In vivo Acetylation of Natural Amino Acids. (17393)

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Interest in the acetylation of natural amino acids stems from the suggestion that acetylamino acids may be intermediates in protein synthesis.[1] Bloch and Borek[2] demonstrated acetylation of leucine and phenylalanine in liver slices. So far, acetylation of natural amino acids has not been shown *in vivo* probably because in most cases such derivatives are further metabolized rapidly and do not accumulate sufficiently to permit isolation. Knoop[3] found that the unnatural alpha amino acid, phenylamino butyric acid, is readily acetylated in the dog.

Luck and coworkers[4] observed that most of the acetyl-DL-tryptophan administered intravenously to human subjects is excreted unchanged although excretion is negligible when the substance is given orally.[5] Preliminary experiments showed that rats also excrete acetyltryptophan in considerable amounts when it is given intravenously. We have accordingly taken advantage of this behavior to trap any labeled acetyltryptophan formed *in vivo*.

Experimental methods. Standard methods were used for the preparation of acetyl derivatives of DL- and L-tryptophan,[6] and acetylglycine.[7] Acetylryptophan was determined colorimetrically by the reaction with p-dimethylaminobenzaldehyde.[4]

Isolation of acetyltryptophan. Urine was made alkaline to phenolphthalein and extracted continuously with ether for 24 hours. It was next acidified to congo red and reextracted with ether for another 24 hours. The second ether extract was evaporated to dryness, the residue dissolved in hot water, and decolorized with charcoal. Crystals of acetyltryptophan appeared readily on cooling. Usually, two recrystallizations were enough to give a pure product. Further recrystallizations did not lower the specific radioactivity. The products isolated in experiments I to IV melted at 205-207° (reported 206°) and that in experiments V and VI at 188-189° (reported 190°). The melting points showed no depression on mixing with authentic derivatives. Kjeldahl nitrogen, found 11.28 to 11.46%; theory 11.37%. In order to establish that the radioactivity was due entirely to tryptophan, the isolated acetyltryptophan was hydrolyzed[8] and the convenient formaldehyde derivative of tryptophan prepared.[9] In experiment V 20 mg of acetyl-L-tryptophan was added to the urine as carrier. The specific activity per mole was unchanged (Table I). The derivatives melted sharply at 309-310°. Nitrogen, found 12.88 to 12.99%; theory 12.96%.

Isolation of acetylglycine. The method was similar except that ethyl acetate was used instead of ether. Crystallizations have to be carried out with greater care since acetylglycine is more soluble in cold water than acetyltryptophan. The acetylglycine was recrystallized to constant specific activity. It melted at 206°, showed no depression in m.p. on mixing with pure acetylglycine and had a nitrogen content of 11.87% (theory 11.96%).

The samples were deposited on aluminum discs from water or petroleum ether suspension and counted with a thin mica-window Geiger-Muller Counter.

The tryptophan used in the experiments was a racemic mixture labeled with C^{14} on the

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TABLE I. *In vivo* Acetylation of Tryptophan and Glycine.

Exp. No.	Compounds injected	Cts./min. administered, × 1000	Acetyl amino acid excreted, mg	Activity cts./min./mg acetyl amino acid	Activity cts./min./mg methylene tryptophan
I†	Acetyl-DL-tryptophan, DL-tryptophan*	100	75	30.7	37.6
II†	Acetyl-DL-tryptophan, DL-tryptophan* L-tryptophan (100 mg)	100	58	150	164
III†	Acetyl-DL-tryptophan, DL-tryptophan* DL-tryptophan (200 mg)	100	40	15.8	18.1
IV	Acetyl-DL-tryptophan, acetate*	780	49	4.4	5.5
V	Acetyl-L-tryptophan, acetate*	780	8.7	3.7	4.1
VI	Acetyl-L-tryptophan, alanine*	530	54	5.0	5.3
VII†	Acetylglycine, glycine*	240	28‡	3.2	

* Indicates the radioactive compound. † The data represent the average results from 2 rats. In the rest one rat was used.
‡ Final amount recovered pure.

beta-carbon and had an activity of 106,000 counts per minute per mg. The alanine was also the racemic compound labeled on the alpha-carbon (600,000 counts per minute per mg). The acetate and glycine were labeled on the carboxyl carbons (210,000 counts per minute per mg and 1,000,000 counts per minute per mg respectively).

In Experiments I to VI, 100 mg of the acetyl-tryptophan (DL- or L- dissolved in 3.8 ml water (pH 7.4) were injected intravenously by the jugular vein immediately followed by intraperitoneal injections of the other compounds mentioned in Table I. In Experiment VII, the rat was injected intravenously with 300 mg of acetylglycine in 3 ml water followed by the labeled glycine solution (0.2 ml) injected intraperitoneally. The urine was collected for the next 8 hours in all cases, the bladder being emptied at autopsy if required.

Acetyl-DL-tryptophan and acetyl-glycine were incubated with labeled tryptophan and glycine respectively for 8 hours in phosphate buffer at pH 7.4 at 37° for 8 hours. The acetylamino acids isolated from the mixture contained no C^{14} showing there was no chemical exchange.

Results. The results presented in Table I show that natural amino acids are acetylated *in vivo* in rats. In experiments II and III, the inert L- or DL-tryptophan was added to dilute the specific radioactivity with a view to determining whether the excreted radioactive acetyl derivative was predominantly of the L- or D- configuration. This is of interest since there is no biological mechanism for the deacetylation of D-amino acids.[10] The first four experiments, however, do not definitely decide this question. The subsequent experiments in which acetyl-L-tryptophan was used, leave no doubt that the L-isomer can be acetylated *in vivo*. In the last experiment with acetylglycine, the problem of optical isomerism does not exist.

The radioactivity in the isolated acetyl-L-tryptophan and acetylglycine is rather low. This does not mean that acetylation of amino acids is necessarily a reaction of little im-

[10] Bloch, K., *Physiol. Rev.*, 1947, **27**, 574.

portance. Lack of rapid and complete equilibrium between the newly formed and injected acetylamino acids would also yield low radioactivity in the excreted product. Both acetate and alanine (pyruvate) carbons can be used in this acetylation.

Summary. Intravenously injected acetyltryptophan and acetylglycine are excreted in part by rats. Advantage was taken of this behavior to trap any radioactive acetylamino acids that are formed *in vivo*. The results show that natural amino acids can be acetylated *in vivo*.

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Distribution of Bacitracin in the Body.* (17394)

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Bacitracin is an antibiotic with a wide antibacterial spectrum. It was discovered in the Bacteriological Research Laboratory of the Department of Surgery of Columbia University. The active principle is produced by the Tracey I strain of *B. subtilis*.[1] Bacitracin has been used clinically in the treatment of many infections which had failed to respond to other antibiotic and chemotherapeutic agents.[2] Because of its clinical importance, it seemed to the authors worth while to determine the distribution of the drug in the different tissues and fluids of the experimental animal following intravenous administration.

First series of experiments. Eleven normal rabbits weighing from 2 to 4 kilos were selected. Bacitracin in a dosage of 3,000 units per kilo of body weight, dissolved in 3 or 5 cc of physiological saline, was injected slowly into the ear vein of each rabbit. One, 2 or 3 hours after the injection the animals were bled to death under nembutal anesthesia. The whole organ or a portion of the tissue was then removed from the skin, heart, lung, liver, skeletal muscle, brain, bone marrow, pancreas, kidney, intestines and the spleen. Each kind of tissue was ground up in a mortar with an equivalent weight of physiological saline. It was then filtered through cotton. Each filtrate was then assayed for the concentration of bacitracin by the penicylinderplate method against *Micrococcus flavus*.[3] The cerebrospinal fluid, blood, bile and urine were also collected and their bacitracin content similarly determined. The results are presented in Table I.

Discussion. Although there are some wide variations, composite figures as expressed in averages give an approximate value for the various tissues. The figures seem to indicate that the antibiotic is widely distributed throughout the body, but its concentration is somewhat variable in different tissues. For example, the chorioid plexus apparently acts as a barrier to the passage of bacitracin. None or only a trace of bacitracin was found in the cerebrospinal fluid or in the brain tissues after intravenous or parenteral injection. This is also true of penicillin and streptomycin.[4,5]

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The bacitracin used was supplied by the Commercial Solvents Corporation of Terre Haute, Ind.

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