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Evidence That Serotonin Has a Role in Brain Function

The finding of serotonin (5-hydroxytryptamine) in brain (*1, 2*) has aroused speculation that it may have a role in brain function (*3*), perhaps as a neurohumoral agent (*4*). Studies from our laboratory that support this view have shown that lysergic acid diethylamide (LSD), a hallucinogenic agent, suppresses an action of serotonin on the central nervous system (*5*), while reserpine, a drug used in treatment of mental disorders, liberates serotonin from body depots, including intestines (*6*) and platelets (*7*).

The development of a specific fluorometric assay for serotonin in brain (*2*) has now made it possible to test our hypothesis that reserpine action may be mediated through the liberation of serotonin in brain. The serotonin content of brain, normally about 0.55 μg/g, declined rapidly after the intravenous injection of 5 mg/kg of reserpine. Within 30 minutes the total brain serotonin declined by about 75 percent and within 4 hours it declined by about 90 percent. The low level persisted for about 24 hours and then increased slowly, attaining the normal value after about 7 days. The brain is particularly sensitive to the serotonin-releasing properties of reserpine—doses as low as 0.1 mg/kg appreciably lowered the serotonin content.

Reserpine measured fluorometrically was no longer detectable in brain 12 hours after administration (*8*), whereas sedative effects and changes in brain serotonin persisted longer than 48 hours. The sedative effects thus seem to be related to the change in brain serotonin rather than to the concentration of reserpine. This may be interpreted as further evidence that reserpine acts through liberation of serotonin. Throughout the period of low serotonin content in brain, 5-hydroxyindoleacetic acid, the metabolic product of serotonin, appeared in urine in appreciable amount. Presumably, therefore, serotonin was still being formed in the body but was not accumulating in brain tissue. Thus it seems that reserpine causes an alteration in the serotonin-binding capacity of brain cells that persists long after reserpine can no longer be detected.

Our present concept, on the basis of the available facts, is as follows: serotonin in brain is normally present mainly in a bound form, thus being protected from the highly active enzyme, monoamine oxidase (*9*). After reserpine administration, brain cells lose, in part, their capacity to retain serotonin. As a result, serotonin is liberated and metabolized by the action of monoamine oxidase. Although reserpine rapidly disappears from the brain, the effect on the capacity of cells to retain serotonin persists. Since serotonin is still being formed during this period, much of it is presumably present in a free or physiologically active form. This free form of serotonin is considered as the mediator of the prolonged reserpine action. Since free serotonin is rapidly metabolized, the total serotonin remains at a low level.

The data described in this communication are in accord with the view that serotonin has an important role in brain function, possibly as a neurohumoral agent.

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Systematic Effect of C^{14}-Labeling on Ion-Exchange Chromatography of Amino Acids

In the course of studies on amino acid metabolism in mammalian cells in tissue culture (*1*), protein hydrolysates from cells fed C^{14}-labeled glucose or glutamine were chromatographed on an ion exchange column. It was observed that the peaks of radioactivity in the effluent did not precisely coincide with amino acid peaks located by the ninhydrin color reaction, but in every case followed them closely. The two curves were identically shaped, and activity did not occur elsewhere in the effluent. It seemed likely that the heterogeneity was the result of the partial resolution of labeled from unlabeled amino acid. This was shown to be the case by chromatography of known C^{14}-labeled amino acids. Some preliminary observations concerning the nature of the effect are reported in this paper.

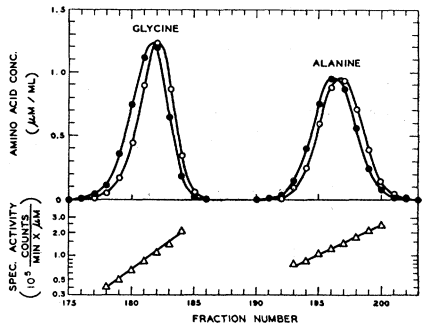

Fig. 1. Portion of the effluent curve from the chromatography of C^{14}-labeled amino acids on a 100- by 0.9-cm column of Dowex 50: ●, amino acid concentration as determined by ninhydrin; ○, amino acid concentration as determined by C^{14} count assuming 100 percent recovery; and △, calculated specific activities.

The ion-exchange separation used was a modification of the methods of Moore and Stein (*2, 3*). A 100- by 0.9 cm column of Dowex 50 (*4*), operated at 50°C and 6 ml/hr throughout the run, was employed. Elution was started from a reservoir containing 250 ml of a 0.37*M* citrate buffer (0.25*N* in sodium citrate) of *p*H 3.10. A continuous *p*H gradient was produced by the addition of 0.25*N* sodium hydroxide to the buffer to give a ratio of flow rates into and out of the reservoir of 1 to 5.7. After about 170 ml of effluent had been collected, the ratio of flow rates was changed to 1 to 1. The degree of resolution provided was significantly greater than it was in the original Moore and Stein procedure (*2*) with a 100 cm column and in some respects was equal to that of their newer method (*3*) employing a 150 cm column.

Approximately 4 μmoles each of L-aspartic, L-threonine, L-serine, L-proline, glycine, L-alanine, and L-valine (*5*), all uniformly labeled with C^{14} at a level of about 0.1 μc/μmole, were chromatographed. One-milliliter fractions were collected and 3 ml of water were added to each. One milliliter was taken for determination of amino acid concentration with ninhydrin (*6*). Carbon-14 was determined on a 0.5-ml portion of the remainder after diluting 1 to 50, except at the leading and trailing edges of the peaks, where 0.5 ml of undiluted sample

was taken. The samples were dried in 1-cm copper planchets and counted in a gas flow counter. Corrections for self-absorption were not necessary on the diluted samples. On the others, a known amount of the labeled amino acid was added to occasional samples; these were then dried and counted, and the absorption factor was calculated. This value did not vary significantly within any one peak. That part of the effluent curve showing glycine and alanine is reproduced in Fig. 1, together with the calculated specific activities for the individual fractions. Qualitatively similar results were obtained with all the amino acids studied.

It is apparent that the presence of C^{14} in an amino acid molecule resulted in slower movement on the column. Consequently, there was a progressive increase in the specific activity of the fractions within a peak. In large part, the observed differences involved singly labeled molecules, for the amino acids were uniformly labeled only in a statistical sense, and the level of radioactivity in the source material used for their biosynthesis corresponded to a C^{14} to C^{12} ratio of approximately 1 to 10 (*5*). It follows that in the case of glycine, for example, approximately 18 percent of the total counts would have been contributed by doubly labeled compound.

If it is assumed that each pair of curves can be represented by the same distribution curve with different means, an equation relating specific activity to fraction number can be derived from the ratio of the two distribution curves (*7*). For a normal distribution, the logarithm of the specific activity proves to be directly proportional to the fraction number in the region where the peaks overlap. The slope constant describing this linear relationship is a measure of the degree of resolution of the labeled and unlabeled compounds. The slopes for the seven known amino acids here studied are given in Table 1. Also included are the slopes obtained from the chromatography of a hydrolysate of total protein from cells fed uniformly labeled glucose. It is apparent from Table 1 that there was an extremely close relationship between the ratio of C^{14} to total C in a labeled molecule and the degree of resolution. Using the slopes listed in Table 1 (the average when two figures were available) the correlation coefficient was in fact 0.96. The correlation coefficient between slope and molecular weight was −0.74. This suggests that the effect of a C^{14} atom on chromatographic behavior may depend on its position in the molecule, rather than solely on its mass. The larger the number of carbon atoms, the smaller would be the chance that a single C^{14} atom would appear in one of the more effective positions. This working hypothesis is supported by the observation that the specific activity slopes of glutamic acid and proline derived from glutamine-2-C^{14} were more than twice those observed for the randomly labeled compound.

Table 1. Slope of curve relating the logarithm of the specific activity of randomly labeled amino acids to fraction number. The slopes are given in arbitrary units; A, known amino acid mixture; B, protein hydrolysate of cells fed labeled glucose.

Amino acid	Ratio* of C^{14} to total C	Slope	
		A	B
Glycine	1/2	100	108
Serine	1/3	62	51
Alanine	1/3	59	66
Aspartic acid	1/4	57	54
Threonine	1/4	32	
Valine	1/5	24	
Proline	1/5	21	
Glutamic acid	1/5		16

* Ratio per labeled molecule, assuming a single label.

Isotope effects have been noted in a number of biological and chemical systems (*8*) as well as in behavior on ion-exchange chromatography (*9*). A slight concentration of natural isotopes by ion exchange was first reported for Li^6 and Li^7, K^{39} and K^{41}, and N^{14} and N^{15} by Taylor and Urey (*10*) and by Brewer (*11*), who used 35-ft and 100-ft columns of a natural zeolite. Recently, a 780-fold concentration of N^{15}-labeled ammonia to 74 mole percent N^{15} has been effected by repeated cycling through 5-ft columns of Dowex 50 for a total of 600 ft (*12*). Tritrium-labeled organic acids have shown a similar isotope effect on partition chromatograms (*13*). In contrast, the same technique failed to change the isotope ratio of C^{14}-labeled formic acid significantly. The difference in chromatographic behavior between the labeled and unlabeled amino acids in the present experiments was small; but it is possible that with much larger columns and repeated cycling, an isotopic enrichment could be developed similar to that accomplished for $N^{15}H_3$ (*12*).

Coincidence of radioactivity and ninhydrin color has been widely used as a criterion of identity in the study of labeled amino acids and related compounds. This seems to be valid for paper chromatography, but it is not valid for the newer methods of ion exchange chromatography with their extremely high resolving power. However, a straight line relationship, with the correct slope, between the logarithm of the specific activity and the fraction number would serve as a criterion of homogeneity. There is a large possible error if a single fraction, rather than the entire peak, is used for the determination of specific activity.

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7. Thus,

$$\ln S = \left(\frac{m_1 - m_2}{\sigma^2}\right) x + \frac{m_2^2 - m_1^2}{2\sigma^2}$$

where S is the specific activity, m_1 is the mean of the activity curve, m_2 is the mean of the ninhydrin color curve, σ is the standard deviation, and x is the fraction number.
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28 September 1955

Thallium Acetate in the Diagnosis of Chronic Respiratory Disease of Chickens

Thallium acetate (TA) was first recommended in 1947 as a bacteriostatic agent in the isolation of pleuropneumonialike organisms (PPLO) in liquid medium and on plate cultures (*1*). In primary isolations where contamination was usual, TA in proper concentration inhibited ordinary bacteria and allowed PPLO to grow in pure culture. In another study of PPLO of human origin (*2*), TA used as a bacterial inhibitor in liquid and solid media did not produce "L" forms from bacteria, possessed a wide bacterial spectrum, inhibited both gram-positive and gram-negative organisms, and had a selective action independent of the total protein content of the culture medium.

Chronic respiratory disease (CRD) of chickens, caused by a PPLO (*3*), is diagnosed by isolation of the agent in em-