increase in viscosity as well as the drop in the optical density at 260 mμ which was reported recently by Warner.[2] From this viscous solution, tough, glassy fibers can be drawn which are negatively birefringent, $\Delta n = -0.10$.

These fibers produce a well-oriented X-ray diffraction pattern with a distribution of intensity which is characteristically helical. The helical pitch varies from about 32 Å. at low relative humidity to 36 Å. at high relative humidity. The molecules are packed in hexagonal array, with an intermolecular spacing varying from 26 Å. at low humidity to over 32 Å. at high humidity. From the strong near meridional reflections in the range 3.0–4.2 Å., it can be shown that the number of residues per turn is approximately ten. Thus, this diffraction pattern has many similarities to that exhibited by desoxyribose nucleic acid (DNA).[3] However, a major difference is found in the first layer line, which is very strong for this molecule, and quite weak for DNA.

We have interpreted these results in the following way. The molecule is a two-stranded helix containing one strand of polyadenylic acid and one of polyuridylic acid. The bases adenine and uracil make two hydrogen bonds with each other in the same manner as that postulated for adenine and thymine in DNA,[4] with the base pairs stacked above each other roughly perpendicular to the fiber axis. The strong first layer line indicates that the angular separation of the ribose-phosphate backbones viewed from the helical axis is less than in DNA. This may be due to a parallel arrangement of the backbones, or to an antiparallel arrangement (as has been postulated for DNA) but with a greater radius than exists in the DNA molecule. It is anticipated that further studies of the Fourier transforms of these alternatives will permit us to decide between them.

These results show for the first time that it is possible for the ribonucleic acid (RNA) backbone to assume a configuration not unlike that found in DNA, using the same complementarity in the base pairs. This implies that there may exist a form of the RNA molecule similar to that of DNA and that this could be the form in which RNA carries out its implied molecular duplication in the plant and smaller animal viruses.

Finally, we would like to point out that this method for forming a two-stranded helical molecule by simply mixing two substances can be used for a variety of studies directed toward an understanding of the formation of helical molecules utilizing specific interactions.

We would like to thank Professor S. Ochoa for supplying us with some of the polynucleotide polymers used in this work, and Dr. F. H. C. Crick for helpful discussion.

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## THE ENZYMATIC SYNTHESIS OF RUBBER[1]

*Sir:*

Studies of the mechanism of biosynthesis of rubber in seedlings and cultured tissues of guayule have established that the carbon of the isoprenoid chain can be derived exclusively from acetate[2]; and flax enzyme preparations have indicated that the branched chain acids, β-methylcrotonate and β-methyl β-hydroxyglutarate are probable intermediates in the formation of the basic isoprenoid unit.[3] However, the requirement for intact plants or tissue cultures has made detailed studies of rubber biosynthesis difficult. We have now observed that incubation of latex with $C^{14}$-labeled acetate results in the incorporation of radioactivity into rubber. Latex, which may be obtained in quantity by "tapping" of *Hevea brasiliensis* bark, is a viscous cell-free liquid containing rubber particles, proteins and particulate cellular components, and constitutes the raw material for the natural rubber of commerce.

The time course of incorporation of acetate into rubber is shown in Fig. 1. Following incubation,

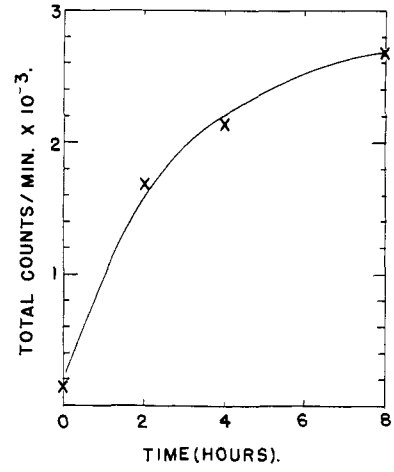


Fig. 1.—Time course of incorporation of 1-$C^{14}$-acetate into rubber: each incubation tube contained in micromoles, adenosine triphosphate 1, magnesium fructose diphosphate 2, CoEnzyme A 0.01, diphosphopyridine nucleotide 0.01, ethylenediamine tetraacetate 1, potassium phosphate 1, sucrose 60, acetate 2 (containing approximately $5 \times 10^6$ c.p.m.), and latex 0.1 ml.; total volume 0.3 ml.; *p*H 7; incubation at 37° for the indicated time.

the latex reaction mixture was coagulated with acetone and the rubber purified for counting by acetone and water extraction, solution in benzene–trichloroacetic acid, and precipitation as the micro-crystalline rubber bromide $(C_5H_8Br_2)_n$. The addition of cofactors, as listed in the legend for Fig. 1, stimulated the rate of acetate incorporation into rubber approximately ten-fold. Latex was also incubated with $C^{14}$-labeled sucrose, carbon

(1) Contribution jointly from Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture and Michigan State University, under contract with Quartermaster Corps., United States Army, using facilities at the Federal Experiment Station, Mayaguez, Puerto Rico.

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dioxide, β-methylcrotonate and pyruvate. It was found that β-methylcrotonate and pyruvate were incorporated at a considerably lower rate than acetate, and that sucrose and carbon dioxide were not incorporated.

The dependency of acetate incorporation into rubber upon latex concentration in the incubation mixture is illustrated by the data of Table I. Latex was added to all tubes at the end of the incubation period and immediately inactivated by the addition of acetone so that equal amounts of rubber were present in all tubes for isolation and counting.

TABLE I

EFFECT OF ENZYME (LATEX) CONCENTRATION OF ACETATE INCORPORATION INTO RUBBER[a]

| Latex, ml. | Radioactivity, (counts/minute/mg. carbon) |
|---|---|
| 0 | 2.6 |
| 0.02 | 2.8 |
| .04 | 4.5 |
| .06 | 14 |
| .08 | 38 |
| .10 | 120 |
| .13 | 210 |

[a] Reaction conditions as for Fig. 1 except for latex concentration as indicated; incubation time, 4 hours.

That the radiocarbon to acetate was in fact incorporated into rubber was demonstrated by degradation of a sample of the enzymatically synthesized rubber to levulinic acid. The specific activities of rubber bromide isolated as in the above experiments, of rubber bromide isolated after preliminary alcohol purification of the rubber, and of levulinic acid 2,4-dinitrophenylhydrazone prepared from the purified rubber were 220, 200, and 230 counts per minute per milligram of rubber carbon.

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## THE ENZYMATIC SYNTHESIS OF TRIGLYCERIDES

*Sirs:*

Previous reports from this Laboratory have described the function of cytidine coenzymes in the biosynthesis of lecithin and of phosphatidylethanolamine.[1,2,3,4] The synthesis of lecithin in isolated enzyme systems proceeds by the following reactions:

CTP + phosphorylcholine ⟷ Cytidine diphosphate choline + inorganic pyrophosphate (1)

Cytidine diphosphate choline + D-α,β-diglyceride ⟷ Lecithin + CMP (2)

Enzyme preparations from chicken liver catalyze the net synthesis of lecithin when supplemented with cytidine diphosphate choline and D-α,β-diglyceride.[4] It also has been shown that D-α,β-diglycerides may be formed by the enzymatic dephosphorylation of phosphatidic acids.[4]

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These results suggested that D-α,β-diglycerides might be common intermediates in the biosynthesis of phospholipides and of neutral fat. It has now been found that the same chicken liver enzyme preparations which carry out the synthesis of lecithin also catalyze the enzymatic net synthesis of triglyceride when supplemented with D-α,β-diglyceride and palmitoyl-S-Co A labeled with palmitic acid-1-$C^{14}$ (Table I). Extensive incorporation of labeled palmitic acid into the triglyceride fraction was noted with the complete system. No significant incorporation was observed if the enzyme was previously boiled. Omission of D-α,β-diglyceride or substitution of equivalent amounts of palmitate + CoA for the palmitoyl-S-Co A reduced incorporation to low levels.

TABLE I

ENZYMATIC NET SYNTHESIS OF TRIGLYCERIDE

| | Experimental conditions | Radioactivity of triglyceride fraction, total counts | Total triglyceride, micromoles |
|---|---|---|---|
| 1 | Complete system | 210,000 | 2.46 |
| 2 | Complete system, boiled enzyme | 610 | .72 |
| 3 | D-α-β-Diglyceride omitted | 12,200 | .79 |
| 4 | Palmitate + CoA in place of palmitoyl-S-Co A | 3,350 | .61 |

The complete system contained 1000 micromoles of Tris buffer of *p*H 7.4, 60 micromoles of $MgCl_2$, 40 micromoles of D-α,β-diglyceride derived from purified egg lecithin by treatment with lecithinase of *Cl. perfringens*,[5] 4 micromoles of palmitoyl-S-Co A labeled with palmitate-1-$C^{14}$ (140,000 cts./micromole), 20 mg. of "Tween-20" (polyoxyethylene sorbitan monolaurate),[6] 80 micromoles of cysteine, and 1.0 ml. of a suspension of mitochondria from chicken liver in a final volume of 10 ml. The tubes were incubated for two hours at 40°. The lipides, after extraction from the enzyme system with ethanol, were taken up in carbon tetrachloride and repeatedly washed with aqueous ammoniacal ethanol to remove unreacted palmitate and palmitoyl-S-CoA. The triglyceride fraction was isolated from the lipid mixture by the chromatographic method of Borgström.[7] The radioactivity was measured by direct plating and counting of a small aliquot. The total triglycerides were estimated by the hydroxamic method.[8]

Chemical measurement of the total amount of triglyceride at the end of the experiment showed that the incorporation of radioactivity was not due to simple exchanges processes, since a considerable net increase of triglyceride was observed in the complete system, compared to the control with boiled enzyme.

These results support the conclusion that the synthesis of triglycerides in these preparations proceeds by the reaction:

D-α,β-diglyceride + palmitoyl-S-Co A ⟶ Triglyceride + CoA-SH (3)

A close interconnection is thus indicated between the enzymatic synthesis of phospholipides and of neutral fat.[9]

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(9) The following abbreviations are used in this paper: CTP = cytidine-5′ triphosphate; CMP = cytidine-5′ phosphate; CoA = coenzyme A