulted in a marked inhibition of the mitochondrial ATPase activity.

The effects of various PCP concentrations on liver mitochondria isolated from control and PCP-injected rats were also analyzed (Fig. 2). In agreement with the results of Weinbach,[5] in normal mitochondria the activation by PCP appeared to be evident already at 1×10^{-7} M, and progressively increased to a maximum at 1×10^{-5} M. At higher concentrations the activation diminished and, at 1×10^{-3} M, a clear inhibition of the ATPase activity was observed. The behaviour of mitochondria isolated from PCP-injected rats was quite different: even at the lowest concentrations tested, 1×10^{-7} M, PCP not only did not stimulate, but inhibited the ATPase activity, and increasing concentrations of the compound provoked higher inhibitions of this enzymic activity. The effect of Mg^{2+} on ATPase activity was also investigated: the slight activation found remained practically unmodified during the entire period of PCP intoxication. The recovery of the ATPase activity to the normal values was reached some hours after the end of pyrexia, and it was parallel to that of the respiratory control (Table 1).

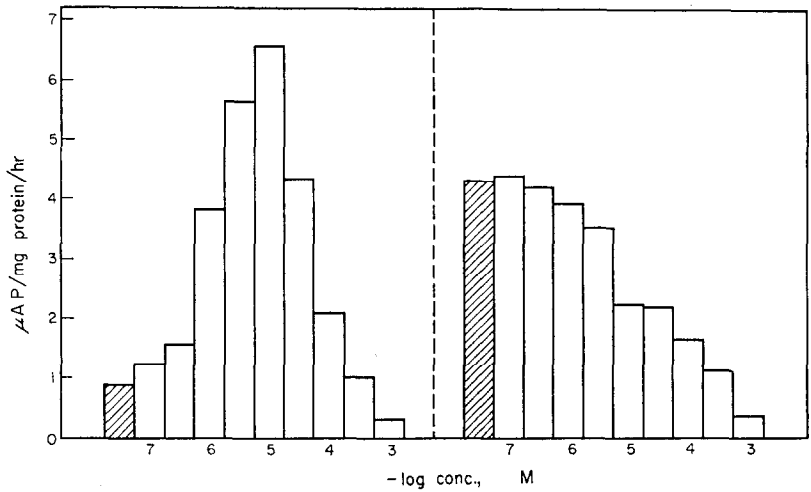

FIG. 2. Effect of pentachlorophenol on adenosine triphosphatase activity of rat liver mitochondria. Left, mitochondria from control rat; right, mitochondria from a rat injected with 20 mg pentachlorophenol/kg body weight per 90 sec. Shaded columns, activity without activators.

The effect on energy-rich phosphates

As a consequence of the impairment of oxidative phosphorylation, the concentration of energy-rich phosphates from liver tissue was significantly lowered. As shown in Table 5, this decrease became manifest some time after the biochemical lesions were evident in the mitochondria. Shortly after, the ~P level gradually rose and it returned within the normal ranges subsequent to the end of pyrexia.

DISCUSSION

The biochemical behaviour of mitochondria isolated from PCP-injected rats raises the question whether they were specifically or non-specifically injured, i.e. whether their altered functioning was depending on a biochemical lesion caused by PCP, or on some

generic structural change induced by the intoxication. The following evidence supports the former hypothesis. (a) The Mg^{2+} stimulated ATPase activity remained practically unmodified during the entire course of intoxication. Now, inasmuch as liver mitochondria are concerned, this activity is regarded as a rather sensitive index of mitochondrial structural integrity.[18] (b) The substrate-linked phosphorylation appeared to be unaffected. In fact the P:O ratios for L-glutamate and α-oxoglutarate remained in the range of 1 even at the most severe stages of intoxication, while the ratios for the substrates giving rise only to respiratory chain phosphorylation were annulled or nearly so.

TABLE 5. EFFECT OF PCP ON PHOSPHATES CONTENT OF RAT LIVER TISSUE

Time of intoxication	Δ Rectal temperature	Orthophosphate (μmole/g wet tissue)		
		P_i	$P/_T$	$\frac{P/_{T'} \times 100}{P_i + P/_{T'}}$
Controls	—	6·56 (15)	7·26 (15)	52·3
1 min	0	6·24 (7)	7·60 (7)	54·0
3 min	0	7·78 (4)	4·45 (4)	36·3
7 min	0	8·39 (4)	5·29 (4)	38·7
10 min	+0·5	7·73 (4)	5·79 (4)	42·9
60–90 min	+2·5	7·85 (6)	5·90 (6)	43·4
2·5–5 hr	0	7·11 (6)	6·08 (6)	45·7
3·0–6 hr	0	7·12 (8)	6·82 (8)	48·4
18–20 hr	0	4·75 (5)	7·04 (5)	61·6

The rats were injected with 20 mg/kg body weight. In parentheses, the number of determinations.

However, a certain degree of reversible structural damage probably also coexisted. In fact the increase of the oxidation rate with some substrates (pyruvate, fumarate, and L-glutamate) was possibly depending on a modified permeability condition of the mitochondria. Further, the inhibition of citrate oxidation seemed to be due to a lack of cofactors, since it was restored by adding NAD, NADP or a yeast concentrate. On the other hand, the PCP-induced release of nucleotides from liver mitochondria which has been shown by Weinbach to occur *in vitro*, might account for the observed diminution of succinate oxidation rate, in agreement with recently proposed mechanisms of succinate oxidation in liver mitochondria.[19]

The observed changes could be accounted for by the presence of PCP within the mitochondria. Attempts were made to identify the compound in the mitochondria obtained from the poisoned rats, but the results were equivocal. However, the behaviour of the ATPase activity in the course of intoxication and the effects upon this activity caused by PCP added *in vitro* to the injured mitochondria were indicative of the presence of the inhibitor in the particles isolated from the PCP-injected animal. In fact our results are in line with those by Weinbach[5] on normal liver mitochondria tested *in vitro* with different concentrations of PCP. This suggestion is further reinforced by the fact that the inhibition of oxidative phosphorylation caused by PCP *in vitro* in rat liver mitochondria cannot be reversed by washing.[8] The mitochondria isolated from liver of PCP-injected rats showed the same biochemical changes as normal liver mitochondria after addition of PCP *in vitro*. Thus it seems reasonable to conclude that PCP acts on mitochondria *in vivo* as it does *in vitro*. The fact that the

concentration of energy-rich phosphates in liver tissue markedly decreases during PCP-intoxication, provides additional evidence that the changes found in the isolated mitochondria occurred also *in vivo*.

The sudden and marked hydrolysis of energy-rich phosphates and the augmented rate of cell respiration, partially uncoupled, caused by PCP, indicates that an increase of heat production must occur in the liver of the poisoned rat. The question then arises whether the same phenomenon occurs also in other tissues and, especially, in muscle, in view of the role played by this tissue in the maintainance and regulation of body temperature. The mitochondria isolated from striated muscle of PCP-injected rats, contrary to those from liver, have been shown not to be biochemically injured.[8] This cannot be taken as a demonstration that they are not damaged *in vivo* by PCP. Since the uncoupling effects that PCP causes *in vitro* on muscle mitochondria can be reversed by washing,[8] it seems probable that during the isolation procedure the PCP, present in muscle mitochondria of poisoned rats, is washed away and their biochemical activities restored. On the other hand, following the PCP injection the concentration of energy-rich phosphate compounds undergoes a rapid and marked diminution in muscle,[8] and this leads to the conclusion that also in this tissue a relevant increase of heat production is taking place as a result of PCP-administration. This conclusion is substantiated by the reported stimulation of actomyosin ATPase activity by PCP *in vitro*,[20] and by the observation that the respiration of the rat increased during PCP-intoxication.[3] Thus it seems a well established fact that hyperthermogenesis does occur in the PCP-injected rat.

It remains to be decided whether this overproduction of heat is *per se* sufficient to explain the observed increase of body temperature. It is a widely accepted view that the hyperthermia caused in animals by 2:4-dinitrophenol and similar uncoupling agents is peripheral in origin and due to an excessive heat production. From the observed facts it is extremely probable that the increased heat production occurring in PCP-injected rats plays a decisive role in the pathogenesis of this hyperthermia. It cannot be excluded that mechanisms depending on the central nervous system also intervene in the complex phenomenon of PCP pyrexia. One observation might be of relevance in this connection: the recovery of body temperature occurs suddenly and it is not associated with a parallel change of the biochemical behaviour of liver mitochondria. In fact when the temperature falls to normal levels, the mitochondria are still "abnormal", and in liver tissue there is still over-production of heat.

Injected PCP diffuses in all tissues and most probably it causes similar biochemical lesions in cells of all kinds. However, the biochemical lesions will variously impair the physiology of the cells affected. It is therefore possible that PCP causes in the organism both hyperthermogenesis and also disturbance to some central physiological mechanism involved in the regulation of body temperature.

It is remarkable that the phosphorylative ability of liver mitochondria can be annulled or nearly so for some minutes without causing permanent damage to mitochondria. It is further noticeable that 1 hr after the end of pyrexia the energy-rich phosphates level was within the normal range, in spite of the fact that the phosphorylative ability of the mitochondria was still markedly reduced. This might mean that this level of mitochondria activity was sufficient to maintain the normal $\sim$P concentration, in conditions of low expenditure of energy as in the experimental animals during

the experiments, or that some other compensating phosphorylative mechanism was in action.

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