TABLE II. Effect of Insulin upon Incorporation of Amino Acids into Protein by Isolated Adipose Tissue Cells.

	Cell protein (CPM)*				
	Control	800 μunits/ml insulin	Mean increase of cell protein in response to insulin		
Exp No.	Mean ± SEM†	Mean ± SEM	CPM	%‡	"P"§
1	1,531 ± 56	1,914 ± 42	383	25	<.01
2	706 ± 27	835 ± 18	129	18	<.01
3	957 ± 33	1,139 ± 27	182	19	<.01
4	3,428 ± 63	4,169 ± 53	741	22	<.01
5	2,210 ± 95	2,722 ± 37	512	23	<.01
6	1,443 ± 34	1,631 ± 17	188	13	<.01
7	1,526 ± 52	1,800 ± 25	274	18	<.01

* Counts per minute per vial.
† Mean ± standard error of mean of 4 determinations following 2-hr incubation.
‡ % Increase of insulin (mean CPM) compared with control (mean CPM).
§ Probability of significance of difference between means of control and insulin computed by Student "t" test. "P" from Fisher's Tables.

units/ml. Krahl(4) emphasizes that insulin has a specific stimulatory effect on adipose tissue protein synthesis that cannot be simulated by other substances which increase glucose uptake or lipid synthesis in fat.

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Entry of L-Phenylalanine-C^{14} into Brain and Cerebrospinal Fluid.* (31054)

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Previous studies from this laboratory have indicated that elevated plasma levels of L-phenylalanine inhibit the entry of 5-hydroxytryptophan-C^{14} into cerebrospinal fluid (CSF) of dogs(1). In order to assess the significance of the entry of labelled amino acid into CSF, it was felt necessary to obtain data correlating radioactivity of brain and CSF.

In the present experiments, L-phenylalanine-C^{14} was administered to anesthetized dogs and levels of radioactivity in CSF were compared with simultaneously determined levels in the trichloracetic acid (TCA) insoluble and TCA soluble fractions of several anatomical regions of the brain. The results form the basis of the present report.

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Methods. Operative procedures. Eight purebred beagle dogs of either sex were utilized in these experiments. The animals were fasted overnight prior to the experiment. They were anesthetized with 30 mg/kg of intravenous pentobarbital. Respirations utilizing room air were maintained with a mechanical respirator attached to an oral cuffed endotracheal tube. Three μc/kg, L-phenylalanine-UL-C^{14} (sp act 190 mC/

mmole) was injected into a cannulated femoral vein and arterial blood for plasma determinations was obtained from a cannulated femoral artery. Small samples (0.2 ml) of CSF were periodically obtained from an indwelling spinal trocar inserted into the cisterna magna. The animals were sacrificed by removal of the brain which was washed free of superficial blood with physiological saline and dissected as rapidly as possible. Portions of temporal cortex, parietal cortex, mid-brain, thalamus, hypothalamus and white matter from the centrum ovale were dissected from the brain. The samples were stored at —15°C until prepared for analysis.

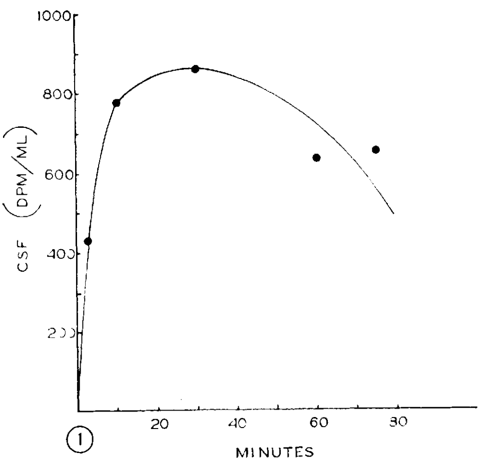
TCA insoluble proteins were prepared from each brain area(2). The TCA soluble material, after treatment with Dowex-2 to remove the TCA, was lyophilized and stored until analyzed. The amount of blood present in brain tissue was determined(3) and since its contribution was negligible, no correction was applied to the data.

All samples containing radioactivity were determined by conventional means using a Nuclear-Chicago liquid scintillation spectrometer(4).

The quantitative determination of the amino acids in the TCA soluble and insoluble fractions (after hydrolysis in 6 N HCl at 110° for 22 hours) was carried out with an automatic amino acid analyzer(5). To identify the specific amino acids in which the radioactivity existed, the effluent from the analyzer was passed through a Nuclear-Chicago Chromacell attached to the liquid scintillation spectrometer.

Results. The radioactivity in the TCA soluble fractions of the different brain areas and in the CSF increased in a parallel manner (Fig. 1 and 2). After reaching a peak, the radioactivity decreased steadily during the remaining time intervals studied. A curve of similar configuration was obtained when samples of CSF were followed at the same time intervals in different dogs. In contrast, the peak of radioactivity in the TCA insoluble fraction occurred later (Fig. 3). Maximum incorporation of radioactivity occurred in the TCA insoluble fraction at 70 minutes compared to 30 minutes for the acid soluble fractions and CSF.

The regional brain analyses indicate a small increase in incorporation of radioactivity into protein of cerebral cortical areas when compared to subcortical areas. These differences may reflect variability of the phenylalanine and tyrosine content in the ana-



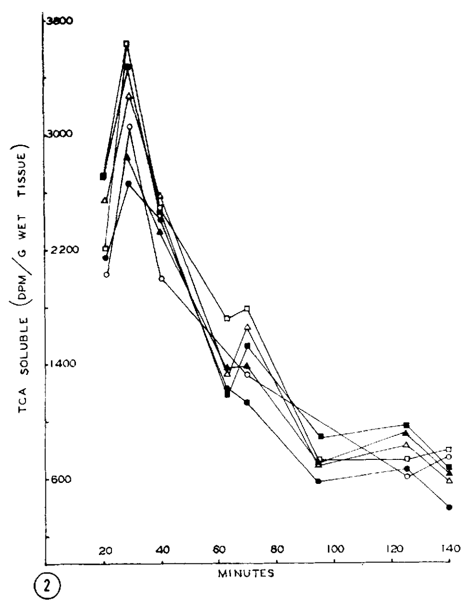


FIG. 1. Entry of L-phenylalanine-C^{14} into CSF - Serial samples from one dog.

FIG. 2. Entry of L-phenylalanine-C^{14} into TCA soluble fractions of dog brain. Each vertical series of points represents 6 regional brain areas from one dog. ●——● midbrain; □——□ white matter; ▲——▲ thalamus; ○——○ hypothalamus; △——△ temporal cortex; ■——■ parietal cortex.

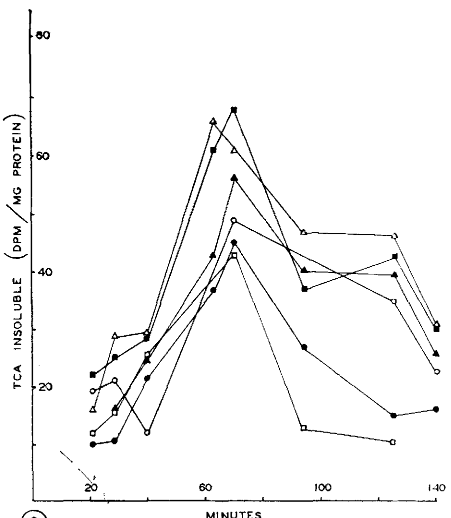


FIG. 3. Incorporation of L-phenylalanine-C^{14} into TCA insoluble fractions. Legends as in Fig. 2.

tomical regions. The variations between different cortical and subcortical areas were too small to have significance.

To establish the identity of the radioactive amino acid present in the protein, the hydrolyzate of the protein of each brain area of the 70-minute sample was subjected to column chromatography and the effluent passed through a flow cell attached to a liquid scintillation spectrometer. This technique demonstrated that all of the radioactivity existing in the hydrolyzates occurred as phenylalanine or tyrosine. A similar finding was observed for the free amino acids in the TCA soluble fraction at thirty minutes.

Discussion. The data reported demonstrate that intravenously administered L-phenylalanine-C^{14} enters into regional brain areas and CSF in a similar manner. This supports the previously stated opinion that CSF may be looked upon as representative of a central nervous system extracellular fluid. It is also of interest that all brain areas studied showed a similar radioactivity time curve indicating that the pattern of amino acid entry and incorporation into brain protein is alike for a number of anatomically distinct areas. However, it should be kept in mind that heterogeneity of brain protein does exist(6) so that differences in incorporation into various proteins might be missed in this study since individual proteins were not isolated.

Although the identity of the radioactive amino acid incorporated into protein was not established at each time interval studied, it was demonstrated that at the peak of incorporation the activity existed as phenylalanine and tyrosine in the TCA insoluble protein. The presence of radioactive tyrosine in the cerebral protein can best be explained as a conversion of phenylalanine to tyrosine by the liver and subsequent uptake by brain. It has already been demonstrated that tyrosine can be taken up by rat brain and probably a similar situation exists here(8).

The results of this study confirm and extend previous studies in which phenylalanine-C^{14}, leucine-C^{14}, and isoleucine-C^{14} were injected subcutaneously into the mouse and TCA precipitate of the cortex was evaluated for the incorporation of the amino acid(7). The peak of the radioactivity occurred in the pool prior to the protein and the time of maximum radioactivity agreed with the work reported herein.

The assessment of the kinetics of entry of an isotope into the central nervous system following a single injection is limited because of the rapid decrease in blood levels during the experiment. More precise information would be obtained if relatively constant blood levels could be maintained. Attempts in this direction are now being made by administering the isotope by constant infusion rather than by a single injection.

Summary. There is a close resemblance of the time curves of phenylalanine-C^{14} in CSF and the acid soluble fractions of several regional brain areas in the anesthetized dog. This supports the view that *in vivo* studies of CSF provide information relevant to the entry of isotopically labelled amino acids into the brain.

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Effects of Diuretic Agents on Serum and Tissue Electrolytes in Rats.* (31055)

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The physician's ability to manage patients with hypertensive cardiovascular disease and patients with edema has been aided in part by the synthesis of chlorothiazide(1) and its derivatives(2-5). However, the frequent occurrence of hypokalemia in patients during the course of thiazide administration has disturbed clinicians(6,7). The effect of thiazides upon potassium metabolism has been deduced from balance studies(8), from serial measurements of exchangeable potassium with K^{42} (9,10). and from direct tissue analyses(11, 12). These studies have shown that potassium is lost from the body during the first few days of therapy, potassium balance may then become positive, while hypokalemia persists. Since the mechanism of production of the hypokalemia was obscure, a series of experiments was designed to test the hypothesis that the hypokalemia occurring during thiazide administration did not reflect a deficit of body potassium.

Methods. Eighty Sprague-Dawley rats including equal numbers of males and females weighing from 175-250 g were randomly assigned to one of 4 coded diet programs (with 20 rats in each group): I. Normal rat diet,† II. Normal diet with chlorthalidone‡ added to supply a daily dose of 2.1 mg/kg, III. Normal diet with Hydrochlorothiazide‡ added to supply a daily dose of 1.4 mg/kg, IV. Normal diet with polythiazide§ added to supply a daily dose of 0.028 mg/kg.

These diets were prepared elsewhere and were delivered to our laboratory in coded containers. All animals were individually caged, food intake was estimated by weighing the food containers 3 times weekly, and the weight gain of individual animals was determined by weighing each animal once weekly. These data demonstrated that food intake by the 4 groups of animals was consistent and that all 4 groups gained equal amounts of weight during the period of the study. The animals were permitted distilled water *ad libitum* during the 4-week treatment period. At the end of that time, the animals were anesthetized with ether, a sample of blood was drawn from the abdominal aorta and then both quadriceps femoris muscles were removed for tissue analysis. After the blood had been allowed to clot under oil at room temperature, the samples were centrifuged and the serum removed for analysis.

Serum water was determined by drying aliquots of serum to constant weight at 110°C, chloride by Cotlove titration and sodium and potassium by an internal standard flame photometer. Tissue analyses were perfomed by methods previously described(14) except that an internal standard flame photometer was used for determination of sodium and potas-

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† Diets were obtained from Rosner-Hixson Laboratories, Chicago, Ill.

‡ Chlorthalidone and Hydrochlorothiazide used in this study were supplied by Dr. John D. Sproul, Geigy Pharmaceuticals, Yonkers, N. Y.

§Polythiazide used in this study was supplied by Dr. Findlay Crowe, Pfizer Laboratories, New York.