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REFERENCES

1. L. SPOLTER and W. MARX, *Fedn Proc.* **20**, 162 (1961).
2. W. MARX and L. SPOLTER, *Helv. physiol. Acta* **19**, C85 (1961).
3. L. SPOLTER, L. I. RICE and W. MARX, *Biochim. biophys. Acta* **69**, 583 (1963).
4. L. I. RICE, L. SPOLTER, Z. TOKES, R. EISENMAN and W. MARX, *Archs Biochem. Biophys.* In press.
5. J. FURTH, P. HAGEN and E. W. HIRSCH, *Proc. Soc. exp. Biol. Med.* **95**, 824 (1957).
6. W. MARX, L. I. RICE, J. SHARON, A. S. BALASUBRAMANIAN and L. SPOLTER. In preparation.
7. B. L. STREHLER, in *Methods of Enzymatic Analysis* (Ed. H. U. BERGMEYER), p. 563 (1963).
8. N. D. GOLDBERG, J. L. DAHL and R. E. PARKS, *J. biol. Chem.* **238**, 3109 (1963).
9. S. SUZUKI and J. L. STROMINGER, *J. biol. Chem.* **235**, 257 (1960).

Biochemical Pharmacology, 1967, Vol. 16, pp. 232–235. Pergamon Press Ltd., Printed in Great Britain.

The effect of the dietary lipids upon the ability of chlorpromazine to inhibit oxidative phosphorylation in liver mitochondria

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IT HAS been previously reported that fatty acid compositions of tissue lipids in animals[1–3] and in man[4] can be made to differ by feeding nutritionally adequate diets containing lipids of different fatty acid composition. These changes in the lipoprotein structure of the cellular membranes and the tissue enzymes can be quite substantial, and it appeared reasonable to anticipate that cellular and subcellular functions and the response of enzyme systems to drugs and chemical reagents could be influenced by feeding different fats to growing animals.

The marked effect of dietary lipids on the appearance of various signs of vitamin E deficiency in different animal species has been documented.[5–9] The swelling of liver mitochondria in response to various toxic substances has been shown to be affected by the lipid fed to experimental animals.[10] Chlorpromazine was also shown to depress the uptake of ^{32}P into rat brain phospholipids *in vivo* at different rates in rats fed diets containing cod liver oil, corn oil, or beef fat.[11] Walker and Kummerow[12] reported that erythrocytes from rats fed coconut oil were more readily hemolyzed by glycerol and thiourea than if the animals were fed corn oil; and Tepperman and Pownall[13] found that feeding a saturated fat to rats resulted in increased activities of liver hexose monophosphate and malic dehydrogenases. The present investigation describes the inhibition of chlorpromazine on the uptake of inorganic phosphate in an oxidative phosphorylating system as a function of the lipid fed to the rat.

EXPERIMENTAL

Weanling male Sprague–Dawley rats were fed semisynthetic diets containing 15 per cent beef fat, 15 per cent corn oil, or 7 per cent cod liver oil for 7–10 weeks, as previously described,[14] except that

"salt mixture 446"[15] was used instead of "salts USP 14", and 0·38 ppm of chromium and 0·16 ppm of selenium were added. Vitamins A and D were supplemented orally, and *d*-α-tocopheryl acetate was added to the oils to provide 4·0 mg/100 g diet. Duplicate liver mitochondrial suspensions equivalent to 250 mg tissue/ml in 0·25 M sucrose were prepared from each rat. Concentrations of the six mitochondrial preparations for each day were made approximately equal by optical density measurements[10] and appropriate dilutions. Components added to glass-stoppered flasks in a total of 1·8 ml were 100 μmoles glycylglycine, 40 μmoles K_2HPO_4, 0·03 μmole cytochrome *c*, 26 μmoles sodium pyruvate, 4 μmoles malic acid (neutralized with KOH), 2·5 μmoles potassium ATP (neutralized with KOH), 16 μmoles $MgCl_2$, 10 μmoles NaF, and 0·5 ml mitochondrial suspension. The final pH was 7·4. Other substrates when substituted for malate and pyruvate were added in 26-μmole amounts. Chlorpromazine was added as indicated in Fig. 1. The flasks were preincubated for 2 min in a water bath shaker at 27°, followed by the addition of 3 mg hexokinase (Sigma, type III) and 50 μmoles dextrose in 0·2 ml. Samples were analyzed for inorganic phosphate by a modified Lowry–Lopez procedure[16] at zero time, and after 25 and 40 min of incubation. Per cent inhibition by the drug was determined for

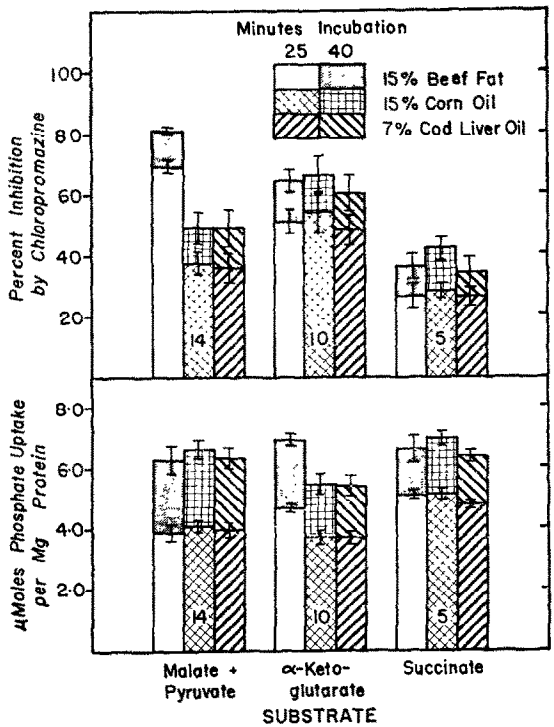

FIG. 1. Rate of inorganic phosphate uptake and relative inhibition by chlorpromazine in liver mitochondria from rats fed different dietary lipids. Concentration of chlorpromazine was 6×10^{-5} M when malate plus pyruvate or α-ketoglutarate was the substrate, and 9×10^{-5} M when succinate was the substrate. Numbers of animals per experimental diet are indicated for each groups of bars. Values are shown ± standard errors of the means.

each animal as the average inhibition obtained with two mitochondrial preparations from each liver. Mitochondrial protein was determined by a modified biuret reaction.[17] P:O Ratios of approximately 2·7 to 2·9 were obtained in preliminary determinations in which oxygen uptake was also measured in Warburg vessels. For the purposes of this study, only specific uptake determinations of inorganic

phosphate were necessary, since P:O ratios were usually not as sensitive an indicator of depression of phosphorylation.[18, 19]

RESULTS

Inhibition of phosphorylation by 6×10^{-5} M chlorpromazine was significantly higher ($P < 0{\cdot}001$) in mitochondria from animals fed beef fat, as compared with preparations from rats fed corn oil or cod liver oil, with malate plus pyruvate as the substrate (Fig. 1). With α-ketoglutarate as the substrate, there were no significant differences in per cent inhibition of phosphorylation with 6×10^{-5} M chlorpromazine among the mitochondria from rats fed beef fat, corn oil, or cod liver oil. Similarly, no difference was noted in inhibition by 9×10^{-5} M chlorpromazine with succinate as the substrate.

When 6×10^{-5} M dinitrophenol was incubated with mitochondria and malate plus pyruvate was the substrate, about 30 per cent inhibition was observed in the rate of phosphate uptake; but no differences were observed in the inhibition by 2,4-dinitrophenol in preparations from animals fed beef fat, corn oil, or cod liver oil.

With α-ketoglutarate as the substrate, the specific rate of inorganic phosphate uptake was significantly higher ($P < 0{\cdot}01$) with mitochondria from rats fed beef fat, as compared with activities of similar preparations from animals fed corn oil or cod liver oil (Fig. 1). These differences were observed in both the 25- and 40-min incubation periods. Mitochondrial activities were not significantly different among the experimental groups with either malate plus pyruvate or succinate as substrate.

DISCUSSION

The results presented here support the hypothesis that cellular and subcellular functions can be affected by the fatty acids furnished by the diet, and in turn by the composition of fatty acids found in cellular and subcellular lipoprotein structures and membranes. It is possible that the enzymes involved in the specific coupled phosphorylation reactions are themselves lipoproteins whose fatty acid composition can be altered by dietary means, resulting in different rates of phosphorylation, or in a difference in sensitivity to the depressant action of chlorpromazine, dependent upon the experimental conditions chosen. Dawkins *et al.*[20] proposed that chlorpromazine inhibits the enzymes involved in the transfer of high-energy phosphate coupled with electron transport between NADH and cytochrome *c*, whereas the action of 2,4-dinitrophenol is described as non-enzymatic uncoupling,[21] and is therefore not expected to be affected by changing the fatty acid compositions of tissues. Further investigations would be necessary to clarify this point, as well as to elucidate other changes in tissue functions and pharmacological effects resulting from feeding different dietary lipids.

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REFERENCES

1. L. A. WITTING, C. C. HARVEY, B. CENTURY and M. K. HORWITT, *J. Lipid Res.* **2**, 412 (1961).
2. B. CENTURY, L. A. WITTING, C. C. HARVEY and M. K. HORWITT, *Am. J. clin. Nutr.* **13**, 362 (1963).
3. R. T. HOLMAN, *Fedn Proc.* **23**, 1062 (1964).
4. M. K. HORWITT, *Am. J. clin. Nutr.* **8**, 451 (1960).
5. H. DAM and E. SØNDERGAARD, *Z. Ernährungsw.* **2**, 217 (1962).
6. B. CENTURY and M. K. HORWITT, *Archs Biochem. Biophys.* **104**, 416 (1964).
7. B. CENTURY and M. K. HORWITT, *J. Nutr.* **72**, 357 (1960).
8. L. A. WITTING and M. K. HORWITT, *J. Nutr.* **82**, 19 (1964).
9. M. K. HORWITT, *Vitams Horm.* **20**, 541 (1962).
10. B. CENTURY and M. K. HORWITT, *J. Nutr.* **80**, 145 (1963).
11. B. CENTURY and M. K. HORWITT, *Fedn Proc.* **24**, 688 (1965).
12. B. L. WALKER and F. A. KUMMEROW, *Proc. Soc. exp. Biol. Med.* **115**, 1099 (1964).

13. H. M. Tepperman and J. Pownall, *Fedn Proc.* **24**, 210 (1965).
14. B. Century, L. A. Witting, C. C. Harvey and M. K. Horwitt, *J. Nutr.* **75**, 341 (1961).
15. M. S. Mameesh and B. C. Johnson, *J. Nutr.* **65**, 161 (1958).
16. J. L. Peel and B. C. Loughman, *Biochem. J.* **65**, 709 (1957).
17. M. A. Inciosa, Jr., *J. Lab. clin. Med.* **63**, 319 (1964).
18. T. M. Brody and J. A. Bain, *J. Pharm. exp. Biol. Med.* **110**, 148 (1954).
19. B. Century and M. K. Horwitt, *Proc. Soc. exp. Biol. Med.* **91**, 493 (1956).
20. M. J. R. Dawkins, J. D. Judah and K. R. Rees, *Biochem. J.* **73**, 16 (1959).
21. H. A. Lardy and H. Wellman, *J. biol. Chem.* **201**, 357 (1953).