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Increase in Hepatic Mitochondria on Administration of Ethyl α-*p*-Chlorophenoxyisobutyrate to the Rat

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1. The antihypercholesterolaemic drug ethyl α-*p*-chlorophenoxyisobutyrate when fed to the rat orally or mixed with the diet increased the content of mitochondria in the liver by 50–100%. Other subcellular fractions did not show any significant change. 2. In oxidative activity, respiratory control and phosphorylating ability no significant difference was observed between the mitochondria isolated from the livers of the drug-treated rats and those from normal animals. 3. In agreement with earlier reports, administration of the drug depressed the concentration of serum cholesterol and increased liver weight and the liver content of ubiquinone. However, the increase of ubiquinone was greater in the nuclear than in the mitochondrial protein.

The compound CPIB* (Atromid-S or Clofibrate) is being extensively used as a drug for the treatment of hypercholesterolaemia. It was reported from this laboratory that the dietary administration of the drug to the rat resulted in the accumulation of ubiquinone in the liver (Krishnaiah, Inamdar & Ramasarma, 1967*a*). It was also shown that oral administration of ubiquinone increased its content in the liver and concomitantly inhibited the hepatic synthesis of cholesterol (Krishnaiah, Joshi & Ramasarma, 1967*b*). This led to the hypothesis that ubiquinone may be the natural agent that regulates the biogenesis of cholesterol (Ramasarma, Krishnaiah, Inamdar & Aithal, 1969). Ubiquinone in the liver has been shown to be distributed in all the subcellular fractions (Jayaraman & Ramasarma, 1963). The increase in ubiquinone pointed to the possibility that either some subcellular fraction itself or the concentration of ubiquinone in some subcellular fraction may have increased under the influence of the drug. It was therefore thought desirable to investigate the effect of the drug on the subcellular fractions as well as the pattern of distribution of the quinone in the fractions. The results presented in this paper show that concomitant with the depression of serum cholesterol concentration the drug produces an increase in the content of intact mitochondria of the liver. The accumulation of the quinone per mg of protein was greater in the nuclear than in the mitochondrial fraction.

* Abbreviation: CPIB, ethyl α-*p*-chlorophenoxyisobutyrate.

MATERIALS AND METHODS

Animals and administration of the drug. Male albino rats weighing 130–150g from the stock colony of this Institute were used except in one experiment in which female rats of the same weight range were used. The normal diet consisted of casein (20%), peanut oil (5%), cane sugar (10%), salt mixture (*U.S. Pharmacopeia*, 1947; 5%) and starch to make 100%. Every rat received 0.1ml of vitamin B-complex solution (*U.S. Pharmacopeia*, 1947) twice a week. Vitamin A (125μg), Vitamin D (1.0μg), vitamin E (1mg) and vitamin K_3 (25μg) were also given twice a week. Water and food were supplied *ad lib.* After being kept for 7–10 days on the normal diet, experimental animals were given 0.5% (w/w) CPIB mixed with the diet for 5, 10 and 15 days, at the end of which period they were killed. Four animals were used for each time-interval and four animals kept on the normal diet were also killed as controls for each time-interval. Two control and two experimental animals were killed at a time.

In the oral feeding experiments, the rats were dosed with 20mg of the drug. This would be equivalent to 0.2% in the diet on the basis that the rats on average consumed 10g of food/day. Further details are given in the table legends. At the time of killing blood samples were drawn and serum cholesterol was determined as described by Stadtman (1957).

Subcellular fractionation. This was done essentially as described by Johnson & Lardy (1967). The animals were killed by stunning and decapitation. The liver was weighed, chilled and homogenized with a Potter–Elvehjem homogenizer in 0.25M-sucrose, care being taken not to continue the process after the liver tissue was no longer discernible. The homogenate was made up to 10% (w/v)

with the sucrose solution and 30ml (corresponding to 3g of liver) was taken for the separation of subcellular fractions as described by Hogeboom & Schneider (1950). After the removal of the unbroken cells and cell debris by centrifugation at 30–100*g* for 10min, the nuclei and mitochondria were sedimented by centrifugation at 700*g* for 10min and 8250*g* for 10min respectively. The sediment in every case was suspended by homogenization in 10ml of medium, centrifuged at the same speed and the supernatant fraction added on to the next fraction. Since preliminary experiments showed that the microsomal fraction sedimenting at 105000*g* was unaffected by the administration of the drug, no attempt was made to separate the microsomes in these experiments; and the supernatant fraction referred to in Table 2 therefore contains both the microsomal and soluble fractions of the liver. All homogenizations were carried out in an ice bath and centrifugations in a refrigerated Sorvall RC2-B centrifuge at 0–4°C. The sucrose medium was prepared fresh daily in water previously boiled and cooled. The fractions were suspended in the same medium. The protein content of the samples was measured by the biuret method, deoxycholate being used for solubilization (Gornall, Bardawill & David, 1949).

Respiratory control and ADP/O ratio. The uptake of oxygen was measured polarographically (Estabrook, 1967) with the Gilson KM Oxygraph. The reaction medium contained (final concentrations) 0.4M-mannitol, 50mM-tris–HCl buffer, pH7.4, 25mM-potassium phosphate buffer, pH7.4, 25mM-KCl, 5mM-$MgCl_2$, 1mM-EDTA and 1–1.5mg of mitochondrial protein in a total reaction volume of 2ml. The state 2 rate of oxidation (Chance & Williams, 1955) was measured by stirring in 0.04ml of the substrate (0.5M-succinate, 0.25M-pyruvate+0.25M-malate or 0.25M-glutamate). State 3 oxidation was measured by stirring in 400nmol of ADP in 0.04ml. Respiratory control was calculated as the ratio of the oxidation rates in state 3 and state 4 (after the added ADP has been converted into ATP). The uncoupled succinate oxidation was measured by the addition of 0.25mM-2,4-dinitrophenol. The above reactions were carried out with freshly prepared mitochondria at 25°C.

2,4-Dinitrophenol-stimulated adenosine triphosphatase (EC 3.6.1.4) activity was determined in a medium containing (final concentrations) 150mM-tris–HCl buffer, pH7.4, 0.1M-KCl, 10mM-$MgCl_2$ and 0.5–0.75mg of freshly prepared mitochondrial protein in a total reaction volume of 1ml. After equilibration at 30°C for 3min, with or without the addition of 0.25mM-dinitrophenol, the reaction was started by the addition of 6μmol of ATP (in 0.1ml). After 5min the reaction was stopped by the addition of 10% (w/v) trichloroacetic acid (1ml) and the released P_i was measured by the method of Fiske & SubbaRow (1925).

The NAD pyrophosphorylase (EC. 2.7.7.1) activity was determined as described by Kornberg (1955) and glucose 6-phosphatase (EC 3.1.3.9) activity as described by Swanson (1955). DNA was determined by the diphenylamine method (Schneider, 1957). Ubiquinone contents of the cell fractions were determined spectrophotometrically after saponification and extraction (Joshi, Jayaraman & Ramasarma, 1963).

All the reagents used were of analytical grade and were dissolved in water double-distilled in an all-glass apparatus. All solutions were adjusted to pH7.4 before use. The sample of CPIB used was a generous gift from Dr J. M. Thorp, Pharmaceuticals Division, Imperial Chemical Industries, Ltd., Alderley Park, Macclesfield, Cheshire, U.K.

RESULTS

Composition of the liver. Administration of CPIB to the rat has been shown to induce morphological and biochemical changes in the liver. When rats were given CPIB at a dose of 0.5% in the diet there was progressive increase in the weight of the liver (Table 1). In 15 days the liver weight increased by 37% over the controls. This is in agreement with the observations by Hess, Riess & Staubli (1967). The influence of the drug on the protein content of the liver was much less marked. The protein/g of liver was 10–15% higher in the treated animals. Gould, Swyryd, Avoy & Coan (1967) observed that when animals were given

Table 1. *Effect of feeding with CPIB on the liver of the rat*

Rats were given CPIB at a dose of 0.5% (w/w) in the diet. The values are the means ±S.D. of four independent determinations. Succinate oxidation was determined in the presence of dinitrophenol (see the Materials and Methods section).

	Experimental period (days)	Normal	CPIB-fed
Fresh wt. of liver (g/100g body wt.)	5	3.7±0.3	4.5±0.3
	10	3.4±0.1	4.9±0.5
	15	3.3±0.6	5.2±0.6
Protein (mg/g of liver)	5	296±18	330±23
	10	267±4	327±11
	15	273±10	311±13
DNA (μg/mg of protein)	5	3.7±0.3	3.5±0.5
	10	4.1±0.1	3.7±0.2
	15	3.8±0.4	3.2±0.1
DNA (μg/g of liver)	5	1050±46	1135±95
	10	1085±63	1225±80
	15	1038±95	1200±125
Ubiquinone (nmol/100mg of protein)	5	55±7	56±9
	10	64±6	81±9
	15	66±6	112±20
Ubiquinone (nmol/g of liver)	5	115±10	133±9
	10	119±11	186±16
	15	126±19	218±7
Succinate oxidation (ng-atoms of O/min per mg of liver protein)	5	35±4	46±5
	10	43±7	56±5
	15	44±8	62±6
Succinate oxidation (ng-atoms of O/min per g of liver)	5	10333±680	15152±1040
	10	11495±2280	18383±2200
	15	11930±1600	19298±2400

0.3% CPIB for 6–14 days the increase in protein/g of liver was 2%. However, they observed a 14% decrease in the DNA content of the liver (per g of tissue) of the treated animals. This was interpreted to indicate an increase in the size of the cells. The results in Table 1 indicate that the DNA content/mg of liver protein did not show any significant decrease in the experimental animals. However, the DNA/g of liver showed an increase, indicating that the protein content per unit weight of the liver has increased.

The concentration of ubiquinone in the liver increased on administration of the drug. This confirms the observations by Krishnaiah *et al.* (1967*a*), Phillips, Lakshmanan & Brien (1968) and Krishnaiah & Ramasarma (1970). The uncoupled rate of succinate oxidation/g of liver also showed progressive increase and was 62% more than the controls after 15 days of feeding. However, the increase in the rate of oxidation of succinate/mg of protein was only about 40%. Hess, Staubli & Riess (1965) observed that on administration of the drug the cytochrome oxidase activity of the liver increased by 74%.

Protein content in subcellular fractions. The results in Table 1 indicate that the administration of the drug showed a tendency to increase the protein in the liver. This was investigated further by studying the distribution of protein in the subcellular fractions of the liver. For comparison animals kept on the normal diet were killed along with experimental animals to eliminate any variation during the time-intervals employed. The distribution of protein in the subcellular components of the normal and drug-administered (0.5% in the diet) animals is given in Table 2. The results show that on feeding with CPIB the protein content of the mitochondrial fraction increased by about 50% in 5 days and doubled in 10 days of feeding. Further feeding with CPIB up to 15 days did not increase the mitochondrial protein any further. Although in the livers of control rats, under the conditions of homogenization employed, the mitochondrial fraction accounted for about 20% of the total liver protein, in the livers of the CPIB-fed animals it accounted for 31% of the total protein (Table 2).

Table 2. *Effect of feeding with CPIB on the protein content of subcellular fractions of rat liver*

The values given are the means ±S.D. of four independent determinations. The values in parentheses are the percentages of protein, taking the total liver protein to be 100. The 'supernatant' refers to the post-mitochondrial fraction containing both the microsomal and soluble proteins.

Experimental period (days)	Cell fraction	Protein in fractions (mg/g of fresh liver) Normal	CPIB-fed
5	Nucleus	52.9±9.9	52.3±14.8
		(17.7±2.5)	(15.7±3.4)
	Mitochondria	58.0±10.5	81.6±7.4
		(19.5±2.3)	(24.8±2.2)
	'Supernatant'	185.5±2.8	196.0±11.7
		(62.8±4.2)	(59.5±4.2)
10	Nucleus	50.1±4.8	54.9±2.9
		(18.7±1.6)	(16.7±0.8)
	Mitochondria	52.5±3.4	99.8±8.4
		(19.6±1.0)	(30.5±1.7)
	'Supernatant'	164.0±5.2	172.5±3.3
		(61.7±2.5)	(52.8±1.7)
15	Nucleus	47.4±5.8	46.3±4.5
		(17.3±1.7)	(15.0±0.8)
	Mitochondria	55.6±2.2	97.8±12.0
		(20.3±0.9)	(31.5±2.0)
	'Supernatant'	170.0±6.3	166.5±4.5
		(62.4±1.3)	(53.5±3.1)

Table 3. *Effect of feeding with CPIB on the protein content of subcellular fractions of mouse liver*

Mice (5 months old) were given 0.5% (w/w) CPIB in the diet. Six livers weighing about 1.2g each were pooled and used for cell fractionation. The values in parentheses give the percentage distribution in the fractions. The microsomes were sedimented by centrifugation at 100000*g* for 1h in a Vac-60 refrigerated centrifuge.

Experimental period (days)	Cell fraction	Protein in fractions (mg/g of fresh liver) Normal	CPIB-fed
7	Nucleus	35.6	43.0
		(17.4)	(18.0)
	Mitochondria	38.8	48.8
		(19.0)	(20.4)
	Microsomes	32.5	40.3
		(15.8)	(16.8)
	Supernatant	97.5	107.0
		(47.8)	(44.8)
13	Nucleus	39.4	39.0
		(17.4)	(15.5)
	Mitochondria	41.5	62.5
		(18.4)	(24.8)
	Microsomes	39.6	43.0
		(17.5)	(17.1)
	Supernatant	105.5	107.0
		(46.7)	(42.6)
22	Nucleus	39.5	32.1
		(16.8)	(12.4)
	Mitochondria	42.5	69.6
		(18.0)	(26.4)
	Microsomes	47.9	45.2
		(20.2)	(17.1)
	Supernatant	107.0	116.0
		(45.0)	(44.1)

This indicates that the increase in protein content was accounted for by the mitochondrial fraction and that the 10–15% increase in the protein content/g of liver (Table 1) was almost entirely due to the mitochondrial increase on the administration of the drug.

The effect of CPIB administration on the mitochondrial content of the liver was tested in the rat at different dosages and time-intervals. When male rats were given orally 20mg of the drug daily (equivalent to 0.2% in the diet) the protein of the mitochondrial fraction of the livers increased by 40% in 7 days, by 62% in 14 days and by 88% in 21 days. In female rats under the same conditions the liver mitochondrial protein increased by 59% in 14 days and by 73% in 21 days.

Mouse liver mitochondria also increased on the administration of the drug. Thus when 5-month-old mice kept on the normal diet were given CPIB at a dose of 0.5% in the diet the liver mitochondrial protein increased by 26% in 7 days, by 51% in 13 days and by 64% in 22 days (Table 3). As with the rat, the other subcellular fractions did not show any significant change.

Quality of the mitochondria. The extent of nuclear and microsomal contamination of the mitochondrial fraction was determined by routine assays of the fractions for NAD pyrophosphorylase activity, which is localized in the nucleus (Hogeboom & Schneider, 1952), and glucose 6-phosphatase, which is localized in the microsomes (Swanson, 1950). Under the conditions of assay used the mitochondrial fraction of either control or treated livers showed no detectable NAD pyrophosphorylase activity. The glucose 6-phosphatase activity of the 'supernatant fraction' (which included the microsomes) was 150–160nmol of P_i released/10min per mg of protein at 30°C. The mitochondrial fractions under the same conditions showed an activity of 10–12nmol of P_i released/10min per mg of protein, indicating that there was no significant microsomal contamination in mitochondria in either the normal or the treated samples. Part of this activity has been shown to be constitutive of the outer membrane of the mitochondria (Brunner & Bygrave, 1969). The oxidation of succinate by the 'supernatant fraction' was very low (about 2ng-atoms of oxygen taken up/min per mg of protein), indicating that there was practically no contamination of the 'supernatant fraction' by mitochondria.

Administration of the drug produced no deleterious effect on the oxidative or phosphorylative activity of liver mitochondria, as shown by the results in Table 4. The mitochondria isolated from the livers of CPIB-treated rats were similar to normal mitochondria in rate of oxidation, respiratory control and ADP/O ratio with both succinate and NAD-linked substrates. In these mitochondrial preparations the rate of oxygen uptake was low in mitochondria from both control and CPIB-treated rats when a mixture of pyruvate and malate was used as the electron donor. The ADP/O ratio and the respiratory control ratio showed an increase on feeding with CPIB, particularly when glutamate was used as the substrate. In our experience, on occasions when normal mitochondria showed relatively poor respiratory control the mitochondria prepared alongside from drug-treated animals showed good respiratory control, implying that the latter are more refractory to damage during homogenization and isolation. Hess *et al.* (1967) from a comparative study of the behaviour of liver mitochondria from normal and CPIB-fed animals in density-gradient centrifugation concluded that the membrane structure of the latter was probably altered.

It is noteworthy that the specific activity of the enzyme systems involved in energy-linked oxidation is not affected by the administration of the drug (Table 4). This may be contrasted with the sixfold increase in the α-glycerophosphate dehydrogenase (EC 1.1.1.8) activity observed by Hess *et al.* (1965, 1967) in the livers of the drug-treated animals. However, in their opinion, increased

Table 4. *Effect of feeding with CPIB on the oxidative and phosphorylative activity of rat liver mitochondria*

Rats were given CPIB at a dose of 0.5% in the diet (see Table 1). The control values are the means of 12 normal animals. The experimental values are the means of four animals. Values for State 3 oxidation and uncoupled oxidation are given as ng-atoms of O/min per mg of protein; uncoupled oxidation was measured in the presence of dinitrophenol (see the Materials and Methods section). RC, respiratory control.

Experimental period (days)	Succinate				Pyruvate+malate			Glutamate		
	State 3 oxidation	RC ratio	ADP/O ratio	Uncoupled oxidation	State 3 oxidation	RC ratio	ADP/O ratio	State 3 oxidation	RC ratio	ADP/O ratio
Control	153	5.1	1.3	198	46	6.3	2.5	70	10.0	3.2
5	147	5.0	1.5	174	46	5.8	2.5	93	13.0	3.2
10	138	6.2	1.6	180	48	10.0	2.7	65	17.0	3.6
15	135	6.0	1.3	183	44	6.6	2.7	64	15.0	3.1

Table 5. *Effect of feeding with CPIB on the content of ubiquinone in the subcellular fractions of rat liver*

Rats were given CPIB at a dose of 0.5% in the diet. The control values are the means ± S.D. of 12 determinations on the normal animals (given in Table 1). The experimental values are the means ± S.D. of four determinations. The values in parentheses represent the percentages, taking the total in the liver to be 100.

	Nucleus		Mitochondria	
Experimental period (days)	(nmol/ 100mg of protein)	(nmol/ g of liver)	(nmol/ 100mg of protein)	(nmol/ g of liver)
Control	40 ± 4	20 ± 1 (17)	95 ± 9	53 ± 8 (44)
5	68 ± 8	32 ± 10 (25)	93 ± 13	76 ± 16 (58)
10	79 ± 5	41 ± 3 (22)	105 ± 13	105 ± 11 (57)
15	90 ± 8	42 ± 1 (19)	120 ± 13	117 ± 24 (52)

mitochondrial oxidation of α-glycerophosphate may not be directly related to the hypolipidaemic action of the drug. The adenosine triphosphatase activity of the two types of mitochondria also was of the same order (about 8nmol of ATP hydrolysed/min per mg of mitochondrial protein) and was stimulated fourfold in the presence of dinitrophenol.

Distribution of ubiquinone. To find out the pattern of distribution of ubiquinone, the nuclear and mitochondrial fractions were analysed for their ubiquinone content. The results are given in Table 5. In the normal animals the nucleus accounted for 17.0% and the mitochondria 43.5% of the ubiquinone in the liver. These values are in general agreement with the previous report (Jayaraman & Ramasarma, 1963). When CPIB was given to the animals the content of ubiquinone in both the nuclear and the mitochondrial fractions increased, and was doubled in both fractions in 10 days (Table 5). However, the ubiquinone content/mg of mitochondrial protein increased by only about 15%. This indicates that the ubiquinone content of the mitochondrial fraction increased because the mitochondria themselves increased under the influence of the drug. In contrast, the ubiquinone/mg of nuclear protein showed a 50% increase on CPIB administration for 5 days and doubled in another 5 days. The results also show that the ubiquinone concentrations in the microsomal and supernatant fractions were not influenced by the drug.

Pattern of cholesterol decrease and mitochondrial and ubiquinone increase. The pattern of changes in serum cholesterol concentration, liver mitochondrial content and liver ubiquinone content during feeding

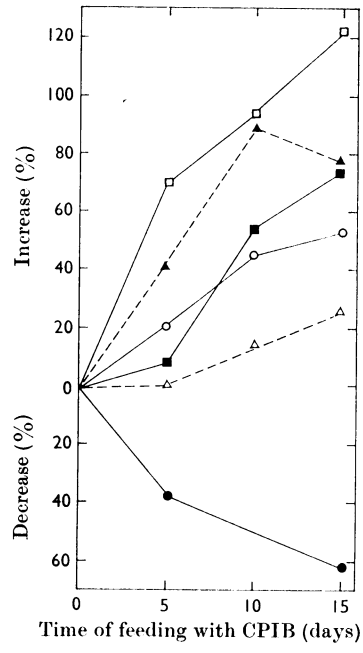

Fig. 1. Effect of feeding with CPIB on serum cholesterol concentration, liver ubiquinone content and liver mitochondrial protein content. The values represent the percentage increases or decreases over the controls. Each point is the mean of four independent determinations. ●, Serum cholesterol concentration; ○, fresh weight of liver; ▲, mitochondrial protein; △, ubiquinone content of mitochondrial protein; □, ubiquinone content of nuclear protein; ■, ubiquinone content/g of liver. The rats were given 0.5% CPIB in the diet for the period indicated.

with CPIB is illustrated in Fig. 1. The decrease in cholesterol concentration and increase in fresh weight of liver were progressive with time and were in agreement with earlier reports (Hess *et al.* 1965). The increase in the mitochondrial content of liver was also progressive with time of feeding up to 10 days. So far as the increase of ubiquinone in the liver was concerned only the nuclear quinone content appeared to follow the same trend as mitochondrial protein or liver weight. The increase of quinone in the whole homogenate as well as in the mitochondrial protein appeared to show an initial lag.

DISCUSSION

Since the discovery by Thorp & Waring (1962) that CPIB was effective in depressing the concentration of serum cholesterol this drug has been in extensive use as an antihypercholesterolaemic agent. Considerable information has accumalated

about the pharmacological and physiological effects of the drug, but relatively little is known about the action of the drug at the cellular and subcellular level. The increase in α-glycerophosphate dehydrogenase and cytochrome oxidase activities observed by Hess *et al.* (1965) indicated the possibility of increased mitochondrial content in the liver of the treated animals. Electron micrographs have also shown a possible increase in the number of mitochondria and lysosomes in the livers of rodents fed on the drug (Paget, 1963). The present results clearly show that the mitochondrial content of the livers of rats and mice is doubled on feeding with the drug and that the mitochondria are metabolically similar to those isolated from the livers of normal animals. Our results therefore indicate that the 74% increase in the cytochrome oxidase activity on administration of the drug observed by Hess *et al.* (1965) may reflect increased mitochondrial content in the liver homogenate, and may not be due to any stimulation of activity as claimed by them. The same, however, cannot be said of the many-fold increase in α-glycerophosphate dehydrogenase activity observed by Hess *et al.* (1965) and Westerfeld, Richert & Ruegamer (1968).

The reason for this increase in the liver content of mitochondria is not clear. It may not be a preventive mechanism developed by the animal to destroy the drug because we have observed that liver mitochondria from normal as well as CPIB-treated animals do not oxidize CPIB (free acid), nor does the free acid exert any significant inhibitory effect on mitochondrial oxidation or phosphorylation. Enhanced oxidative degradation of cholesterol by the increased mitochondrial content may not contribute significantly to the hypocholesterolaemic property of the drug because, even though liver mitochondria have been shown to oxidize the side chain of cholesterol (Lee & Whitehouse, 1963), the process appears to be relatively slow. Moreover, the mechanism of action of the drug has been shown to be the inhibition of some of the enzymic steps in the biosynthesis of cholesterol (Avoy, Swyryd & Gould, 1965).

Although CPIB is not itself thyromimetic, administration of the drug to the rat has been shown to decrease protein-bound iodine in the serum and lead to increase of thyroxine in the liver (Osario, Walton, Browne, West & Whystock, 1965). Experimental evidence both for and against adopting this 'displacement hypothesis' to explain the mode of action of the drug and its stimulatory effect on α-glycerophosphate dehydrogenase has been presented and discussed (Westerfeld *et al.* 1968; Ruegamer, Ryan, Richert & Westerfeld, 1969). Thyroxine has been shown to stimulate mitochondrial enzyme activities, and in skeletal muscle the mitochondrial content (Tata, 1966). However, according to Kadenbach (1966) the stimulation of respiratory activity in hyperthyroid liver was due not to increased mitochondrial content, but to increased synthesis of specific enzymes. He observed no increase in mitochondrial protein in relation to total protein of the liver. Viewed in this light, the reason for the mitochondrial increase in the present case cannot be attributed to accumulation of thyroxine in the liver induced by CPIB because the proportion of mitochondrial protein in relation to the total protein of the liver registers a definite and progressive increase (Tables 2 and 3). Also, according to Hoch (1967) mitochondria isolated from the livers of hypothyroid livers are 'super coupled' and administration of thyroxine to such rats increases the 'state 4' oxidation by the mitochondria. The results presented in Table 4 show that the drug did not produce any increase in the state 4 oxidation, and no decrease in the respiratory control ratio was observed in the mitochondria from CPIB-treated animals.

There are some reports that changes in the content of mitochondria may be brought about by dietary factors. Thus Scarpelli, Chiga & Haynes (1968) observed that starved rats showed a 50% decrease in liver mitochondrial content expressed on a DNA basis. When the rats were then fed, the mitochondrial content was restored to normal. The mitohcondrial content of the queen bee is twice that of the worker bees. This has been traced to the royal jelly, which is eaten only by the queen bee (Osanai & Rembold, 1968). In yeast it has been shown that the mitochondrial content is controlled by the concentration of glucose in the medium (Jayaraman, Cotman, Mahler & Sharp, 1966).

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REFERENCES

Avoy, D. R., Swyryd, E. A. & Gould, R. G. (1965). *J. Lipid Res.* **6**, 369.

Brunner, G. & Bygrave, F. L. (1969). *Eur. J. Biochem.* **8**, 530.

Chance, B. & Williams, G. R. (1955). *J. biol. Chem.* **217**, 409.

Estabrook, R. W. (1967). In *Methods in Enzymology*, vol. 10, p. 41. Ed. by Estabrook, R. W. & Pullman, M. E. New York: Academic Press Inc.

Fiske, C. H. & SubbaRow, Y. (1925). *J. biol. Chem.* **66**, 375.

Gornall, A. G., Bardawill, G. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.

Gould, R. G., Swyryd, E. A., Avoy, D. & Coan, B. (1967). *Prog. biochem. Pharmac.* **2**, 345.

Hess, R., Riess, W. & Staubli, W. (1967). *Prog. biochem. Pharmac.* **2**, 325.

Hess, R., Staubli, W. & Riess, W. (1965). *Nature, Lond.*, **208**, 856.

Hoch, F. L. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 506.

Hogeboom, G. H. & Schneider, W. C. (1950). *J. biol. Chem.* **186**, 417.

Hogeboom, G. H. & Schneider, W. C. (1952). *J. biol. Chem.* **197**, 611.

Jayaraman, J., Cotman, C., Mahler, H. R. & Sharp, C. W. (1966). *Archs Biochem. Biophys.* **116**, 224.

Jayaraman, J. & Ramasarma, T. (1963). *Archs Biochem. Biophys.* **103**, 258.

Johnson, D. & Lardy, H. (1967). In *Methods in Enzymology*, vol. 10, p. 94. Ed. by Estabrook, R. W. & Pullman, M. E. New York: Academic Press Inc.

Joshi, V. C., Jayaraman, J. & Ramasarma, T. (1963). *Indian J. exp. Biol.* **1**, 113.

Kadenbach, B. (1966). In *Regulation of Metabolic Processes in Mitochondria*, p. 508. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.

Kornberg, A. (1955). In *Methods in Enzymology*, vol. 2, p. 670. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Krishnaiah, K. V., Inamdar, A. R. & Ramasarma, T. (1967*a*). *Biochem. biophys. Res. Commun.* **27**, 474.

Krishnaiah, K. V., Joshi, V. C. & Ramasarma, T. (1967*b*). *Archs Biochem. Biophys.* **121**, 147.

Krishnaiah, K. V. & Ramasarma, T. (1970). *Biochem. J.* **116**, 321.

Lee, M. J. & Whitehouse, M. W. (1963). *Biochem. J.* **89**, 189.

Osanai, M. & Rembold, H. (1968). *Biochim. biophys. Acta*, **162**, 22.

Osario, C., Walton, K. W., Browne, C. H. W., West, W. & Whystock, P. (1965). *Biochem. Pharmac.* **14**, 1479.

Paget, G. E. (1963). *J. Atheroscl. Res.* **3**, 729.

Phillips, W. E. J., Lakshmanan, M. R. & Brien, R. L. (1968). *Can. J. Physiol. Pharmac.* **46**, 81.

Ramasarma, T., Krishnaiah, K. V., Inamdar, A. R. & Aithal, H. N. (1969). *Adv. exp. Med. Biol.* **4**, 395.

Ruegamer, W. R., Ryan, N. T., Richert, D. A. & Westerfeld, W. W. (1969). *Biochem. Pharmac.* **18**, 613.

Scarpelli, D. G., Chiga, M. & Haynes, E. jun. (1968). *J. Cell Biol.* **39**, 119A.

Schneider, W. C. (1957). In *Methods in Enzymology*, vol. 2, p. 680. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Stadtman, T. C. (1957). In *Methods in Enzymology*, vol. 3, p. 392. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Swanson, M. A. (1950). *J. biol. Chem.* **184**, 647.

Swanson, M. A. (1955). In *Methods in Enzymology*, vol. 2, p. 541. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Tata, J. R. (1966). In *Regulation of Metabolic Processes in Mitochondria*, p. 489. Ed. by Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C. Amsterdam: Elsevier Publishing Co.

Thorp, J. M. & Waring, W. S. (1962). *Nature, Lond.*, **194**, 948.

Westerfeld, W. W., Richert, D. A. & Ruegamer, W. R. (1968). *Biochem. Pharmac.* **17**, 1003.