

theoretical studies by Powell and Eyring¹ and Gee,² that liquid sulfur contains chain-type polymers. The point of view adopted by Krebs³ that large rings are the primary type of polymeric material is probably incorrect.

The sulfur was purified by the method of Bacon and Fanelli,⁴ but after being subjected to repeated cycles of heating and cooling until the elapsed time of heating approached about 30 hours, a few black particles were observed in the sample, and the width of the resonance line had increased by about a factor of two. In addition, the sample showed a very weak paramagnetic absorption at room temperature whereas no absorption had been detected at room temperature before the extended period of heating. It is evident that greater care must be taken to insure a high degree of purity, and therefore our present results cannot be relied on for quantitative validity.

Our measurements⁵ were made on sulfur which had been degassed by 15 cycles of melting and freezing under vacuum (about 10^{-3} mm.). The concentration of radicals was found to increase by a factor of from 100 to 200 in the range from 190 to 375°; the absolute value of the intensity corresponds to a radical concentration of the order of 10^{-5} mole/l. at 200°. The value of ΔH for breaking a S-S bond in a long chain that is derived from these data is consistent with the value obtained by Gee² from the viscosity data of Bacon and Fanelli,⁵ and the observed radical concentration at 200° corresponds in order of magnitude to that estimated by Gee. The width of the resonance line is about 15 gauss at 190° and appears to increase to about 35 gauss at 375°. The spectroscopic splitting factor (g -value) is 2.02.

Bacon and Fanelli⁶ reported that several varieties of C.P. sulfur were blackened after they were subjected to boiling for 2-3 minutes over a free-flame. We have found that N. F. sublimed sulfur flowers supplied by the Amend Drug Co. yields black particles after even less severe heat treatment. These black particles are paramagnetic at room temperature; the line width is about 20 gauss and the g -value is 2.01. It should be emphasized that in the absence of black particles the observed intensity of paramagnetic absorption was reversible with temperature and paramagnetism was not detected below about 190°.

Precise measurements will require not only more highly purified sulfur than has been used, but also a reliable standard of paramagnetic intensity usable over the entire temperature range. In addition to the value of ΔH for a S-S bond, such measurements can be used with data like that of Hammick, *et al.*,⁷ for the weight fraction of polymer to obtain

- (1) R. E. Powell and H. Eyring, *THIS JOURNAL*, **65**, 648 (1943).
- (2) G. Gee, *Trans. Faraday Soc.*, **48**, 515 (1952).
- (3) H. Krebs, *Angew. Chem.*, **66**, 293 (1953).
- (4) R. F. Bacon and R. Fanelli, *THIS JOURNAL*, **65**, 639 (1943).
- (5) A preliminary account of our instrument, which employs a type 2K25 Klystron at a wave length of 3.2 cm., has been published: J. M. Hirshon, R. L. White and G. K. Fraenkel, *Rev. Sci. Instr.*, **23**, 772 (1952). A detailed account has been submitted for publication by J. M. Hirshon and G. K. Fraenkel to *Rev. Sci. Instr.*
- (6) R. F. Bacon and R. Fanelli, *Ind. Eng. Chem.*, **34**, 1043 (1942).
- (7) D. L. Hammick, W. Cousins and E. Langford, *J. Chem. Soc.*, 797 (1928).

the degree of polymerization as a function of temperature. Present limitations on sensitivity preclude measurements at temperatures much lower than 190°.

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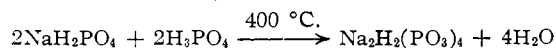
RECEIVED OCTOBER 20, 1954

NEW SODIUM PHOSPHATES

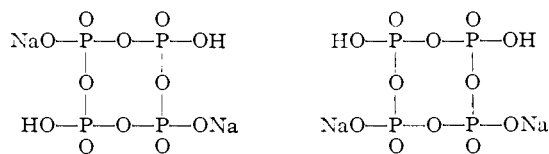
Sir:

A new series of sodium hydrogen phosphates has been discovered and two new compounds have been isolated. At 300° and above, monosodium orthophosphate reacts with orthophosphoric acid to yield sodium acid metaphosphate melts and when the melts are slowly cooled crystalline acid metaphosphates are formed. The crystallization is hastened if the melt is stirred for two or three minutes after the sample reaches the required composition.

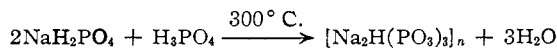
A tetrametaphosphate results from the reaction:



The crystalline acid metaphosphate has a unique X-ray pattern, it melts near 400° and has one of two alternate structures



A second reaction



yields a compound, the crystals of which are fibrous. The disodium monohydrogen acid metaphosphate also has a unique X-ray pattern and melts near 420°. The structure of this compound is not yet known but indications are that it is a long chain compound (polyphosphate). The reactions involving other ratios of monosodium orthophosphate to orthophosphoric acid also yield acid metaphosphates but are more difficult to isolate in a pure condition than those mentioned above.

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THE LINKAGE OF GLUCOSE IN COLIPHAGE NUCLEIC ACIDS¹

Sir:

Independent reports^{2,3} of two unusual properties of the deoxyribonucleic acids (DNA) of the related T6 and T4 coliphages led to the present investigation. The inability of pancreatic deoxyribonuclease (DNase) and intestinal phosphatase to hydrolyze T6 DNA to a reasonable quantity of hydroxy-

- (1) Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.
- (2) S. S. Cohen, *Symp. Quant. Biol.*, **18**, 221 (1953).
- (3) M. Jesaitis and W. F. Goebel, *ibid.*, **18**, 205 (1953).

methyl cytosine (HMC) nucleoside, in the face of good yields of the deoxyribonucleosides of the other three bases, was reported by Cohen,² while the seemingly unrelated work of Jesaitis and Goebel³ indicated the presence of a significant quantity of glucose in T4 DNA. With the notion that the presence of glucose in the DNA molecule somehow inhibited the action of the combined enzymes, we have attempted to separate the products of such enzymatic hydrolysates by ion-exchange and to identify them. Such experiments have led us to the conclusion that glucose is associated mole for mole with HMC in T4 DNA and, further, that the glucose is probably linked as an O-glycoside to the 5-hydroxymethyl group of HMC, rather than substituted for deoxyribose in the DNA sugar-phosphate chain. We have extended the observation of Jesaitis and Goebel of the presence of glucose in T4 to T2 and T6 DNA's (but not T1 DNA) and have noted that the ratio of glucose to DNA phosphorus is about 0.17 in these phages.

The course of hydrolysis of 40 mg. of highly purified T4⁺ DNA by pancreatic DNase was followed by electrometric titration. A maximum of 11% of the phosphoryl linkages was hydrolyzed by the enzyme, this value being about half of that obtained by a similar digestion of DNA from other sources.⁵ Half of the digest was hydrolyzed with whole *Crotalus adamanteus* venom (containing both diesterase and 5'-monoesterase) and the other half was hydrolyzed with a diesterase preparation essentially free of monoesterase activity.⁶ Both hydrolyses were again followed by titration and reached an end-point when an additional 70% of the phosphoryl linkages of the DNase-produced polynucleotides was broken.

With calf thymus DNA such procedures yield nucleosides and 5'-nucleotides, respectively, in quantitative yield.⁶ With T4 DNA, however, many polynucleotides remain.

The ion-exchange analyses⁷ of the two digests are summarized in Table I. It is clear that the diesterase does not cleave most of the HMC nucleotide linkages. However, a variety of nucleo-

sides, nucleotides and polynucleotides containing HMC was obtained. Within each such compound or mixture of compounds, HMC was determined by spectrophotometric observations and glucose by the anthrone test.⁸ Wherever HMC was present, glucose was also present in equimolar quantity. In addition, there was at least one HMC residue per polynucleotide.

Glucose was identified as such after liberation from T4 DNA by boiling for 7 hours with a sulfonic acid cation exchanger (Dowex-50-H). The identification was made by anion-exchange chromatography in a borate system,⁹ and by the hexokinase-firefly luminescence system.¹⁰ These analyses agree with each other and with the colorimetric anthrone method⁸ in indicating about 90% release of the glucose as such.

Indirect evidence on the mode of linkage of glucose to HMC in T4 was given by conventional acid hydrolysis (1 *N* HCl, 1 hour, 100°), which yielded 88% of the glucose as free sugar whereas 87% of the HMC remained in mononucleotide, nucleoside diphosphate and polynucleotide form. From the latter observation, glucose cannot be the sugar involved in internucleotidic linkage of HMC. Furthermore, its appearance without simultaneous HMC destruction is more consistent with the properties of O-glycosides than with those of pyrimidine-N-glycosides, which are markedly acid-stable. The sugar is therefore tentatively allocated to the 5-hydroxymethyl group.

NOTE ADDED IN PROOF.—Recently a report of similar findings with T2 bacteriophage DNA has appeared by Sinsheimer, *Science*, **120**, 551 (1954), dealing with the identification of glucose associated with BMC mononucleotide. Our results confirm this and extend the observation to the polynucleotide-bound BMC.

(8) D. L. Norris, *Science*, **107**, 254 (1948).

(9) J. X. Khyam and L. P. Zill, *THIS JOURNAL*, **74**, 2090 (1952).

(10) B. L. Strehler and J. R. Totter, *Arch. Biochem. and Biophys.*, **40**, 28 (1952).

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¹⁴C AND ¹³C INTRAMOLECULAR ISOTOPE EFFECTS IN THE DECARBOXYLATION OF LIQUID MALONIC ACID AT 140.5°

Sir:

The theoretical value for the ratio of C¹⁴ to C¹³ isotope effects in reactions involving rupture of C-C, C-N or C-O bonds is near 2.¹ Reported experimental values are: decarboxylation of mesitoic acid² -2.8 ± 0.3; dehydration of oxalic acid³ -2.0 ± 0.2; enzymatic hydrolysis of urea⁴ -3.2 ± 0.4; non-enzymatic hydrolysis of urea⁵ -1.8 ± 0.2; decarboxylation of malonic acid⁶ -3.5 ± 0.4. Only for malonic acid are other results available for the C¹⁴ isotope effect; Roe and Hellman⁷ gave a figure of 6 ± 2% for the intramolecular

(1) J. Bigeleisen, *J. Phys. Chem.*, **56**, 823 (1952).

(2) W. H. Stevens, J. M. Pepper and M. Lounsbury, *J. Chem. Phys.*, **20**, 192 (1952).

(3) A. Fry and M. Calvin, *ibid.*, **56**, 901 (1952).

(4) J. A. Schmitt, A. L. Myerson and F. Daniels, *ibid.*, **56**, 917 (1952).

(5) J. A. Schmitt and F. Daniels, *THIS JOURNAL*, **75**, 3564 (1953).

(6) P. E. Yankwich and E. C. Stivers, *J. Chem. Phys.*, **21**, 61 (1953).

(7) A. Roe and M. Hellman, *ibid.*, **19**, 660 (1951).

TABLE I

PER CENT RECOVERY OF THE BASES OF T4 DNA BY ION-EXCHANGE ANALYSIS FOLLOWING THE COMBINED ACTION OF PANCREATIC DNASE AND (a) WHOLE SNAKE VENOM AND

(b) SNAKE VENOM DIESTERASE

Base	(a) Whole venom		
	Per cent recovered as		
	Nucleoside	Mononucleotide	Polynucleotide
(HMC) ^b	4	12	84
Guanine	79	0	21
Thymine	72	0	27
Adenine	78	0	21
(b) Venom diesterase			
(HMC) ^b	..	17	82
Guanine	8	74	18
Thymine	9	64	26
Adenine	8	72	19

^a Nucleoside formation with diesterase is ascribed to residual 5'-monoesterase action. ^b 5-hydroxymethylcytosine.

(4) R. M. Herriott and J. L. Barlow, *J. Gen. Physiol.*, **36**, 17 (1952).

(5) M. Kunitz, *ibid.*, **33**, 363 (1949-1950).

(6) R. O. Hurst and G. C. Butler, *ibid.*, **193**, 91 (1951).

(7) E. Volkin and W. E. Cohn, *ibid.*, **205**, 787 (1953).