The experimental work was carried out in the Bio-organic Division of the University of California Radiation Laboratory during the tenure of a Fellowship from the National Foundation for Infantile Paralysis. The author acknowledges his gratitude to Dr. EDWARD L. BENNETT for his counsel during these studies.

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Zusammenfassung

Der Umbau von 4,6-C^{14}-Adenin in Ribo- und Desoxyribonukleinsäure wurde im Gewebe von jungen Mäusen vom Stamm Webster 17 h nach intraperitonealer Injektion von markiertem Purin analysiert. Das Verhältnis der spezifischen Aktivität von RNA zu DNA variiert von 1,1 bis 1,6 für sämtliche Gewebe, ausgenommen Leber, wo ein Verhältnis von 6,4 gefunden wurde.

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Do Any Morphological Pictures of Separated Excretion of Histamine and Heparine in Tissue Mast Cells Exist?

Recently a notice was published in this journal by HILL[1] on the morphological effects of histamine and mucopolysaccharide secretion in the tissue mast cells. The author assumed that the releasing of both substances proceeds in two stages: first, histamine is released and thereafter heparine. With the first stage, a change in the stainability of the granulations is involved. As this statement is of great importance in the histophysiological and histopathological interpretation of the morphological pictures, I decided to examine in a model whether or not the existence of histamine has an influence on the staining properties of heparine, and whether a destruction of the mucopolysaccharide has a releasing effect on the histamine.

We have prepared mixtures of hyaluronic acid, heparine and histamine each substance alone or two together, and have stained them with toluidine blue. The binding of histamine to any of the mucopolysaccharides examined has no influence on the staining properties of the latter.

In a second series of experiments, we have observed the process of histamine-heparine molecule destruction using hyaluronidase (Hyalase-Benger Lab. Ltd.) or heparinase prepared by us. We have stated that, despite the destruction of the heparine molecule, a significant amount of histamine is still bound to the latter. The process of releasing histamine in the mast cells may proceed another way, of course, but we suppose that a separate secretion of histamine and heparine is not probable.

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Zusammenfassung

An Modellpräparaten wurde festgestellt, dass die Färbbarkeit der konjugierten Heparin-Histamin-Moleküle gleich ist wie die des Heparins selbst. Der fermentative Abbau des Heparins führt nicht zur vermehrten Freisetzung von Histamin.

[1] J. HILL, Exper. *13*, 395 (1957).

Phloridzin and Red Cell Phosphate Turnover

It is well known that phloridzin and its aglycone, phloretin, inhibit the movement of sugars into erythrocytes and other cells. The action has been attributed to interference with phosphorylation but this explanation meets the difficulty that sugar phosphates do not themselves penetrate red cells easily, if at all (LEFEVRE[1], WILBRANDT[2]). If, however, the mechanism of sugar movement involves a series of temporary combinations with a succession of phosphate groups forming part of the cell structure then the slow movement of ready-made sugar phosphate would not be inconsistent with the requirement for particular phosphorylations to take place. If the phosphate groups of the cell are concerned with sugar movement then substances which slow sugar movement might act by reducing the rate of turnover of the phosphate. This was tested by using P 32 labelled phosphate incorporation as an indicator of the phosphate turnover.

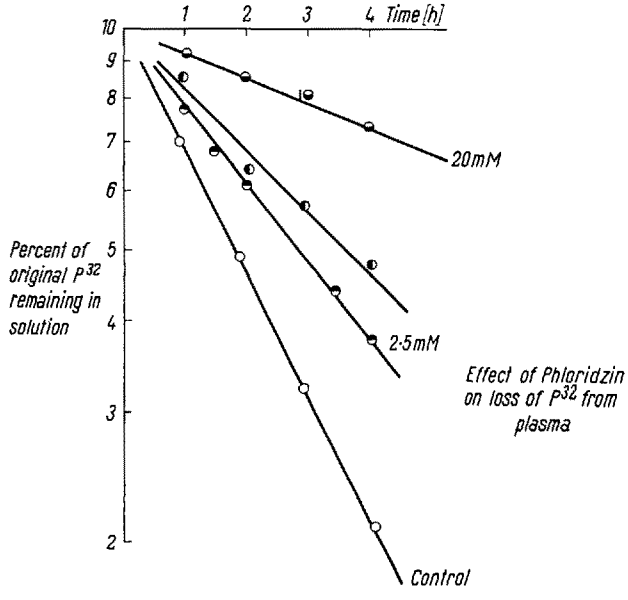

Fig. 1.—The fall of the P 32 phosphate content of plasma during incubation of the plasma with red cells, compared with and without phloridzin. In these experiments the P 32 phosphate was added to whole heparinised blood. The P 32 uptake by the cells is limited by the falling P 32 concentration in the medium.

Experiments were made either (a) by adding a trace of P 32 phosphate to whole heparinised blood and measuring the P 32 remaining in the plasma at intervals according to the method of PRANKERD and ALTMAN[3] or (b) by adding P 32 and carrier phosphate (1·2 *mM*) to a suspension medium in which the haematocrit was only 5% so that the P 32 level in the medium remained nearly constant, the activity associated with the cells being measured at intervals. Both methods showed that incorporation of the P 32 into the cells was reduced in the presence of phloridzin (Fig. 1 and 2). Using method (b) an inhibition by 0·03% w/v phloretin was found (Fig. 2). The phosphate esters and 'inorganic' phosphate of the cells after the method (a) were separated chromatographically and their radioactivities were measured (Table). Although the con-

[1] P. G. LEFEVRE, Symp. Soc. exp. Biol. *8*, 118 (1954).
[2] W. WILBRANDT, Symp. Soc. exp. Biol. *8*, 137 (1954).
[3] T. A. J. PRANKERD and T. ALTMAN, Biochem. J. *58*, 622 (1954).

Relative specific activity (RSA) and phosphate ester content of cells after 4 h incubation in control or phloridzin media

	Control		Phloridzin 2·5 *mM*		Phloridzin 20 *mM*	
	RSA	Quantity μgP/ml cells	RSA	Quantity μgP/ml cells	RSA	Quantity μgP/ml cells
Diphospho-glycerate	214, 250	135, 165	110, 94	150, 143	71	165
Inorganic phosphate	125, 143	35, 27	47, 36	42, 32	27	59
Adenosine triphosphate	195, 204	45, 58	56, 71	51, 46	17	36

tents of the fractions were little affected by the phloridzin the amount of P 32 incorporated was reduced, even in the 'inorganic' fraction. From these results we conclude that the red cell phosphate is in a dynamic state and that the turnover is sensitive to phloridzin and phloretin.

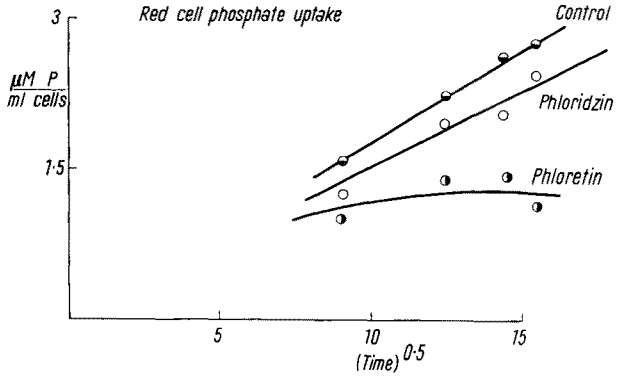

Fig. 2.—The uptake of P 32 by red cells from a large volume of medium containing 1·2 *mM* P 32 labelled phosphate. The test media contained either 6 *mM* phloridzin or 0·03% w/v phloretin. The P 32 concentration in the medium remained nearly constant.

(Time measured in minutes)

In the same experiments analyses for Na and K were carried out. These showed that no changes of Na or K content took place during 4 h exposure to phloridzin or phloretin. One sample of cells was cold stored overnight so that the Na content was 36 meq/l cells and the K content 72·5 meq/l. During subsequent incubation the Na content fell to 26 meq/l and the K content rose to 79 meq/l in both control, phloridzin, and phloretin media showing that net cation movement is unaffected. From this it appears that sufficient sugar still enters the poisoned cells to maintain that part of the metabolism which energises conversion from the low K-high Na to the high K-low Na condition.

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Résumé

L'addition de phlorizine ou de phlorétine à une suspension d'hématies humaines ralentit l'allure d'incorporation du radio-phosphate dans les cellules. Celles-ci contiennent les mêmes quantités d'esters phosphoriques après l'action de la phlorizine. Les deux drogues ne modifient pas non plus les concentrations en Na et en K des cellules.

Lipase Activity in Insect Flight Muscle

GEORGE and JYOTI[1] showed that flying birds and bats utilize fat as the chief fuel for long and sustained muscular activity. WEIS-FOGH[2] established that in the locust, during sustained flight, about two-thirds of the total energy expended is derived from fat. The importance of lipase in such muscles which utilize fat as fuel for their activity has been demonstrated by GEORGE and SCARIA[3]. They have shown that the flight muscles of birds indulging in sustained flight and the heart muscle of all vertebrates possess a high concentration of lipase. They correlated the lipase concentration of the muscle with the extent of fat utilization depending on the activity of the muscle. It was therefore thought desirable to study how far this correlation could be extended to the flying insects which exhibit various degrees of muscular activity during flight.

Aqueous extracts of the flight muscles of the following insects were used for the study: the desert locust (*Schistocerca gregaria*), the dragon fly (*Pentala flavescens*) and the bumble bee (*Xylocopa* sp.). The enzyme was prepared in the following manner: Weighed quantities of the flight muscles were extracted in known quantities of distilled water in cold for 1 h by scrubbing with sand in a test tube; it was then centrifuged at 2500 r.p.m. for 5 min and the supernatant used for the study. The method used for the assay was a manometric method adopted from MARTIN and PEERS[4] using the Warburg apparatus in a bicarbonate carbon dioxide buffer system of pH 7·4 at 37°C using tributyrin as substrate. The reaction flask contained 1·5 ml 0·025 *M* bicarbonate solution, 0·5 ml distilled water and 0·5 ml of the enzyme solution in the main chamber and 0·5 ml 4% (v/v) tributyrin in 0·0148 *M* bicarbonate (emulsified by shaking with a drop of Tween 80) in the side arm, thus making up a total volume of 3 ml. The manometers and flasks were gassed for 3 min with a mixture of 95% nitrogen and 5% carbon dioxide from a cylinder. After equilibration for 10 min the substrate was tipped in and the readings taken twice at intervals of 15 min. The manometers were shaken at about 100 oscillations/min allowing an amplitude of 4–5 cm per oscillation.

Lipase activity was calculated on the basis of wet weight and also the protein concentration of the enzyme solution used, and is expressed as the number of μl of CO_2 produced/mg/30 min (Table). Protein was estimated according to the colorimetric method for total proteins[5].

[1] J. C. GEORGE and D. JYOTI, J. Anim. Morph. Physiol. *2*, 1 (1955); *4*, 2 (1957).

[2] T. WEIS-FOGH, Proc. roy. Soc. [B] *237*, 640 (1952).

[3] J. C. GEORGE and K. S. SCARIA, J. Anim. Morph. Physiol. *3*, 2 (1956); *4*, 2 (1957).

[4] H. F. MARTIN and F. G. PEERS, Biochem. J. *55*, 523 (1953).

[5] P. B. HAWK, B. L. OSER, and W. H. SUMMERSON, *Practical Physiological Chemistry* (McGraw-Hill Book Company Inc., 1954).