whether a similar state of affairs exists in *Fusarium*, the mycelial extract was made particle-free by either filtering it through kieselguhr[9] or spinning the extract at 19000 *g* in a refrigerated centrifuge for 30 min at 2°C. When such extracts were dialyzed and then tested for transaminase activity, essentially the same results were obtained as with ordinary extracts. This might mean that transaminases, at least in fungi, are not confined to the particulate fraction as in animal[5].

I thank Prof. P. MAHESHWARI, Head of the Botany Department for providing laboratory facilities.

B. D. SANWAL

Department of Botany, University of Delhi (India), April 8, 1958.

Zusammenfassung

Aus *Fusarium lycopersici* wurde ein Enzympräparat gewonnen, welches Glutaminsäure durch Transaminierung aus α-Ketoglutarsäure bildete. Von den geprüften 20 Aminosäuren dienten die folgenden als Donatoren der Aminogruppe: Tyrosin, Ornithin, Alanin, Methionin, Leucin, Isoleucin, Prolin, Phenylalanin, Tryptophan, Valin und Asparaginsäure. Das Transaminasesystem scheint nicht an Strukturen des Cytoplasmas gebunden zu sein. Die Resultate stimmen im Prinzip mit jenen überein, welche bei *Neurospora* und bei Hefen gefunden wurden.

Specific Rotation of Phenylacetylglutamine Excreted by Cancer Patients and Normal Subjects

According to KÖGL[1], tumour tissue proteins contain substantial amounts of the 'unnatural' forms of some α-amino acids, particularly D-glutamic acid. These findings have been disputed[2], but recent work of HILLMANN *et al.*[3] lends further support to the possibility.

It was thought that the following procedure might throw some light on the question of the occurrence of D-glutamic acid in cancer patients.

When phenylacetic acid is ingested by humans, conjugation with glutamine occurs (other mammals utilise glycine[4]) and laevorotatory phenylacetylglutamine, $C_6H_5CONHCH(COOH)(CH_2)_2CONH_2$, is excreted in the urine[5]. If D- as well as L-glutamic acid is available for protein synthesis in cancer patients, it seemed possible that both 'natural' and 'unnatural' forms of glutamine might also occur in these patients. Thus the specific rotation of phenylacetylglutamine (PAG) excreted by cancer patients should be lower than that of the conjugate from normal subjects by an amount determined by the quantity of D-glutamine available for conjugation. Since phenylacetic acid in small doses is well-tolerated by humans[6] (PAG has been observed as a normal metabolite in humans[7]) it was decided to compare polarimetrically conjugates from normal persons and from cancer cases who ingested small amounts of phenylacetic acid.

In the experiment now reported, 2 male cancer patients and 1 male normal subject each received by mouth 1 g of phenylacetic acid (as sodium salt; 100 ml of bicarbonate solution) and the dose was repeated 3 h later. Urine was collected under toluene for 24 h from the start of the experiment. The urines were concentrated at 35–40°C *in vacuo*, acidified with sulphuric acid and extracted continuously with ethyl acetate following, with a few modifications, the method of THIERFELDER and SHERWIN[5]. The products, which were obtained as silky white flakes after one crystallisation from ethyl acetate, melted indefinitely at about 110°C and decomposed vigorously at ~ 120°C. Despite the similarity in melting point behaviour, nitrogen analyses (see Table, col. 3) showed that the substances were not pure PAG (for which calculated N% = 10·6), and manometric studies[8] (Table, col. 5) indicated that they contained appreciable amounts of urea. THIERFELDER and SHERWIN[5] obtained, in addition to pure PAG, a complex consisting of 1 mole PAG + 1 mole urea for which they recorded a specific rotation of – 14°. In our experience, it was found that without taking steps to remove urea before extraction of the urine with ethyl acetate, PAG products containing up to several moles of urea could be isolated.

For the present experiment, no attempt was made to eliminate urea from the once-crystallised products, the rotations of which were measured in distilled water at ~ 20°C. For calculation of the specific rotation [α], the weight of PAG in each product was estimated by subtracting the weight of urea as determined (a) by manometry and (b) by calculation from N% (assuming that the product contained PAG and urea only; no glucuronide

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Specific rotations of phenylacetylglutamine excreted by cancer patients and a normal subject

Subject	Condition	PAG complex from urine						
		Found N%	Calculated urea %	Manometric urea %	g complex/100 g water	α	$[\alpha]_D^{20}$ corrected for urea % as	
							calculated	determined manometr.
L	Myeloid leukaemia	16·5	16·4	—	2·0087	– 0·302	– 17·97	—
M	Teratoma of testis (operated). Extensive secondaries in para-aortic region and widespread deposits in lungs	21·1	29·2	29·4	1·9785	– 0·241	– 17·45	– 17·45
N	Normal	17·1	18·0	20·3	1·9646	– 0·285	– 18·34	– 18·17

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.

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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]).

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