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.

If the mononucleotides thus obtained are fractionated by ion-exchange chromatography at *p*H 4.3 (*3*), five peaks are obtained. The last three of these are readily recognized as thymidylic, desoxyadenylic, and desoxyguanylic acids. The first two, temporarily named H1 and H2 (Fig. 1), are new nucleotides, cystosine-like in spectra. The proportions of the five mononucleotides are given in Table 1. Data on certain ultraviolet spectroscopic ratios of the two new nucleotides are given in Table 2.

While differing in spectra at *p*H 4.3, nucleotides H1 and H2 are virtually identical in spectra at *p*H 7 or *p*H 1. Upon hydrolysis in 6*N* HCl for 3 hr at 100°C, both nucleotides yield the pyrimidine, 5-hydroxymethylcytosine (HMC) (*4*), as evidenced by its chromatographic behavior in water-ammonia-propanol solvent (*5*) and its ultraviolet absorption spectrum (*6*). One mole of HMC per mole of phosphorus is obtained from each nucleotide.

Both nucleotides are dephosphorylated by prostatic phosphomonoesterase. Both are dephosphorylated by the 5-nucleotidase of rattlesnake venom, which is known not to attack 3′-desoxyribonucleotides (*7*), although it should be noted that the action of this enzyme on H1 is considerably slower than on H2.

Upon applying the Stumpf (*8*) test for desoxyribose, both H1 and H2 produce an incomplete reaction, very nearly parallel in time course to that of desoxycytidylic acid. Upon applying the Dische test (*9*), H2 produces in 24 hr an absorption peak at 380 mμ, characteristic of desoxyribose; H1, however, produces an immediate reaction, characteristic of hexoses, with an absorption maximum at 410 mμ. In 24 hr the spectrum shifts to that obtained from a mixture of hexose and desoxypentose.

If H1 is hydrolyzed with 1*N* HCl for 2 hr at 100°C, 0.92 mole of reducing sugar (*10*) is released per mole of P. Similar treatment of H2 released no reducing sugar (under these conditions, desoxyribose is converted to levulinic acid and does not titrate as a reducing sugar).

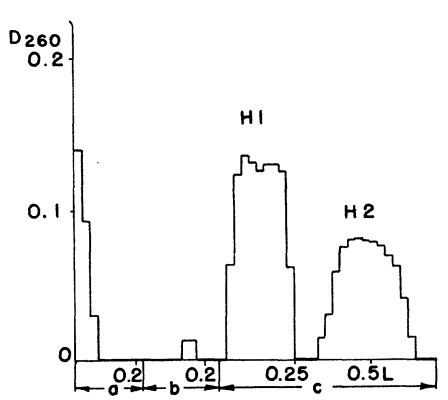

Fig. 1. Chromatographic fractionation of H1 and H2 from 60 mg of T2r⁺ DNA. Column Dowex-1-8x, 10cm $\times \pi/4$ cm². *a*, 0.01*N* NH_4OH; *b*, 0.01*M* acetate buffer, *p*H 4.3; *c*, 0.06*M* acetate buffer, *p*H 4.3.

Table 1. Mononucleotides obtained by enzymatic degradation of T2r⁺ DNA.

Nucleotide	Ultraviolet absorption at 260 mμ (%)	P (%)
H1	1.78	3.0
H2	1.25	2.2
Thymidylic	20.0	22.2
Adenylic	34.5	22.8
Guanylic	15.9	12.5
Total	73.4	62.7

Table 2. Spectroscopic ratios.

*p*H	Ratio (mμ/mμ)	H1	H2	Desoxycytidylic
4.3	260/280	0.66	0.60	0.64
	270/290	1.45	1.28	1.60
6.5	260/280	0.83	0.82	1.02
	270/290	1.96	1.97	4.2

Paper chromatographic analysis of such a 2-hr hydrolysate of H1, using a water-pyridine-butanol solvent (3 : 4 : 6 parts by volume) and staining with aniline phthalate reagent (*11*), indicated the presence of an aldohexose that migrates as does glucose. Spraying with ninhydrin reagent gave a negative reaction.

After hydrolysis of H1 for 1 hr in 1*N* HCl at 100°C, which released 0.61 mole of reducing sugar per mole of P, the digest was fractionated on Dowex-1 resin at *p*H 4.3. Three peaks were recovered, a pyrimidine base, undegraded H1 (0.10 mole/mole initial H1), and H2 (0.25 mole/mole initial H1). The pyrimidine base had the spectrum of HMC and migrated with HMC in paper chromatography. However, it gave a positive hexose reaction in the Dische test (HMC does not) to the extent of 0.27 mole hexose (as glucose) per mole initial H1. The undegraded H1 plus the hexose of the pyrimidine base thus accounts for the residual, unreleased hexose of the initial H1. The H2 recovered must have been derived from the H1 by splitting off reducing sugar (glucose).

Similar hydrolysis of H2 yielded only HMC and undegraded H2 (0.41 mole/mole initial H2).

It thus appears that H2 is 5-hydroxymethyldesoxycytidylic acid, while H1 is a glycosyl substituted 5-hydroxymethyldesoxycytidylic acid. The mixed pyrimidines recovered after acid hydrolysis of H1 are HMC and glycosyl substituted HMC. The position of the glucose substitution has not been established, although the 5-hydroxymethyl position seems most likely in view of the near identity of the spectra of H1 and H2.

Thymidylic, desoxyadenylic, and desoxyguanylic acids from T2r⁺ nucleic acid give only the desoxyribose reaction in the Dische test.

It thus appears that the nucleic acid of T2r+ is an example of an unusual desoxyribonucleic acid with glucose side chains.

It may be noted that the nucleotides H1 and H2 differ notably in both the sensitivity and nature of their response to irradiation with ultraviolet (2537 A) irradiation.

References and Notes

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1. R. L. Sinsheimer and J. F. Koerner, *J. Biol. Chem.* **198**, 293 (1952).
2. R. O. Hurst, A. M. Marko, and G. C. Butler, *ibid.* **204**, 847 (1953).
3. R. L. Sinsheimer and J. F. Koerner, *Science* **114**, 42 (1951).
4. G. R. Wyatt and S. S. Cohen, *Biochem. J.* (*London*) **55**, 774 (1953).
5. A. D. Hershey, J. Dixon, and M. Chase, *J. Gen. Physiol.* **36**, 777 (1953).
6. We are indebted to C. S. Miller of Sharp and Dohme Division, Merck and Co., for a gift of synthetic 5-hydroxymethylcytosine.
7. A. M. Michelson and A. R. Todd, *J. Chem. Soc.* (1954), p. 34.
8. P. K. Stumpf, *J. Biol. Chem.* **169**, 367 (1947).
9. Z. Dische, *ibid.* **181**, 379 (1949).
10. J. T. Park and M. J. Johnson, *ibid.* **181**, 149 (1949).
11. L. Hough, J. K. N. Jones, and W. H. Wadman, *J. Chem. Soc.* (1950), p. 1702.

Lewis J. Stadler, Geneticist

IN the death of Lewis J. Stadler on 12 May 1954 the science of genetics lost one of its most gifted and talented votaries. For more than 30 years Stadler conducted genetic experiments notable for their significance and clarity of conception, yet his influence on the course of genetical theory cannot be properly assayed from the published record alone. His penetrating and logical mind and his unexcelled ability to grasp the essential and discard the trivial made him a masterful critic and analyst. His friendly counsel and advice were constantly sought, for the extraordinary quality of his intellect was widely recognized. It is truly lamentable that death should come at the relatively early age of 57 when he was at the height of his intellectual powers and at a time when he was engrossed in fruitful studies.

Stadler attended the University of Missouri for 2 years before transferring to the University of Florida, where he obtained the degree of B.S. Agric. in 1917. Returning to Missouri for graduate work, he was awarded his A.M. in 1918 and then spent the following year at Cornell before coming back to Missouri to finish his graduate studies in 1922. Stadler was an assistant and instructor in the Department of Field Crops during the period of his graduate work, and after obtaining his Ph.D. he was made an assistant professor.

Except for a year spent at Harvard and Cornell (1925–26) as a National Research Council fellow and for sojourns as visiting professor at the California Institute of Technology in 1940 and at Yale in 1950, Stadler remained at Missouri until his death. From 1930 to 1954 he was jointly employed by the University of Missouri and the U.S. Department of Agriculture. Under Stadler's leadership the genetics laboratory at Missouri became a world-renowned center that attracted fellows from many lands.

Beginning with 1921 his maiden publications dealt with field plot technique and related agronomic problems, and it was not until 1925 that his first genetical paper, a study of variation in linkage values in maize, appeared. A more extensive analysis of the same phenomenon was published in 1926. Even today these papers constitute the most critical and intensive study of variation in recombination values in maize and are noteworthy contributions to genetic knowledge. However, it was at the 1927 meeting of the AAAS at Nashville that Stadler attracted wide attention by his confirmation of Muller's (1927) prior announcement of the mutagenic effect of x-rays. As is so often true, the same type of experimentation is conducted coincidently and wholly independently in more than one laboratory; this was true for the genetic effects induced by x-rays. It in no way detracts from the significance of Muller's work to say that Stadler was also a pioneer investigator of the genetic effects of short-wave irradiation.

From 1926 on Stadler was primarily concerned with studies of gene mutation, and it is with this area of genetic research that his name is commonly associated. The critical nature of Stadler's mind is best revealed by his approach to the nature of induced mutation. Immediately following the disclosure that x-rays produced gene mutations it was widely held that induced mutations were similar to those occurring spontaneously and that at last the geneticist had a powerful technique that would eventually lead to a solution of the nature of the gene. Stadler was one of the few, and the foremost among these, who felt that a comparative study of spontaneous and induced mutations was essential in order to evaluate the usefulness of this new tool in the determination of gene structure. He therefore began a long and extensive series of experiments in which he compared spontaneous and induced mutation at selected loci in maize. These brilliantly planned and executed investigations led him to conclude that in maize, at least, all x-ray induced mutations were extragenic in origin and that x-rays did not produce the kind of germinal changes arising spontaneously. Some geneticists are loath to believe that Stadler's conclusions apply to all forms of organic life, but no one can question the ineluctable force of his closely reasoned arguments. In Stadler's studies on the mutational process, we have an example of scientific experimentation at its best. They are superb in their clarity of conception and design, in