Milder conditions of hydrolysis have been to no avail in producing a peptide longer than the tetrapeptide. The peptide bond between the fourth and fifth amino acids clearly is a labile one. The work of Synge[3] and of Desnuelle and Casal[4] indicates that if the fifth amino acid were serine or threonine the bond would indeed be labile and it may also be that tryptophan which is sensitive to acid would behave similarly.

Several authors[5,6] have concluded that lysozyme contains one or at most two peptide chains. Thompson[7] after applying corrections could account for little more than half an end-group per molecule. In previous work[1] and in the present study, the actual uncorrected amount of α,ϵ-di-DNP-lysine which was isolated accounted for more than 0.6 end-group per molecule. Our analyses of the peptides which were isolated from DNP-lysozyme and also of model synthetic peptides demonstrate that 25 to 40% of α,ϵ-di-DNP-lysine may be destroyed during hydrolysis. Our results agree with those of Thompson[7] that a complete hydrolysate of DNP-lysozyme contains no DNP-amino acids other than α,ϵ-di-DNP-lysine and ϵ-DNP-lysine. We may, therefore, conclude that lysozyme has a single polypeptide chain and, from the present work, that the sequence on the amino end of this chain is lysyl-valyl-phenylalanyl-glycyl—.

(3) R. L. M. Synge, *Biochem. J.*, **39**, 351 (1945).
(4) P. Desnuelle and A. Casal, *Biochim. Biophys. Acta*, **2**, 64 (1948).
(5) J. C. Lewis, N. S. Snell, D. J. Hirschmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **186**, 23 (1950).
(6) H. Fraenkel-Conrat, A. Mohammed, E. D. Ducay and D. K. Mecham, THIS JOURNAL, **73**, 625 (1951).
(7) A. R. Thompson, *Nature*, **168**, 390 (1951).

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A SECOND CRYSTALLINE MODIFICATION OF POLYTHENE

Sir:

We have obtained conclusive evidence of the occurrence of a second crystalline modification of polythene in films of this polymer subjected to the mechanical process of redrawing.[1] The new modification is characterized by the appearance of two strong crystalline interferences in the X-ray pattern, corresponding to spacings of 4.23 and 4.55 Å. Four to seven successive redrawings were necessary to obtain a high degree of sharpness and intensity in the new diffraction peaks. The two strong peaks, 110 and 200, of the usual orthorhombic modification of polythene,[2] with spacings to 3.78 and 4.17 Å., remained strong, indicating only partial conversion to the new modification.

A polythene film[3] subjected to seven redrawings was mounted successively in each of three orientations with respect to the X-ray beam: (A) beam normal to the film plane; (B) beam parallel to the film plane but normal to the last direction of draw; (C) beam parallel to the film plane and to the last direction of draw. Flat camera photographs of moderate exposure were taken for each orientation. Orientation A showed only the two equatorial spacings 3.78 and 4.17 Å. characteristic of the well-known modification. Orientation B showed only the two equatorial spacings 4.23 and 4.55 Å., characteristic of the new modification, while orientation C showed all four spacings, with 3.78 and 4.17 Å. doubled on equatorial lines about 65° each side of the single equator of the 4.23 and 4.55 Å. spacings. This suggests that the familiar modification had undergone twinning by a glide process similar to that observed by other investigators[4,5]; in this case the glide was probably parallel to the two sets of 110 planes.

Additional spacings corresponding to the new modification have not yet been obtained; hence, it is not possible to determine either the shape or the dimensions of the unit cell. A more detailed report of our work will be submitted to THIS JOURNAL at the conclusion of the research.

(1) Cold drawing of the specimen along a direction 90° with respect to that of previous cold drawing has been termed redrawing: W. M. D. Bryant, *J. Polymer Sci.*, **2**, 558 (1947).
(2) C. W. Bunn, *Trans. Faraday Soc.*, **35**, 482 (1939)
(3) The specimen was coated on both sides with powdered sodium chloride to provide a simultaneous calibration.
(4) A. Brown, *J. Applied Phys.*, **20**, 552 (1949).
(5) I. L. Hopkins, W. O. Baker, J. B. Howard, *ibid.*, 206 (1950).

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ABSENCE OF DETECTABLE POLY-*CIS* FORMS FROM HEAT-ISOMERIZED LYCOPENE SOLUTIONS

Sir:

While poly-*cis* lycopenes, $C_{40}H_{56}$, are occasionally found in nature,[1] it has not been possible so far to obtain such forms *in vitro*, by submitting all-*trans* lycopene to any of the well known stereoisomerization methods.[2] Since, however, all pertinent experiments had been carried out with only small amounts of starting material so far, we endeavored to investigate the thermic *trans* → *cis* isomerization of lycopene on an unusually large scale, under conditions which would allow the recovery of even a trace of poly-*cis*-lycopene formed.

Thirty grams of analytically pure and chromatographically homogeneous lycopene was prepared from 180 kg. of commercial tomato paste.[3] Two-gram portions of this pigment were refluxed in 2 l. of benzene (per portion) in diffuse daylight for one half hour. A subsequent resolution on fifteen, slightly conical percolators (50 × 24 cm.) filled with lime–celite, yielded 30 l. of a weakly colored chromatographic filtrate that was free of all-*trans* or *neo* forms which were held strongly by the adsorbent. This filtrate underwent further resolutions on alu-

(1) L. Zechmeister, A. L. LeRosen, F. W. Went and L. Pauling, *Proc. Nat. Acad. Sci.*, **27**, 468 (1941); A. L. LeRosen and L. Zechmeister, THIS JOURNAL, **64**, 1075 (1942); L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, **144**, 315 (1942); L. Zechmeister and J. H. Pinckard, THIS JOURNAL, **69**, 1930 (1947).
(2) L. Zechmeister, *Chem. Rev.*, **34**, (1944); *cf.* also L. Pauling, *Helv. Chim. Acta*, **32**, 2241 (1949).
(3) The following method was adapted for large-scale isolation work: A. Sandoval and L. Zechmeister, "Biochemical Preparations," J. Wiley and Sons, Inc., New York, N. Y., Vol. I, p. 57, and London: Chapman and Hall, 1949.

mina–lime–celite and yielded, finally, four pigments which, on the basis of their adsorption affinities, could have belonged to the poly-*cis* lycopene series. However, none of them gave the very sensitive optical test described earlier, *viz.*, the conspicuous migration of the spectral bands, upon iodine catalysis, from 470 and 443.5 mμ to 502, 471.5 and 443 mμ, for example, as observed in hexane solutions of prolycopene in the Loewe–Schumm Grating Spectroscope.

Since this test became strongly positive upon the addition of 10 μg of prolycopene to any of the four pigment solutions, no inhibitor of the steric rearrangement was present. Further controls showed that quantities of prolycopene as small as 10 μg, when washed through a 50 × 24 cm. column with a few liters of benzene, could be easily recovered to the extent of 60% from the first liter of the chromatographic filtrate.

We conclude that, although about 40% of a lycopene sample is converted into a mixture of *cis* isomers by refluxing, the order of magnitude of poly-*cis* forms in such equilibria must be 1 part in 3 million (or more) parts of this total pigment.

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DI-DESOXYRIBONUCLEOTIDES[1]

Sir:

Among the products of the action of pancreatic desoxyribonuclease upon highly polymerized calf thymus desoxyribonucleic acid (prepared according to the method of Mirsky and Pollister[2]) are a set of dinucleotides.[3] It has been found possible to separate several of these into apparently homogenous fractions by ion exchange chromotography.[4] Figure 1 is a portion of an ion exchange elution diagram, indicating the resolution of the first three fractions, which came off the column after the mononucleotides.[5]

The ultraviolet absorption spectra of these fractions indicate that P1 contains only desoxycytidylic acid, and P3 contains desoxycytidylic and desoxyadenylic acids, in equimolar proportion. (P2 appears to be a mixture of nucleotides and is under further investigation.) These spectroscopic analyses of P1 and P3 have been completely confirmed by quantitative degradation to mononucleotides by means of a purified phosphodiesterase preparation from rattlesnake venom,[7] followed by quantitative determination of the mononucleotides by ion exchange separation and ultraviolet absorption measurement.

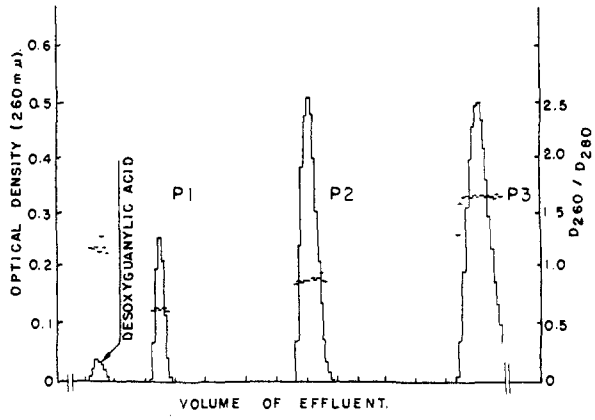

Fig. 1.—Elution diagram of first three dinucleotides.[6]

That these two fractions are dinucleotides is adduced from the fact that, in each case, exactly half of the total phosphorus of the fraction is monoesterified, being quantitatively removable by an alkaline phosphatase of bone, which previously had been freed of phosphodiesterase activity.[8] Following the action of this enzyme, a product can be recovered by ion exchange fractionation (P3′ of Fig. 2), which has an ultraviolet absorption similar to that of the dinucleotide, one-half the original phosphorus content, and which migrates considerably more rapidly in the ion exchange chromatogram, as illustrated in Fig. 2.

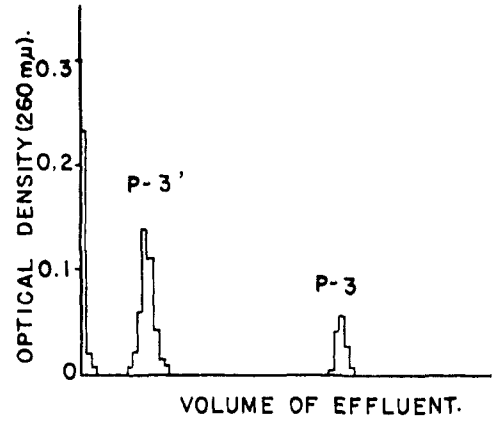

Fig. 2.—Elution diagram after incomplete degradation of P-3 by alkaline phosphatase.

In the case of P_3, the possibility of isomerism exists, and it has not been determined whether the fraction is truly homogeneous or is a mixture of the two isomers. For P1 (di-desoxycytidylic acid), this question does not arise.

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RECEIVED OCTOBER 22, 1951

(1) Aided by a grant from the Rockefeller Foundation.

(2) A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.*, **30**, 117 (1946).

(3) Previous titrimetric work (J. A. Little and G. C. Butler, *J. Biol. Chem.*, **188**, 695 (1951) and reference therein) had demonstrated that the products of this degradation, are, on the average, tetranucleotides. Since the products described here amount to only some 7% of the total digest, our results are not considered to be in conflict with the earlier observations, but emphasize their average character.

(4) W. E. Cohn, THIS JOURNAL, **72**, 2811 (1950).

(5) R. L. Sinsheimer and J. F. Koerner, *Science*, **114**, 42 (1951).

(6) A column of Dowex-1 resin, 250–500 mesh, 18 cm. × π sq. cm., initially in the acetate form, was used. The eluting medium was 0.05 *M* acetate buffer *p*H 4.3 plus an increasing concentration of chloride anion. Over the portion of the elution diagram shown, the Cl^- concentration varied from 0.02 to 0.05 *M*.

(7) R. L. Sinsheimer and J. F. Koerner, to be published.

(8) J. M. Gulland and E. M. Jackson, *Biochem. J.*, **32**, 590 (1938).