A striking feature of the oviduct chromatogram is the predominance of uridine nucleotides. For example, adenosine triphosphate has not been found, and the amount present must be extremely small compared to any one of the uridine nucleotides. A major function of oviduct is the synthesis of glycoproteins containing N-acetylglucosamine and mannose[9]**. It also contains mucopolysaccharides (including both hyaluronic acid and chondroitin sulfate[10]). These facts suggest that these nucleotides may be the means of activation of glycosyl residues for incorporation into protein, and for synthesis of some mucopolysaccharides.

JACK L. STROMINGER

Laboratory of Pharmacology and Toxicology,
National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health, Bethesda, Md. (U.S.A.)

1 J. T. PARK, *J. Biol. Chem.*, 194 (1952) 877, 885, 897.
2 J. L. STROMINGER, *Federation Proc.*, 12 (1953) 277.
3 E. CABIB, L. F. LELOIR AND C. E. CARDINI, *J. Biol. Chem.*, 203 (1953) 1055.
4 R. B. HURLBERT AND V. POTTER, *J. Biol. Chem.*, 209 (1954) 1.
5 E. E. B. SMITH AND G. MILLS, *Biochim. Biophys. Acta*, 13 (1954) 386.
6 W. T. J. MORGAN AND L. A. ELSON, *Biochem. J.*, 28 (1934) 988.
7 M. A. JERMYN AND F. A. ISHERWOOD, *Biochem. J.*, 44 (1949) 402.
8 H. G. PONTIS VIDELA, *Ciencia e Investigacion*, 10 (1954) 236.
9 cf. A. L. ROMANOFF AND A. J. ROMANOFF, *The Avian Egg*, John Wiley and Sons, New York, 1949, p. 918.
10 S. SCHILLER AND A. DORFMAN, Personal communication.

Received March 26th, 1955

* A mannose containing nucleotide, guanosine-5′ pyrophosphate mannose, has also been identified in oviduct (J. L. STROMINGER, *Federation Proc.*, 13 (1954) 307). This compound is identical to the compound isolated from yeast by CABIB AND LELOIR (*J. Biol. Chem.*, 206 (1954) 779).

Effect of substrate structure on the aldolase equilibrium*

The purpose of this communication is to call attention to a relationship between the structure of the aldehyde component and the equilibrium constant of aldolase reaction systems.

In the aldolase reaction

(1) D-3-phosphoglyceraldehyde + dihydroxyacetone phosphate $\rightleftharpoons$ D-fructose-1,6-diphosphate

various aldehydes may replace 3-phosphoglyceraldehyde (GAP) but dihydroxyacetonephosphate (DAP) appears to be specific[1,2,3,4,5]. When "foreign" aldehydes react with DAP, corresponding ketose-1-phosphates are formed (*i.e.* D-fructose-1-phosphate from D-glyceraldehyde[1,3] and D-xylulose-1-phosphate from glycolaldehyde[3]). MEYERHOF *et al.*[1] observed that the aldolase reaction was fully reversible with GAP or acetaldehyde. On the other hand, fructose-1-phosphate, readily formed from D-glyceraldehyde and DAP in muscle extracts, was not detectably split when added to such extracts, suggesting "irreversible" formation. More recent work[2,6] has shown that crystalline muscle aldolase does effect some cleavage of fructose-1-phosphate which is greatly enhanced by trapping or enzymic removal of reaction products.

We have examined the effect of "foreign" aldehydes on the equilibrium system resulting from the action of aldolase + isomerase on fructose-1,6-diphosphate. The course of the reactions toward new equilibria was followed by estimating DAP, other ketose-1-phosphates, and free aldehydes[1,7,8]. From the new equilibria reached the aldehydes tested fell into two categories: (A) aldehydes causing complete disappearance of DAP from medium (DL-glyceraldehyde, β-hydroxypropionaldehyde, and β-hydroxybutyraldehyde); and (B) aldehydes which caused establishment of a new equilibrium in which significantly large amounts of DAP remained (formaldehyde, acetaldehyde, propionaldehyde, β-methoxypropionaldehyde, DL-lactic aldehyde, and D-glyceraldehyde-3-phosphate).

If the "foreign" aldehydes condense with DAP in strict analogy with the "natural" aldolase reaction (no exceptions are known to date) then it is seen that the new ketose-1-phosphates formed from aldehydes of group (A) may exist in the stable pyranose configuration since a free hydroxyl group is present at carbon 6 (*i.e.*, D-fructose (L-sorbose) 1-phosphate from DL-glyceraldehyde; 5-desoxyfructose-1-phosphate from β-hydroxypropionaldehyde, *etc.*). However, the ketose-1-phosphates produced from aldehydes of group (B) have no hydroxyl at carbon 6 and must exist as the less stable furanoses, or as acyclic structures (*i.e.* fructose-1,6-diphosphate from D-glyceraldehyde-

* Supported by grants from the National Institutes of Health, Nutrition Foundation, Inc., and the American Cancer Society, recommended by the Committee on Growth, National Research Council.

3-phosphate; 5,6-didesoxyfructose-1-phosphate from propionaldehyde, *etc.*). These findings thus suggested that thermodynamic stabilization afforded by formation of cyclic hemiacetal forms of the aldol product is a major factor affecting the equilibrium constant of aldolase reaction systems.

To test this idea crystalline muscle aldolase, free of triose phosphate isomerase, was allowed to act on three representative 6-carbon ketose-1-phosphates of known high purity: the *pyranose* fructose-1-phosphate (synthetic[2,9]), the *furanose* fructose-1,6-diphosphate (chromatographically purified[10]), and the presumably *acyclic* 5,6-didesoxyfructose-1-phosphate (prepared enzymically and isolated as crystalline Ag salt[10]). The equilibrium constants (K_{eq}) were determined by following the reactions in the direction of cleavage by measuring formation of alkali-labile P or free aldehyde[7] (Table I).

TABLE I

ALDOLASE EQUILIBRIUM CONSTANTS

Substrate	*Probable configuration*	K
D-Fructose-1-phosphate	Pyranose	$2.8 \cdot 10^{-6}\ M$
D-fructose-1,6-diphosphate	Furanose	$1.18 \cdot 10^{-4}\ M$
5,6-didesoxyfructose-1-phosphate	Open chain	$6.9 \cdot 10^{-3}\ M$

Reaction systems contained substrates in range from 0.006 to 0.030 M and from 0.13 to 3.3 mg crystalline isomerase-free aldolase per ml at pH 7.0. Temperature, 37°. Figures are averages from at least 3 experiments run to apparent equilibrium. The considerably greater extent of cleavage of fructose-1-phosphate seen by HERS AND KUSAKA[6] could not be confirmed with our synthetic sample.

It is clear that K_{eq} for the three substrates varies in accordance with the generalization made above. The sugar having the most stable configuration (pyranose), fructose-1-phosphate, showed an extent of cleavage at apparent equilibrium which was very slight but easily measurable. K_{eq} for fructose-1,6-diphosphate, the most stable possible form of which is *furanoid*, agreed well with values already reported[8,11]; it is nearly two orders of magnitude higher than that of fructose-1-phosphate. K_{eq} for 5,6-didesoxyfructose-1-phosphate, which can exist only in the open-chain form (or as a very unstable four- or three-membered cyclic hemiacetal) is substantially higher than that of fructose-1,6-diphosphate, in good agreement with K_{eq} for cleavage of 5-desoxyxylulose-1-phosphate (also acyclic) calculated from data given by MEYERHOF[1,8].

The aldolase equilibrium may thus consist of two separate reversible reactions: (a) enzymic aldolization of dihydroxyacetone phosphate and an aldehyde to yield an open-chain ketose-1-phosphate, and (b) formation of pyranose, furanose, or other, less stable, ring forms from the open-chain structure. The latter reaction probably occurs non-enzymically, although enzymic catalysis is not excluded. The formation of the stable pyranose ring is thus an effective "trap", accounting for the relative "irreversibility" of formation of fructose-1-phosphate in the aldolase system. Only when the cleavage products are removed, either enzymically or by carbonyl trapping agents, is fructose-1-phosphate readily and extensively dealdolized.

K_{eq} for the three substrates tested should reflect the relative thermodynamic stability of pyranose, furanose, and open-chain forms of fructose-1-phosphate, for which no direct measurements have been reported. Aqueous fructose solutions at equilibrium (20°) contain about 80% pyranose, and 20% furanose[12,13,14]. The equilibrium concentration of open-chain form is less certain, but calculations based on the few data available[15,16,17] suggest an amount of the order of 0.1%. If the equilibrium concentrations for pyranose, furanose, and acyclic forms of fructose-1-phosphate are assumed to be approximately similar to those of fructose, then the equilibrium constants listed in Table I differ from each other in the expected directions and by magnitudes consistent with the explanation offered.

The foregoing thermodynamic interpretation of the aldolase equilibrium is based on the energy content of the initial and final states. It is therefore independent of the source or substrate specificity[18] of the enzyme or catalyst, the rates of the reactions involved, and the reaction mechanism.

ALBERT L. LEHNINGER*
JEAN SICÉ
ELWOOD V. JENSEN

The Ben May Laboratory for Cancer Research, University of Chicago, Chicago 37, Illinois (U.S.A.)

* Present address: Department of Physiological Chemistry, The Johns Hopkins School of Medicine, Baltimore 5, Maryland.

[1] O. MEYERHOF, K. LOHMANN AND P. SCHUSTER, *Biochem. Z.*, 286 (1936) 301, 319.
[2] T.-C. TUNG, K. H. LING, W. L. BYRNE AND H. A. LARDY, *Biochim. Biophys. Acta*, 14 (1954) 488.
[3] W. L. BYRNE AND E. A. LARDY, *Biochim. Biophys. Acta*, 14 (1954) 495.
[4] L. HOUGH AND J. K. N. JONES, *J. Chem. Soc.*, (1951) 1122, 3191; (1952) 4047, 4052; (1953) 342. P. A. J. GORIN AND J. K. N. JONES, *ibid.*, (1953) 1537, 2140.
[5] F. C. CHARALAMPOUS AND G. C. MUELLER, *J. Biol. Chem.*, 201 (1953) 161.
[6] H. G. HERS AND T. KUSAKA, *Biochim. Biophys. Acta*, 11 (1953) 427.
[7] L. A. GREENBERG AND D. LESTER, *J. Biol. Chem.*, 154 (1944) 177.
[8] O. MEYERHOF, in *The Enzymes*, ed. J. B. SUMNER AND K. MYRBACK, Academic Press, New York, (1951), Vol. II, part I, p. 162.
[9] F. LEUTHARDT AND E. TESTA, *Helv. Chim. Acta*, 34 (1951) 931.
[10] A. L. LEHNINGER AND J. SICÉ, *J. Am. Chem. Soc.*, Submitted for publication.
[11] D. HERBERT, H. GORDON, V. SUBRAHMANYAN AND D. E. GREEN, *Biochem. J.*, 34 (1940) 1108.
[12] H. S. ISBELL AND W. W. PIGMAN, *J. Research Natl. Bur. Standards*, 20 (1938) 773.
[13] A. GOTTSCHALK, *Austral. J. Exptl. Biol. Med. Sci.*, 21 (1943) 133, 139.
[14] R. H. HOPKINS AND M. HORWOOD, *Biochem. J.*, 47 (1950) 95.
[15] W. BEDNARCZYK AND L. MARCHLEWSKI, *Biochem. Z.*, 300 (1938) 42.
[16] F. LIPPICH, *Biochem. Z.*, 248 (1932) 280.
[17] S. M. CANTOR AND Q. P. PENISTON, *J. Am. Chem. Soc.*, 62 (1940) 2113.
[18] F. LEUTHARDT AND H. P. WOLF, *Helv. Chim. Acta*, 37 (1954) 1734.

Received April 13th, 1955

Calcium citrate in an insect

The oothecae of all Praying Mantids so far examined contain small rectangular crystals adhering to or embedded in the protein membranes of which the ootheca is formed. The crystals originate in a large gland located in the body wall of the seventh ventral sternite or sub-genital plate, and are added to the secretions from the colleterial glands at the time of laying. The purpose of this note is to describe the chemical nature of the crystals, each of which is contained in a protein envelope of complicated structure.

Under the microscope the crystals within the ventral body-wall gland appear identical with those found in the oothecae. They vary in size but average dimensions are $5 \times 3 \times 1\ \mu$, and when allowed to settle on a slide they appear rectangular and strongly birefringent with the optic axis at about 45° to the edge of the crystals. Sometimes the crystals have cleaved parallel to the long edge and these thin plates lie on their sides. In this view they are trapeziform and are birefringent with the optic axis in a plane parallel to the long edges of the crystal. Just before laying, the gland is packed full, *i.e.* there is a volume of several cubic mm of crystals. Within the gland a few crystals of another substance were observed, having no birefringence and a more elongated form.

By X-rays and infra-red the crystals have been identified as calcium citrate. By reaction of calcium chloride and sodium citrate insoluble citrates were obtained that had a variety of structures according to X-ray and infra-red tests. The calcium citrate identical with the crystals in the insect was formed when 150 ml 0.1 *M* calcium chloride was mixed with 100 ml 0.1 *M* sodium citrate and allowed to stand in a tall cylinder, whereupon the calcium citrate slowly precipitated. This precipitate was washed, dried in air, and the rate of dehydration followed over P_2O_5. Changes in the vapour pressure occurred at weights corresponding to the hexahydrate and the tetrahydrate, while heating at 100° C gave an approximately anhydrous product. Three types of X-ray powder diagram were obtained corresponding to the hexahydrate, tetrahydrate and the anhydrous form as just defined. On the basis of their X-ray diagram and infra-red absorption the crystals produced by the Mantis were identified as the hexahydrate.

Several things are noteworthy concerning these crystals. They are formed within protoplasmic membranes. They show that a biological mechanism exists for a very great concentration of citrate as calcium citrate. These special devices suggest that the citrate plays an important part in the transformation of the colleterial gland protein into the highly characteristic ribbon-like elements of the ootheca. In most respects the Mantis ootheca differs markedly from the better known cockroach ootheca; but we may recall that the left colleterial gland in the cockroach contains the structural protein of the ootheca and large quantities of calcium oxalate[1]. The function of this calcium oxalate is unknown—suppression of calcium ions is one possibility. In contrast, the Mantis shows no oxalate (or citrate) crystals within the colleterial glands, but calcium citrate is added from a separate gland during production of the ootheca.

K. D. PARKER
K. M. RUDALL
Dept. of Biomolecular Structure, University of Leeds (England)

[1] *e.g.*, P. BRUNET, *Quart. J. micr. Sci.*, 93 (1952) 47.

Received April 4th, 1955