Amino Acids of Nervous Tissue. (17916)

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In previous work patterns of free amino acids were determined by paper chromatography in various normal mouse tissues and in tumors(1,2). Each normal tissue examined had a distribution characteristic of that tissue, while all of the tumors, regardless of derivation or source, showed similar patterns. Schurr and co-workers(3), using microbiological procedures, have reported differing patterns of free amino acids in the plasma, brain, liver, spleen, and muscle in the rat, but they did not make determinations of glutamic acid, aspartic acid, glutamine, taurine, glycine, alanine, serine, and cystine, the amino acids found in our previous studies to be present in the highest concentrations in the free form in mouse tissues. They were able to account for only approximately 7% of the free α-amino nitrogen of brain. It was therefore of interest to report our observations on the distribution of those amino acids which are present in nervous tissue in the highest concentrations and which undoubtedly account for the greatest proportion of the free amino nitrogen.

Experimental. Animals and preparation of tissues. All mice employed in the present study were killed by dislocation of the cervical vertebrae. The rabbits were usually killed in the same way or deeply anesthetized with intraperitoneal nembutal. Similar tissue removed after either procedure yielded identical results. Whole mouse brains were used. In the case of the rabbits samples were taken from the cerebrum (cerebral cortex), cerebellum, and upper brain stem and samples of sciatic nerve and the lumbosacral spinal cord were also studied. The extracts of the tissues were prepared for analysis essentially as previously described(1). Aliquots corresponding to 75 mg of fresh tissue were employed. The alcoholic extracts gave the same results as dialysates prepared from heat coagulated tissue. The mouse brains were from animals of the C3H, $C57_6$, dba, and Swiss strains. The neuroblastoma was the transplanted tumor C1300 carried in strain A mice at the Jackson Memorial Laboratory. Most of the rabbits used were of the New Zealand White Stock. The ataxic rabbit was of the Sandy Flemish strain. Part of the lumbosacral spinal cord of one of the rabbits was removed 6 days after section of both sciatic nerves.

Chromatography. Suitable volumes of the samples were placed on paper for 2-dimensional paper chromatography by the method of Consden, Gordon, and Martin(4). Phenol saturated with water was used as the first solvent and a mixture of collidine and lutidine saturated with water as the second. The spots were treated with hydrogen peroxide (5) prior to chromatography in a number of instances to convert methionine to the sulfone and cystine to cysteic acid.

Results. Comparison of pattern of free amino acids with that of hydrolyzate of whole brain. A chromatogram made of an acid hydrolyzate corresponding to 10 mg of fresh whole mouse brain (Swiss) is shown in Fig. 1. In addition to the amino acids ordinarily found in proteins, β-alanine, ethanolamine, glucosamine, α-aminobutyric acid and γ-aminobutyric acid were observed. Phenylalanine was poorly separated from the leucines be-

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1. Roberts, E., and Frankel, S., *Cancer Research,* 1949, v9, 645.

2. Roberts, E., and Frankel, S., *Cancer Research,* 1950, v10, 237.

3. Schurr, P. E., Thompson, H. T., Henderson, L. M., Williams, J. N., and Elvehjem, C. A., *J. Biol. Chem.,* 1950, v182, 39.

4. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.,* 1944, v38, 224.

5. Dent, C. E., *Biochem. J.,* 1947, v41, 240.

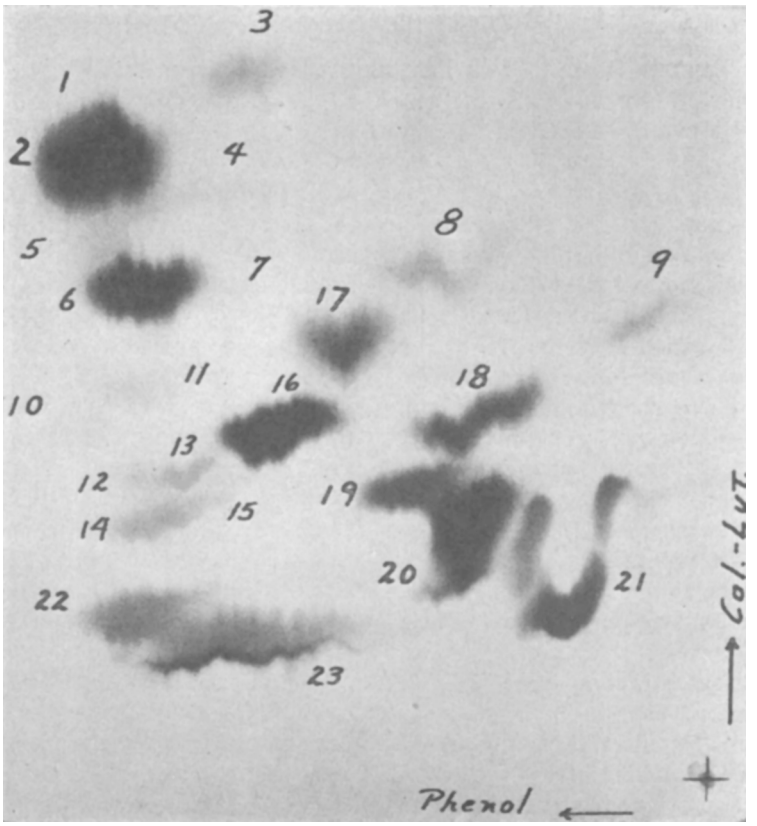

FIG. 1.
Chromatogram of hydrolyzate of 10 mg of whole mouse brain.

Key to numbers on chromatograms: phenylalanine, 1; leucine and isoleucine, 2; tyrosine, 3; glucosamine, 4; ethanolamine, 5; valine, 6; methionine (methionine sulfone), 7; taurine, 8; cystine (cysteic acid), 9; proline, 10; α-amino-N-butyric acid, 11; histidine, 12; hydroxyproline, 13; γ-aminobutyric acid, 14; β-alanine, 15; alanine, 16; threonine, 17; serine, 18; glycine, 19; glutamic acid, 20; aspartic acid, 21; arginine, 22; lysine, 23; glutamine, 24; glutathione, 25; X and X^1 are unknown ninhydrin-reactive substances which disappear on acid hydrolysis; X^{11} and X^{111} are also unknown substances.

cause of the large amounts of the latter. Tryptophan was not detected because it is destroyed during acid hydrolysis. Fig. 2 shows a typical chromatogram of the free amino acids extracted from 75 mg of brain. It is apparent that the pattern does not reflect the amino acid composition of the brain as a whole. A number of the amino acids found in large quantities in the hydrolyzate were present in the free form in amounts below the limit detectable by the chromatographic procedure. Glutamic acid, taurine, aspartic acid, and γ-aminobutyric acid were present in the largest amounts in the free form. The identity of the γ-aminobutyric acid, found at most in traces in tissues other than brain, has been established unequivocally(6,7). Somewhat smaller amounts of cystine, serine, glycine, alanine, and glutamine were found. Free valine, the leucines, threonine, glutathione and β-alanine were detected. On some chromatograms the leucine spot was not found. In addition, two ninhydrin-reactive substances designated x and

6. Roberts, E., and Frankel, S., *Fed. Proc.*, 1950, v9, 219.

7. Udenfriend, S., *Fed. Proc.*, 1950, v9, 240.

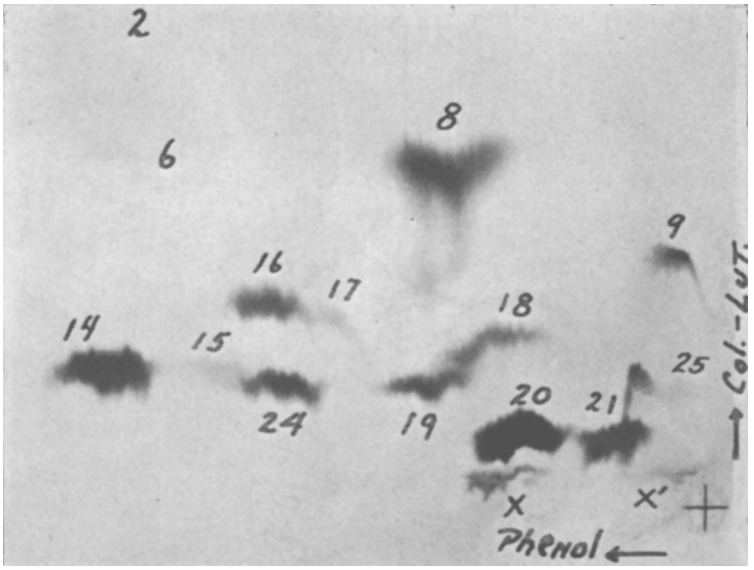

FIG. 2.
Extract of 75 mg of adult mouse brain.

x^1 which disappeared on acid hydrolysis were located below aspartic and glutamic acid on the chromatograms. Some constituents may have been present in quantities below the detectable range. The results of Schurr *et al.*(3) obtained by the more sensitive microbiological methods indicate that probably all of the amino acids found in cellular proteins are present to some extent in tissues in the free form.

Influence of age, sex, and strain on free amino acids of mouse brain. Chromatograms were made of extracts of the brains of mice immediately after birth and one day before birth and were compared with those obtained from the brains of the mother mice. The fetal brains had less glutamic acid and aspartic acid than the brain of the mother, and more taurine, alanine and substance x. Small amounts of arginine, tyrosine, and α-aminobutyric acid were found in the fetal brain, but were not detected in the adult brain. The other constituents were noted in approximately the same amounts in both samples. Extracts of the brains of the newborn mice had less γ-aminobutyric acid, glutamic acid, cystine, and aspartic acid and more taurine and material under glutamic acid than did the extract of the maternal brain. One pooled sample of brain obtained from a litter of 15-day embryos showed no γ-aminobutyric acid and only a trace of glutamine. However, there were considerably larger quantities of glutathione and the intensity of spot x was much greater in the embryonic brain. An examination of the brains of male and female littermate mice (30-37 days old) of the C3H, $C57_6$, and dba strains revealed no essential differences between the sexes. The general impression was obtained that the quantity of detectable amino acids decreased in the order C3H, $C57_6$, dba. However, this will have to be checked by quantitative procedures.

Free amino acids in tumor C1300 (neuroblastoma). Although no direct comparison can be made between the transplantable neuroblastoma and any of the samples of mouse brain examined, it was, nevertheless, of interest to study this tumor, which was the only malignancy of nervous origin available to us. The pattern of free amino acids consistently observed in a number of samples of this tumor was similar to that found for other transplantable mouse tumors(1) and different from that found in the typical brain chromatogram (Fig. 3). The pattern of the

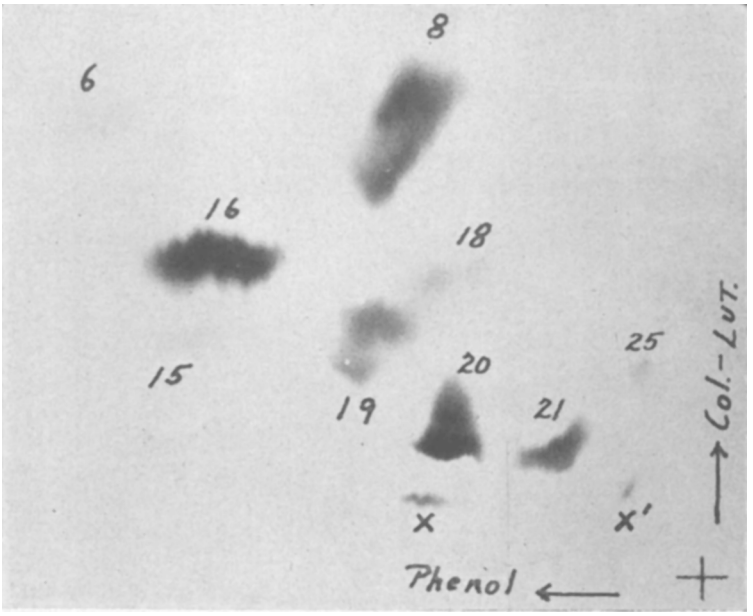

FIG. 3.
Extract of 75 mg of neuroblastoma (tumor C1300).

neuroblastoma was very similar to that of the 15-day embryonic brain, showing no glutamine or γ-aminobutyric acid.

Free amino acids in rabbit nervous tissue. Cerebrum (cerebral cortex) upper brain stem, spinal cord, and sciatic nerve were studied in normal rabbits and in an ataxic rabbit. The distributions of free amino acids in the brain were essentially similar to those found in mouse brains, but the taurine levels of the different parts of the rabbit brain were much lower. Somewhat smaller amounts of some of the constituents were noted in the spinal cord. The sciatic nerve showed much smaller concentrations of most of the detectable constituents and no γ-aminobutyric acid. Relatively large quantities of the latter substance have been observed in every sample of mouse brain and rabbit brain and spinal cord examined. Fig. 4 shows a typical chromatogram for cerebrum (cerebral cortex). The differences in free amino acids of the sciatic nerve and the various parts of the central nervous system may reflect genuine metabolic differences or may be the result of smaller amounts of neuroplasm per unit of fresh weight in the sciatic nerve. The concentrations of free amino acids in the nervous tissues of the moribund ataxic rabbit were much lower than in the corresponding tissues of the normal rabbits. No changes in the distribution of free amino acids in the lumbosacral portion of the spinal cord of the rabbit were noted 6 days after section of both sciatic nerves. It is interesting that in this species relatively little histological change occurs in the spinal cord after severing the sciatic nerves(8).

Homogenate experiments. The high contents of free glutamic and aspartic acids, glutamine, and alanine in brain are consistent with the important roles that these substances play in nitrogen transport in the cell (8). A number of experiments, which will be reported in detail elsewhere, were performed in which homogenates of brain were incubated in 0.05 M phosphate buffer (pH 7.4) at 38°C in an atmosphere of oxygen, with or without added glutamic or aspartic

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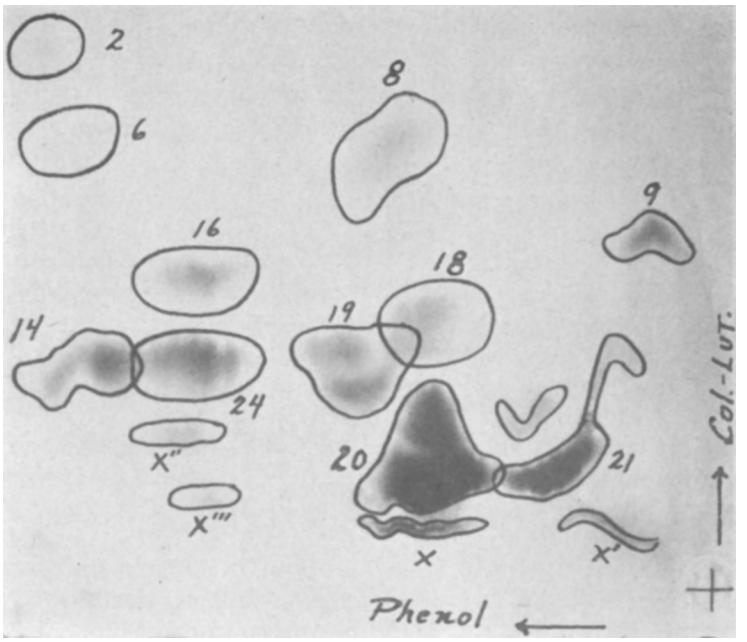

FIG. 4.
Extract of 75 mg of rabbit cerebrum (cerebral cortex).

acids. Suitable aliquots of protein-free extracts of the incubation mixtures were subjected to chromatography. The results clearly showed the presence of enzymatic mechanisms both for the breakdown and synthesis of glutamine. The existence of transaminating mechanisms was shown by the formation of alanine and aspartic acid in the presence of added glutamic acid, and by the increases in the contents of alanine and glutamic acid when aspartic acid was the substrate. It is well known that brain tissue can effect transamination(9) and synthesize glutamine from glutamic acid(10). In addition, γ-aminobutyric acid was found to be formed at an appreciable rate in the presence of added glutamic acid and removed in the presence of added aspartic acid. The latter findings are being investigated further. No notable changes were observed in the contents of amino acids other than those mentioned above under the conditions of the experiments.

Summary. The pattern of free amino acids in mouse brain, as shown by 2-dimensional paper chromatography, does not reflect the amino acid composition of the brain as a whole. The brains of mice of several strains and various parts of the nervous system of the rabbit were studied. Free glutamic and aspartic acids, γ-aminobutyric acid, taurine, glutamine, cystine, serine, glycine, alanine, valine, and the leucines were detected consistently. The variations in these constituents with age, sex, and strain in mice are discussed. The sciatic nerve in the rabbit had considerably smaller quantities of detectable constituents than the various parts of the central nervous system. A transplantable neuroblastoma in mice had a pattern of free amino acids which was similar to that found in other malignant tissues and in brains of 15-day embryos and significantly different from that found in all of the other samples of mouse brain examined. The suitability of the chromatographic method for the study of changes in the free amino acids of brain homogenates is indicated.

9. Braunstein, A. E., *Advances in Protein Chem.*, 1947, v3, 1.
10. Krebs, H. A., *Biochem. J.*, 1935, v29, 1951.

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