densation of two molecules of N-acetyl-diiodotyrosine. The N-acetyl group was hydrolysed with 5 *N* hydrochloric acid in the presence of iodide to prevent deiodination. Only the racemic forms of thyroxine, diiodotyrosine and tyrosine could be prepared by the above method. All attempts to obtain the L-forms by the action of L-amino-acid de-acetylase from pig kidney or penicillium extracts have so far failed.

We are indebted to Dr. R. Pitt-Rivers for help and guidance.

J. R. TATA*
A. D. BROWNSTONE
National Institute for Medical Research,
Mill Hill, London, N.W.7.

* Beit Memorial Fellow.

[1] Pitt-Rivers, R., and Tata, J. R., "The Thyroid Hormones" (Pergamon Press, London, 1959).
[2] Lipner, H. J., Barker, S. B., and Winnick, T., *Endocrinol.*, **51**, 406 (1952).
[3] Saul, J. A., and Trikojus, V. M., *Biochem. J.*, **42**, 80 (1948).
[4] Pitt-Rivers, R., *Biochem. J.*, **43**, 223 (1948). Pitt-Rivers, R., and James, A. T., *ibid.*, **70**, 173 (1958).

Inhibition of Lipid Peroxidation in Microsomes by Vitamin E

THE requirement for vitamin E is largely represented by a need for a biologically active lipid antioxidant. Vitamin E-deficient animals show *in vivo* lipid peroxidation and a wide variety of metabolic changes[1]. How metabolic changes are related to lipid peroxidation is a major problem.

Recently, we found[2] that isolated mitochondria deteriorate by hæmatin-catalysed lipid peroxidation. In addition, mitochondria isolated from the livers of vitamin E-deficient rabbits had undergone lipid peroxidation *in vivo* as measured by the thiobarbituric acid reaction. When isolated these mitochondria were more labile to lipid peroxidation *in vitro* than those from controls. This suggests that the free-radical intermediates of lipid peroxidation damage the mitochondria structurally and functionally, thus bringing about deranged metabolism.

Microsomes should be labile to lipid peroxidation for the same reasons as mitochondria. The microsomal fraction contains 30–40 per cent total lipid, mainly phospholipides, and contains twice the amount of polyunsaturated lipids on a nitrogen basis as do mitochondria. Some of these lipids are in close molecular proximity to cytochrome b_5. Cytochromes are among the most potent lipid peroxidation catalysts. Vitamin E is the only known lipid antioxidant in microsomes where it would function to inhibit lipid peroxidation.

Following the same course of research with microsomes as with mitochondria[2] it was found that the microsome fraction from rat livers deteriorated by lipid peroxidation at a rate of 0·1 ml. oxygen reacted/mgm. nitrogen/hr. at 37° C. Increase in thiobarbituric acid reactants paralleled oxygen absorption during the first 2 hr. Added α-tocopherol at 0·25 mgm./mgm. nitrogen gave 72 per cent inhibition of oxygen absorption and 69 per cent inhibition of thiobarbituric acid reactants. Since 0·01 *M* cyanide and 0·1 *M* methylene blue give 71–94 per cent inhibition as measured by oxygen absorption or thiobarbituric acid reactants, the lipid peroxidation appears to be catalysed by cytochrome b_5 and other hæmochromes present. Cytochrome b_5 destruction in a 4-hr. reaction was 80 per cent. To determine if the microsomes of vitamin E-deficient animals undergo lipid peroxidation *in vivo* they were isolated from the livers of vitamin E-deficient and control rabbits. The packed volume yield was one-third and the total nitrogen/ml. was six times higher for the microsomes from the vitamin E-deficient as compared to controls. The significance of these differences is at present unknown. Microsomes from vitamin E-deficient rabbits had 5·7 times as much thiobarbituric acid reactants per ml. as the controls. This highly significant difference ($P = 0·001$) indicates that the microsomes had undergone lipid peroxidation *in vivo*.

Lipid peroxidation damage to the microsomes of vitamin E-deficient animals may account for degeneration of microsomes as observed by electron microscopy[3], increased turn-over rate of some nucleic acids[4], effects on protein synthesis[1], and decreased ascorbic acid synthesis[5].

This research was supported in part by grant *A*–1672 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

A. L. TAPPEL
H. ZALKIN
University of California,
Davis, California.

[1] Harris, P. L., and Mason, K. E., in "Vitamin E", 3rd International Congress on Vitamin E, Venice, Italy (1955).
[2] Tappel, A. L., and Zalkin, H., *Arch. Biochem. Biophys.*, **80**, 326, 333 (1959).
[3] Piccardo, M. G., and Schwarz, K., in "Liver Function, a Symposium on Approaches to the Quantitative Description of Liver Function" (Amer. Inst. Biological Sci., Washington, D.C., 1958).
[4] Dinning, J. S., *J. Biol. Chem.*, **212**, 735 (1955).
[5] McCay, P. B., Carpenter, M. P., Kitabchi, A. E., and Caputto, R., *Arch. Biochem. Biophys.*, **82**, 472 (1959).

Biosynthesis of Glycerides in the Mucosa of the Small Intestine

IT is generally agreed that a mixture of free fatty acids, glycerol, monoglycerides and diglycerides is absorbed by the intestinal cells during digestion of fat and that the chyle secreted by these cells contains mainly triglycerides[1]. The present communication describes some studies on the biosynthesis of triglycerides by mitochondrial preparations from the mucosa of the small intestine of the rabbit and the rat.

For the preparation of the mitochondria, the small intestine was removed and washed with ice-cold potassium chloride (1·3 per cent). The mucosa was scraped off, homogenized in 10–15 vol. of ice-cold sucrose (0·25 *M*), and the mitochondrial fraction obtained by the usual sedimentation methods[2]. The washed mitochondria were suspended in potassium phosphate buffer (0·05 *M*, *p*H 7·0). 1·0 ml. of this suspension (containing between 1 and 4 mgm. protein per ml.) was incubated for 1 hr. at 37° C. with palmitic acid labelled with carbon-14 and various co-factors (see Table 1). Pure DL-α-glycerophosphate and labelled palmitic acid were used in aqueous solution as their potassium salts. α-Monostearin and α,β-dipalmitin were emulsified with small amounts of potassium oleate (0·8 μmole per 16 μmoles of α-monostearin; 1·2 μmoles per 4·0 μmoles of α,β-dipalmitin). All co-factors were at *p*H 7·0.

Reaction was stopped by the addition of 1/10 vol. of 50 per cent trichloracetic acid and the precipitate washed with water. The lipids were extracted with organic solvents and purified on alumina columns. The glyceride fractions were taken to dryness and counted as described elsewhere[3].

Experiments 1 and 2 show that α-monostearin, α,β-dipalmitin and α-glycerophosphate all stimulated the incorporation of labelled fatty acid into glycerides