adenylic acid and to Dr. A. M. Michelson for chemically synthesized polyuridylic and polyguanylic acids.

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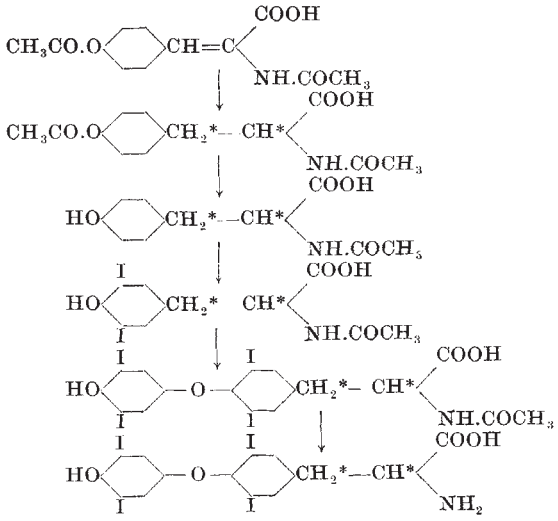
BIOCHEMISTRY

Synthesis of Tritium-labelled Tyrosine, 3 : 5-Diiodotyrosine and Thyroxine

Although much of the progress in thyroid hormone biochemistry is due to the use of thyroxine and 3 : 5 : 3′-triiodothyronine labelled with iodine-131, the high metabolic lability of the iodine atoms makes it impossible to follow the fate of these substances beyond the stage of enzymic deiodination[1]. The low specific activity of thyroxine labelled with carbon-14 [2] has ruled out its use under physiological conditions. Hence, with the availability of pure tritium gas and methods for its measurement, it was decided to prepare tritium-labelled thyroxine. The commonly used methods of exchange could not be used because of the danger of reductive deiodination by tritium gas. The procedure summarized below, and which involved the synthesis of N-acetyl-tyrosine and N-acetyl-3 : 5-diiodotyrosine as intermediates, was finally adopted. All compounds were hence labelled in 2 carbon atoms of their alanine side-chain.



4-Acetoxy-α-acetamidocinnamic acid, synthesized according to Saul and Trikojus[3], was dissolved in dioxan and reduced with tritium gas in the presence of platinum oxide to O,N-diacetyl-tyrosine. (This reaction was carried out by Dr. E. Anthony Evans at the Radiochemical Centre, Amersham.) The specific activity of O,N-diacetyl-tyrosine was 178·8 mc./m.mole. Tritium-labelled tyrosine could be obtained by its hydrolysis.

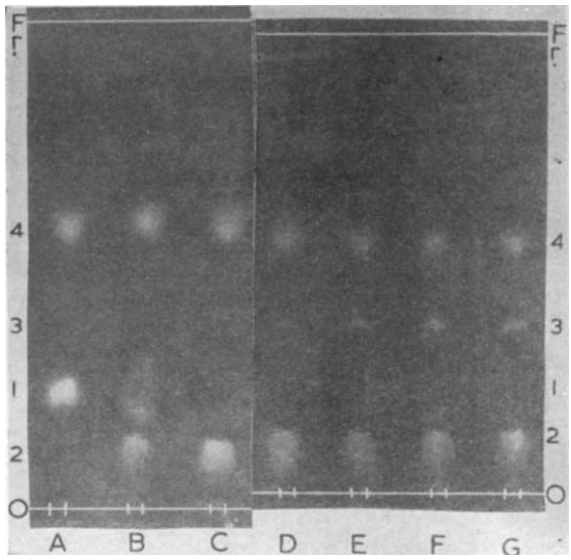


Fig. 1. Autoradiogram of a chromatogram developed to illustrate the reactions leading to the formation of ^{3}H-labelled N-acetyl-thyroxine. *A*: N-acetyl tyrosine in ammoniacal solution; *B*: After the addition of 1·5 m.mole iodine to the ammoniacal solution; *C*: after the addition of 5·0 m.mole iodine; *D*: 24 hr. after the incubation at 37° of reaction products in solution *C*; *E*: 4 days of incubation; *F*: 8 days; *G*: 12 days; 1: N-acetyl-tyrosine; 2: N-acetyl-3 : 5-diiodotyrosine; 3: N-acetyl-thyroxine; 4: unknown tritium-labelled material present as contamination in the original sample of O,N-diacetyl-tyrosine. Chromatographic solvent: *n*-butanol (80)-dioxan (20)-2*N* ammonia; *O*: origin of chromatogram; *Fr*: solvent front. Small amounts of radioactive products formed as a result of self-radiation damage are not visible in the autoradiogram

For the synthesis of labelled diiodotyrosine and thyroxine, 0·150 m.mole O,N-diacetyl-tyrosine was partially hydrolysed in 0·325 ml. 1 *N* sodium hydroxide at room temperature for 30 min. to N-acetyl-tyrosine. 0·350 ml. of concentrated ammonia (sp. gr. = 0·880) solution was added after neutralization of sodium hydroxide with 0·110 ml. 3·5 *N* hydrochloric acid. To this was added dropwise 0·5 m.mole iodine in potassium iodide at 0° and the excess iodine was neutralized with $Na_2S_2O_5$. The N-acetyl-3 : 5-diiodotyrosine formed is separated by preparative paper chromatography if diiodotyrosine is to be obtained. It is not separated if thyroxine is to be prepared; instead the volume of the solution was reduced to 0·3 ml. to which 1 ml. of 0·05 *M* borate buffer, *p*H 7·9, was added. Incubation of this solution at 37° C. led to the coupling of 2 molecules of N-acetyl-diiodotyrosine to 1 molecule of N-acetyl thyroxine, as described by Pitt-Rivers[4]. At the end of 12 days of incubation, the tritiated N-acetyl-thyroxine, the uncoupled N-acetyl-diiodotyrosine and other radioactive products were separated by preparative paper chromatography. Paper chromatography and autoradiography of aliquots withdrawn at different stages of the synthesis were used to follow the course of reaction as shown in Fig. 1.

Autoradiography was found to be a most convenient method for the detection and estimation of tritiated substances on chromatograms. Liquid scintillation counting confirmed the validity of such an estimation. The yield of tritiated N-acetyl-thyroxine was low: 0·009 m.mole (specific activity 56 mc./m.mole). Half the radioactivity would be lost in one alanine side-chain split off in the con-

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.

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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].

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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