In this experiment the formation of PGA from DPGA occurs only in the presence of PBA (Fig. 1, lines Ia, b) to an extent dependent on DPGA concentration if PBA is in excess (Fig. 1, lines IIa, b),

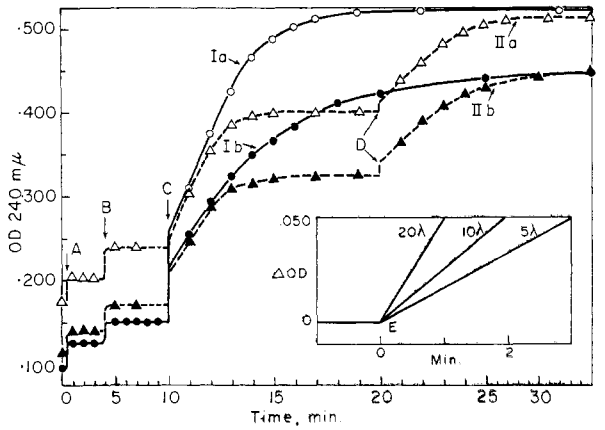

Fig. 1.—Spectrophotometric demonstration of the transfer of phosphate from DPGA to 2PBA and 3PBA: each 1 cm. cuvette contained 1 ml. of 0.1 *M* imidazole-HCl buffer, *p*H 7.0, 0.015 *M* in $MgCl_2$. To Ia and Ib was added 0.2 ml. of 0.015 *M* DPGA; to IIa was added 0.1 ml. of 0.15 *M* 2PBA; and to IIb, 0.1 ml. of 0.15 *M* 3PBA. At time A, enolase was added to each cuvette; then mutase was added at time B. At time C, 0.1 ml. of 0.15 *M* 2PBA was added to Ia, and 0.1 ml. of 0.15 *M* 3PBA was added to Ib. At the same time, 0.1 ml. of 0.015 *M* DPGA was added to both IIa and IIb. A further 0.1 ml. of DPGA was added to IIa and IIb at time D. Insert: Demonstration of the dependence of rate on mutase concentration. Cuvettes contain: 1 ml. of buffer, 0.1 ml. of 0.015 *M* DPGA, 0.1 ml. of 0.15 *M* 2PBA, 0.02 ml. of enolase and 1.76 ml. of H_2O. At E the quantities of mutase indicated on the curves were added and the 240 mμ adsorbance followed with the energy recording adaptor to the Beckman DU spectrophotometer.

and at a rate proportional to the mutase concentration, (Fig. 1, insert). The formation of PEP has been confirmed by paper chromatography of the reaction mixture in 2-propanol, ammonia, water (70:10:20). With the acid molybdate spray, spots corresponding to PEP, PGA-PBA (no separation) and a diphosphate corresponding to synthetic DPBA[10] are found. Synthetic DPBA can replace DPGA as a mutase activator, although ten times the concentration is required for comparable activity.

This series of reactions independently supports the conclusion[1] that DPGA donates one of its phosphate groups during its role in the enzymatic interconversion of the two monophosphoglyceric acid isomers. To show that DPGA donates directly to the enzyme would require the demonstration of phosphorus on the enzyme in equilibrium with the phosphorus on the substrate, but as the formation of a phosphate diester (between DPGA and PGA) as an intermediate in the phosphate transfer appears unlikely,[11] the series of reaction (a) and (b) satisfactorily explains the results.

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$$\text{(a)}\quad \text{DPGA} + \text{E} \rightleftharpoons \text{PE} + \text{PGA} \xrightleftharpoons{\text{enolase}} \text{PEP}$$

$$\text{(b)}\quad \text{PE} + \text{PBA} \rightleftharpoons \text{E} + \text{DPBA}$$

$$\text{(a)} + \text{(b)}\quad \text{DPGA} + \text{PBA} \rightleftharpoons \text{DPBA} + \text{PGA} + \text{PEP}$$

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THE MOLECULAR WEIGHT OF β-LACTOGLOBULIN

Sir:

The generally accepted molecular weight of β-lactoglobulin is in the vicinity of 35,000.[1,2,3,4] In the course of a detailed study of the association properties of this protein as a function of *p*H, it has been found that the weight of its disperse units is strongly dependent on *p*H.[5,6,7] In the *p*H regions below 3.5 and above 7.5, its sedimentation constant, measured at a protein concentration of 10 g./l., decreases from a value of $S_{20,w} = 2.85$ to $S_{20,w} = 2.25$, indicating that there occurs either a decrease in molecular weight or an expansion of the molecule, as in the case of serum albumin.[8,9,10,11,12]

In order to clarify this question, light scattering measurements were carried out on β-lactoglobulin as a function of *p*H. These show[7] that below *p*H 3.5 the molecule of β-lactoglobulin dissociates into units smaller than 35,000, probably into two portions of equal weight. These data are in qualitative agreement with the results of Rands and Tanford,[13] who also observed a similar decrease in the molecular weight of this protein at low *p*H. Because of the rapid re-equilibration of the dissociation with dilution, however, the light scattering data curve strongly upward in the low concentration range, and it is not possible to extrapolate them to a reliable value of the weight average molecular weight. As a result, ultracentrifugal determinations of the molecular weight were carried out by the Archibald technique,[14,15,16] under conditions of strong dissociation.

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TABLE I

pH	Temp., °C.	Speed	Prot. concn., g./l.	Concn. at menisicus, g./l.	$\frac{1}{cx}\frac{dc}{dx}$ extrapd.	Mol. wt.
1.64	24.8	42,040	10.7	0.25	3.30 ± 0.20	16,900 ± 1000
				0.1	3.49 ± .20	17,900 ± 1000
2.01	23.7	39,460	13.2	2.8	3.75 ± .30	20,300 ± 1600
				0.36	3.23 ± .20	17,500 ± 1100
				0.1	3.22 ± .10	17,400 ± 500
2.08	25.0	20,410	12.1	5.4	1.047 ± .02	22,700 ± 500
				4.7	1.054 ± .03	22,900 ± 700

Solutions of β-lactoglobulin were prepared under the desired conditions of *p*H and ionic strength, and centrifuged in a Spinco Model E analytical ultracentrifuge. In each case, the protein solution was centrifuged in one Kel-F cell, while pure solvent was centrifuged in an identical cell used in place of the counterbalance. The menisci of the two were made to coincide by weighing in the proper amount of liquid into each cell, as suggested by Singer.[17] In this manner, the correct base line was obtained directly on each ultracentrifugal pattern. The weight average molecular weight at the meniscus was calculated at various times late in the run, so that the equilibrium be close to total dissociation. Some of the results obtained in a 0.1 Γ/2 NaCl-HCl medium are presented in the table.

These data show that the molecular weight of the dissociated species of β-lactoglobulin is in the vicinity of 17,500, or half of the normally accepted value. It is interesting to note that Bull has obtained a molecular weight of 17,000 from surface pressure measurements under a different set of conditions.[18] These results, together with the reports that β-lactoglobulin contains two identical amino[19] and carboxyl[19,20] end groups, strongly suggest that the molecular unit observed at isoelectric conditions is an aggregate of two identical protein units. It would appear, therefore, that the molecule of β-lactoglobulin consists of a single polypeptide chain with a molecular weight of *ca.* 17,500.

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BOOK REVIEWS

Biochemical Problems of Lipids. Proceedings of the Second International Conference held at the University of Ghent 27–30 July, 1955, organized with the collaboration of the "Vlaamse Chemische Vereniging" of Belgium, under the presidency of Professor R. Ruyssen. Editors, G. POPJÁK and E. LE BRETON. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N. Y. 1956. xvi + 509 pp. 16 × 25.5 cm. Price, $10.75.

This volume contains about 80 papers including a discussion of the nomenclature of the enzymes concerned in fatty acid metabolism. The languages used were English, French and German. There were present about 250 scientists representing 20 nationalities.

The papers are arranged in four sections as they were presented at the meetings: (1) physical and chemical properties of the lipids—methods of separation, structure; (2) metabolism and biosynthesis, enzyme systems; (3) phospholipids and transport; (4) miscellaneous problems.

An excellent outline of the work covered is given in the introduction by President R. Ruyssen. Among his comments are remarks about the complex chemical constitution of the lipids and their behavior as amphophilic substances. The knowledge of structure and composition of the natural phospholipids has much increased due almost entirely to new methods of separation and identification, such as countercurrent distribution, chromatography and spectrophotometry. Knowledge of the enzyme systems and their isolation as discussed in the conference puts us now in position to describe precisely most of the reactions in the fatty acid cycle. The part taken by the phospholipids in the transport of the fatty acids is now due for a reconsideration. Also thereby, new light has been thrown on fat absorption.

It would be impossible in a brief outline to mention the topics presented and their discussion by the attending members. It is sufficient to say that practically every phase of lipid structure, behavior and metabolism has been brought out and examined so that a reader gets a comprehensive picture of the present state of the problems connected with the lipids. A fact which detracts a little from the book as a publication is the repetition of the last three papers of section (1) together with an occasional extra page. Of course, this does not affect the value of the material from a scientific point of view.

The book will be of interest, not only to the purely lipid chemists, but since the lipids in nature combine with most known biochemical substances, forming aggregates of great size and complexity, it should interest all biochemists as well as those whose main interest is in the huge molecules which constitute living matter.

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Microcalorimétrie. Applications Physico-Chimiques et Biologiques. By E. CALVET, Professeur à la Faculté des Sciences de Marseille, and H. PRAT, Professeur a l'Université de Montréal. Masson et Cie., Editeurs, 120, Boulevard Saint-Germain, Paris-6e, France. 1956. viii + 395 pp. 17 × 25 cm. Price, Broché, 4.500 fr. Cartonné toile, 5.200 fr.

Microcalorimetry comprises the measurement of small quantities of thermal power either exothermic or endother-