Biosynthesis of Rubber*

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DESPITE all the knowledge available concerning the production of various types of synthetic rubber, no elastomer has yet been produced that has the low heat build-up or low hysteresis of natural rubber. This property is essential for making large size heavy-duty tires. If it were possible to produce synthetic rubber possessing this desirable property, it would no longer be necessary to maintain a large stockpile of natural rubber. The current carrying charge for the strategic natural rubber stockpile is about $20 million a year. It has been authoritatively stated that "Since the major current requirement for natural rubber is for use in large truck tires, a long-range program to develop and apply synthetic rubber for this purpose presents the only possibility of greatly reducing the Nation's dependence on natural rubber" (*1*).

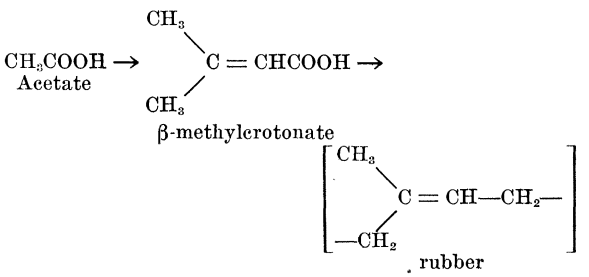
Many chemical approaches have been tried without success in an effort to find a synthetic equivalent for natural rubber. The present program is a biochemical one. We are finding out the processes involved in the formation of rubber in the living plant. It is hoped that the knowledge obtained may give us new and novel approaches to the development of low heat build-up synthetic rubbers.

Two related investigations are currently under way. One at the California Institute of Technology has to do with the study of rubber synthesis by plant enzyme systems. The other, carried on by the U.S. Department of Agriculture, is concerned specifically with rubber synthesis by the rubber tree, *Hevea*. The U.S. Department of Agriculture is also cooperating in the Argonne National Laboratory, Illinois, on production of C^{14}-labeled natural rubber.

Synthesis of rubber in the living plant. Natural rubber is, of course, a polymer made of repeating isoprene units. Each isoprene unit contains one double bond, and in natural rubber these double bonds all have the *cis*-configuration. This structure has not been duplicated outside of the living plant. It is the all-*cis*-configuration, together with the nature of the monomer, that presumably is responsible for the low hysteresis or low heat build-up properties. It may be noted that rubber is only one of a general class of compounds known collectively as the isoprenoids, all of which are based on isoprene as the repeating unit. The isoprenoids include the terpenes, in which 2, 3, 4, or 6 of the 5-carbon isoprene units are bound together in a single molecule; the carotenoids, in which 8 of the 5-carbon units are bound together; and the polyisoprenoids, which include rubber and gutta-percha. All plants synthesize one or another of the isoprenoids. Thus, carotenoids and phytol, the long-chain alcohol that is a component of the chlorophyll molecule, are universal components of higher plants. A few kinds of higher plants, about 4000 of a total of 400,000 or so species, make large quantities of one or another terpene or polyisoprenoid. In some cases, the lower terpenes are accumulated as a so-called "essential oil" (the turpentine of pine trees). In other cases, it is rubber that is accumulated, as the rubber of *Hevea*, guayule, or other species. So far as we can ascertain at the present time, neither rubber nor the lower terpenes have any essential function in the plant (*2*). They appear to represent a storing away of the basic 5-carbon unit in forms that are not usable by the plant. Thus, neither rubber nor the lower terpenes, once made, are utilizable as food material by any higher plant that has been investigated.

If we are to discover how plants make isoprenoids, it is first necessary to discover the 5-carbon monomer used by the plant. The monomer cannot be isoprene itself, since this has not been found to occur in plants. This question was first intensively studied with the guayule, a shrub that produces large quantities of rubber within individual cells. It has been shown (*3, 4*) that it is possible to cause small pieces of the branches of guayule, or even seedlings of this plant, to make additional rubber if they are supplied with appropriate carbon-containing compounds. It has been shown that the simple 2-carbon compound acetate forms the source of all the carbon atoms used in making rubber by the guayule plant. If we supply radioactive C^{14}-labeled acetate to the plant, it makes rubber in which every carbon atom is labeled with C^{14} (*5, 6*). Thus, acetate forms the basic precursor. Yet this 2-carbon compound must somehow be made into a 5-carbon branched-chain compound of the structure of isoprene. The nature of the 5-carbon compound is suggested by the fact that the substance β-methylcrotonic acid pos-

sesses the ability to support efficiently rapid rubber formation in guayule (*3*). That β-methylcrotonate is formed from acetate is indicated by the fact that when plants are fed C^{14}-labeled acetate, they synthesize C^{14}-labeled β-methylcrotonate (*6*). The general course of rubber synthesis in the guayule may therefore be as suggested in the following equation.



One might object that the guayule is only one rubber plant and other species, such as *Hevea,* might synthesize rubber by some different pathway. Experiments similar to those discussed in preceding paragraphs have, therefore, been made with *Hevea* by H. J. Teas at the U.S. Deparament of Agriculture Laboratory in Mayagüez, Puerto Rico. It is not possible to test the effect of added substances on rubber synthesis in *Hevea* by simply injecting the test substance into the latex system and subsequently determining the amount of rubber in the latex collected from a test tap. Compagnon and Tixier (*7*) have shown that injections of such nonspecific substances as copper sulfate greatly increase the flow of latex and, therefore, the yield of rubber in trees being tapped by commercial methods. In the present work, the test substances are applied in holes drilled in squares of bark that have been isolated from the rest of the latex system by deep cuts. After an appropriate period, samples of the bark are removed and analyzed for total rubber. Applications of either acetate or β-methylcrotonate have resulted in apparent increases in rubber synthesis in *Hevea* bark. The increases are considerable, amounting to as much as 50 percent or more. Other substances tested had little or no influence on rubber synthesis.

N. J. Scully and his group at the Argonne National Laboratory have developed facilities that permit the growth of plants under completely controlled environmental conditions and in an atmosphere of radioactive $C^{14}O_2$. Small *Hevea* trees have been grown in this fashion and the latex collected periodically. The uniformly labeled rubber obtained is available for the study of technologic matters. These experiments indicate that there is a time lapse of several hours between the introduction of C^{14} to the leaf and its appearance in the rubber hydrocarbon.

Enzymology of rubber formation. It has been mentioned in a preceding paragraph that β-methylcrotonate is able to support rubber formation in the plant and that this 5-carbon acid may therefore be an intermediate in the formation of the isoprenoid monomer. We now have two problems: (i) how do β-methylcrotonate and the monomer get made from acetate, and (ii) how is the monomer polymerized?

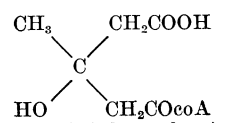
In order to find out more about these matters, it has been necessary to work not with intact plants fed with particular compounds or particular carbon^{14}-labeled radioactive compounds but with isolated plant enzyme systems. It should be possible in principle to trace the path of acetate carbon atoms to rubber by identifying the enzymatic steps responsible for the transformations of acetate on its path to β-methylcrotonate and thence to rubber. It has been found that in the enzymatic system represented by the contents of plant cells, acetate is not further metabolizable until it is first transformed to the derivative acetyl-coA (*8*). The facts available suggest that the path of acetyl-coA to β-methylcrotonate may follow this general outline:

1) $CH_3COOH + coA \rightleftharpoons CH_3COcoA$
Acetate — acetyl-coA

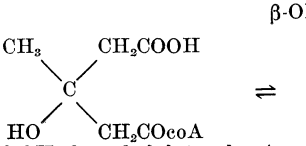
2) $2CH_3COcoA \rightleftharpoons CH_3COCH_2COcoA + coA$
Acetyl-coA — acetoacetyl-coA

3) $CH_3COCH_2COcoA + H_2O \rightleftharpoons CH_3COCH_2COOH + coA$

4) $CH_3COCH_2COOH + CH_3COcoA \rightleftharpoons$
Acetoacetate — acetyl-coA

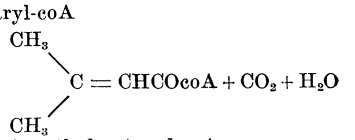


β-OH, β-methylglutaryl-coA

5)


β-OH, β-methylglutaryl-coA

$\rightleftharpoons$



β-methylcrotonyl-coA

In this pathway, acetyl-coA is joined with itself to form a 4-carbon compound acetoacetyl-coA, which is then hydrolyzed to acetoacetate. Another acetyl-coA molecule is joined to acetoacetate to form a 6-carbon compound, and carbon dioxide and water are subsequently cleaved from this material to form β-methylcrotonyl coA.

β-Hydroxy-β-methylglutarate (BOG) was first suspected as an intermediate in this series of reactions when it was tentatively identified as a product of the metabolism of C^{14}-labeled acetate by an acetone powder of spinach leaves (*9*). BOG is now known to be a naturally occurring plant product (*10, 11*) and its metabolism in the plant has been elucidated by J. A. Johnston and D. Racusen. The sequence of reactions 1 through 4 can be consummated with an enzyme system prepared from flax seedlings, a plant that normally accumulates considerable quantities of BOG. Reactions 1, 2, and 3 have been studied in several systems (*12–14*) including those of plants (*8*) and are common to the fatty acid metabolism as well as to isoprenoid syn-

thesis. Reactions 4 and 5 have been investigated conveniently in the reverse direction, using labeled β-methylcrotonate and CO_2 as the substrates.

It is now of interest to know how β-methylcrotonyl units are polymerized and whether it is the β-methylcrotonyl-coA derivative that is involved in rubber formation. It appears possible that union of 5-carbon units is carried on in a manner basically similar to that by which the 2-carbon acetyl-coA fragments are united to form the 4-carbon acetoacetyl-coA. Reduction of the 10-carbon compound, which would result from the initial polymerization, would lead then to a 10-carbon hydrocarbon. But this union necessarily involves several individual enzymatic steps. It involves the introduction of the specificity of the *cis*-bond in each of the two 5-carbon units. We do not yet understand how the responsible enzyme catalyst assures that each unit as it is introduced into the whole will be of the *cis*-configuration, but we do at least have an effective and readily studied system for the working out of these important matters.

It is interesting to note that the pathway by which plants make rubber is not unique to plants but has its parallel in microorganisms and in animal tissues. The participation of acetate and of β-methylcrotonyl units in the syntheses of carotenoid pigments by a variety of lower organisms appears probable (*15*). In these lower organisms, however, as in most higher plants, polymerization of the monomer stops when 8 units are put together. This 8-unit piece is then modified by the introduction of further double bonds until a carotenoid pigment is formed.

The steroid cholesterol appears to be synthesized from acetate in the animal body as is rubber in the plant (*16*). All carbon atoms of cholesterol derive from acetate. Both β-methylcrotonate and the 6-carbon, β-hydroxy, β-methylglutarate are indicated as intermediates in this synthesis (*16, 17*). That the synthesis of β-methylcrotonate by liver proceeds from acetate through β-OH, β-methylglutarate as outlined in reactions 1–5 has been indicated by recent work (*18–21*). It appears possible from the work of Bloch (*16*) that the animal may first synthesize a linear triterpene containing 6 of the 5-carbon monomer units and then cyclize this triterpene to form a precursor of cholesterol which is then modified by elimination of appropriate carbon atoms to form the final carbon skeletal of the substance.

Summary. The problem of how rubber is synthesized in the plant has been divided into two portions: (i) the nature of the monomer used and how this monomer is synthesized, and (ii) the nature of the polymerization reaction by which the monomer is transformed to rubber. With respect to the first, the monomer appears to be the 5-carbon branched-chain compound β-methylcrotonic acid or a derivative thereof. This substance is synthesized in the plant from acetyl-coA. With respect to the second, no information is available, since polymerization of the 5-carbon monomer units to polymers has not yet been achieved outside the living plant.

References and Notes

* Presented at Joint Elastomer Conference, Department of Defense, 12–13 Jan. 1954.
1. Memorandum, Office of Chief of Ordnance, 3 Aug. 1953.
2. J. Bonner and A. W. Galston, *Botan. Revs.* **13**, 543 (1947).
3. J. Bonner and B. Arreguin, *Arch. Biochem.* **21**, 109 (1949).
4. B. Arreguin and J. Bonner, *ibid.* **26**, 178 (1950).
5. J. Bonner, *J. Chem. Educ.* **26**, 628 (1949).
6. B. Arreguin, J. Bonner, and B. J. Wood, *Arch. Biochem.* **31**, 234 (1951).
7. Compagnon and Tixier. *Rev. gén. caoutchouc* **27**, 525, 591, 663 (1950).
8. A. Millerd and J. Bonner, *Arch. Biochem and Biophys.* **49**, 343 (1954).
9. R. S. Bandurski, T. Coyle, and J. Bonner, *Biology 1953* (Calif. Inst. Technol., Pasadena, 1953).
10. R. Adams and B. L. Van Duren, *J. Am. Chem. Soc.* **75**, 2377 (1953).
11. H. Klosterman and F. Smith, *ibid.* **76**, 1229 (1954).
12. E. R. Stadtman, M. P. Doudoroff, and F. Lipmann, *J. Biol. Chem.* **191**, 377 (1951).
13. F. Lipmann *et al., J. Am. Chem. Soc.* **74**, 2384 (1952).
14. H. Beinert *et al., J. Biol. Chem.* **203**, 35 (1953).
15. T. W. Goodwin, W. Lijinsky, and J. S. Willmer, *Biochem. J. (London)* **53**, 208 (1953).
16. K. Bloch, *Harvey Lectures* (Academic Press, New York, 1952–53), p. 68.
17. L. C. Clark *et al., Federation Proc.* **13**, 192 (1954).
18. H. Rudney, *ibid.* **13**, 286 (1954).
19. ———, *J. Am. Chem. Soc.* **76**, 2595 (1954).
20. A. W. Robinson, B. K. Bachhawat, and M. J. Coon, *Federation Proc.* **13**, 281 (1954).
21. J. Rabinowitz and S. Gurin, *J. Biol. Chem.* **208**, 307 (1954).

Nucleotides from T2r$^+$ Bacteriophage*

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If desoxyribonucleic acid, obtained by osmotic shock from T2r$^+$ bacteriophage, and deproteinized, is treated successively with pancreatic desoxyribonuclease and purified venom phosphodiesterase (*1*), 62 percent of the phosphorus (P) of the nucleic acid can be recovered in the form of mononucleotides. This result is in distinct contrast to that obtained with calf thymus or wheat germ desoxyribonucleic acid, which are degraded quantitatively to mononucleotides by this procedure (*1, 2*). The remainder of the P is in the form of enzyme-resistant di-, tri-, and polynucleotides.