or any other substances could be detected in the products used for polarimetry and each solution gave the same u.v. spectrum with absorption peaks at 252, 258, and 264 mμ). The presence of urea would be expected to have little effect on the rotation of PAG and support for this assumption was found in calculations made from [α] values given by THIERFELDER and SHERWIN[5] for PAG:urea (1:1) complexes. Thus for a complex which had $[\alpha]_D^{15} = -13{\cdot}97°$, the specific rotation based on the actual weight of PAG present was −17·3°.

Polarimetric measurements were made with a Hilger standard polarimeter (Model Mk. II. A) using a 1 dcm tube ($l = 1$; capacity ~ 1·25 ml; sodium light). The angular rotation was determined with reference to water blanks. The specific rotation is given by

$$[\alpha]_D^{20°} = 100 \cdot \alpha / c \cdot l \cdot d,$$

where c = g PAG/100 g water and d = density of the solution. In the present experiments the solution densities were not measured and were taken as equal to unity. The results are given in the Table.

THIERFELDER and SHERWIN[5] have quoted values of −17·14°, −18·1° and −18·44° for biosynthetic PAG and of −17·9° for synthetic PAG.

It would appear, therefore, that the ability of cancer patients to conjugate glutamine with phenylacetic acid is unimpaired but that little or no D-glutamine was available for the conjugation reaction. Thus, allowing for the limitations of the present method, KÖGL's findings could not be confirmed.

The writer would like to suggest that some of the positive results previously obtained may have been due to unsuspected contamination of the cancer tissues with micro-organisms such as *B. subtilis*, capsules of which are known to contain appreciable amounts of D-glutamic acid[9].

I should like to thank Dr. H. JACKSON of the Holt Radium Institute, Manchester for his kind cooperation which enabled this experiment to be carried out and Dr. J. W. MINNIS of the Department of Biochemistry, University of Edinburgh for nitrogen analyses. The work was carried out while I was in receipt of a Melville Trust Fellowship in cancer research in the University of Edinburgh.

W. J. P. NEISH

Cancer Research Unit, University of Sheffield, March 10, 1958.

Résumé

On a isolé l'acide phénylacétylglutamine de l'urine de malades atteints de cancer et d'un sujet normal qui avaient absorbé de l'acide phénylacétique. Il n'y a pas de différence entre les rotations spécifiques de ces conjugués. Les résultats montrent que l'isomère optique *non naturel* D-glutamine ne se trouve pas dans certains sujets cancéreux.

[9] C. B. THORNE, Symposium Soc. gen. Microbiol. *6* (*Bacterial Anatomy*) (1956), p. 69.

Incorporation of Adenine-4,6-C^{14} into Nucleic Acids

BENNETT and KRÜCKEL[1] reported on the incorporation of adenine into nucleic acids of a variety of mouse tissues in 4–6 months old C57 mice. The present report extends this study of adenine incorporation into ribo-(RNA) and deoxyribonucleic acid (DNA) to tissues including brain of 5–6 weeks old Webster strain albino mice. Although it was initially planned to pursue this type of investigation extensively, it has had to be discontinued. Hence, it seemed proper to report the results, at this time, in a preliminary form.

Webster strain white mice[2] weighing 16 to 18 g were injected intraperitoneally with 2·0 mg of adenine-4,6-C^{14} containing 2·2 × 10^7 dis/min in 0·5 ml of isotonic saline. After 17 h the animals were sacrificed by cervical fracture and the brain, liver, spleen, small intestine and carcass (including bone), after skinning, immediately removed. The isolation of RNA and DNA and the determination of specific activity of adenine in these fractions were carried out essentially by methods previously described[3].

Specific activity of nucleic acid adenine in various tissues of normal Webster mice*. (dis/min/μg Adenine)

Tissue	RNA	DNA	RNA:DNA
Brain	160	128	1·3
Liver	635	100	6·4
Small Intestine	1040	637	1·6
Spleen	625	456	1·4
Carcass	178	165	1·1

* Mice injected intraperitoneally with 2·0 mg of adenine-4,6-C^{14} containing 2·2 × 10^7 dis/min, and sacrificed 17 h later.

The specific activity of nucleic acid adenine in the various tissues of a mouse analyzed 17 h after administration of adenine-4,6-C^{14} is shown in the Table. The RNA:DNA ratios of these respective tissues of 4 mice similarly analyzed were consistently reproducible. The adenine incorporation into DNA was generally greater here than noted by BENNETT[4] with adult C57 mice, particularly in the liver. The much younger Webster mice employed in the present experiments were still actively growing and hence probably synthesizing DNA and incorporating adenine to a greater extent. It might be noted, in this connection, that in experiments with white rats MARRIAN[5] found a considerably higher uptake of adenine-8-C^{14} in the DNA of resting liver of young rats than that previously observed in experiments with older rats. The RNA:DNA ratio calculated from his results with liver of young rats was 6·1. Of considerable interest, too, was the large incorporation of the labeled adenine into the brain nucleic acids, particularly in view of the so-called blood-brain barrier[6].

Direct comparisons of the ratios observed here for the incorporation of adenine into RNA and DNA with the findings of others are rendered difficult by the wide experimental diversity in animal strains and ages, methods of administration of the labeled purine and time intervals following administration (see review by BROWN and ROLL[7]).

[1] E. L. BENNETT and B. KRÜCKEL, Biochim. biophys. Acta *17*, 503, 515 (1955).

[2] L. T. WEBSTER, J. exp. Med. *65*, 261 (1937).

[3] E. L. BENNETT and B. KRÜCKEL, Biochim. biophys. Acta *11*, 487 (1953); *17*, 503 (1955).

[4] E. L. BENNETT and B. KRÜCKEL, Biochim. biophys. Acta *17*, 503 (1955).

[5] D. H. MARRIAN, Biochim. biophys. Acta *14*, 502 (1954).

[6] L. BOKAY, *The Blood-Brain Barrier* (Thomas, Springfield, Ill., 1956).

[7] G. B. BROWN and P. M. ROLL, *The Nucleic Acids*, vol. 2 (Academic Press, Inc., New York 1955), p. 368.

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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.

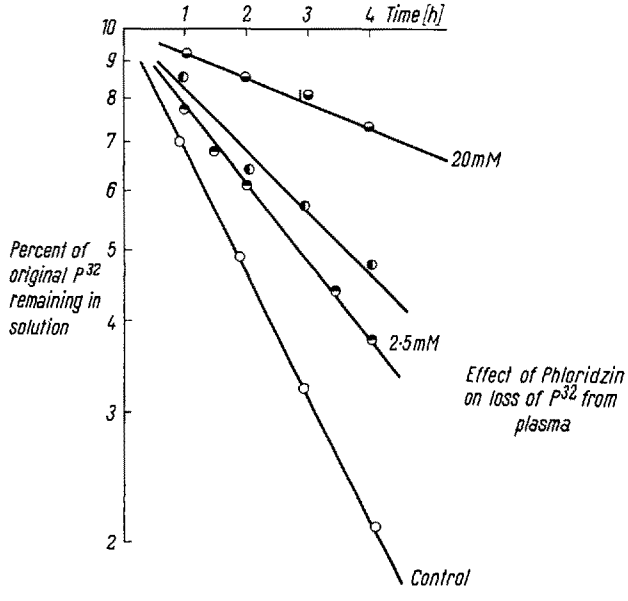

Fig. 1.—The fall of the P 32 phosphate content of plasma during incubation of the plasma with red cells, compared with and without phloridzin. In these experiments the P 32 phosphate was added to whole heparinised blood. The P 32 uptake by the cells is limited by the falling P 32 concentration in the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