rate of H^+ transport approached and exceeded that of the amphibian mucosa. The available evidence does not suggest that generation of an electrical potential difference is necessary for active transport of H^+ by the gastric mucosa: (i) H^+ secretion proceeds even when the potential difference is abolished or reversed by an external current (*1, 2*); (ii) H^+ transport is less sensitive than Cl^- transport to exposure to potent inhibitors of carbonic anhydrase (*6*); (iii) substitution of SO_4^{--} for Cl^- in the bathing medium eliminates the active monovalent anion transport which is responsible for the generation of the potential difference (*7*). Nevertheless, we must inquire why, in most vertebrates secretion of H^+ is associated with a large gastric mucosal potential difference. No explanation for the mechanism of gastric secretion of hydrochloric acid will be adequate until the interdependence of Cl^- and H^+ transport is elucidated (*8*).

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22 December 1958

Free β-Hydroxy-γ-aminobutyric Acid in Brain

Abstract. By using the methods of paper chromatography and high-potential paper electrophoresis and by comparing the pattern of prepared extracts with that of synthesized β-hydroxy-γ-aminobutyric acid, we found that free β-hydroxy-γ-aminobutyric acid exists in the brains of mice, rabbits, cattle, and human beings.

γ-Aminobutyric acid (γ-ABA) has been shown to be converted to β-hydroxy-γ-aminobutyric acid (β-OH-γ-ABA) by beta oxidation and also to glutamic acid by transamination between β-OH-γ-ABA and α-ketoglutaric acid (*1*). On the other hand, hydroxyproline may be converted, in vitro, to β-OH-γ ABA via Δ^1-pyrroline-4-hydroxy-2-carboxylic acid (*2*). Formation of β-OH-γ-ABA through decarboxylation of allo-β-hydroxyglutamic acid has also been shown with an enzyme sample obtained from various strains of *Escherichia coli* (*3*).

β-Hydroxy-γ-aminobutyric acid inhibits nervous transmission in a manner similar to Florey's fraction I (*4*), and also antagonizes the convulsions induced by the injection of sodium glutamate, sodium citrate, sodium phosphate, sodium chloride, and acetylcholine into the motor area of the cerebral cortex. It also antagonizes the convulsion induced by electric stimulation (*5*).

Immediately after fresh brain material was obtained, a homogenate was made and kept frozen until deproteinization by 70 percent ethanol. The deproteinized liquid was subjected to petroleum ether extraction to remove lipids and was evaporated to dryness at 45°C in a vacuum. The residue was dissolved in a small amount of distilled water and applied on Toyo-Roshi No. 131 filter paper for high-potential paper electrophoresis and paper chromatography.

Comparative high-potential paper electrophoresis of the brain extract and of a synthesized sample (*6*) of β-OH-γ-ABA was made. The extract and the synthesized sample were applied in juxtaposition on the starting line of the filter paper and gave the pattern shown in Fig. 1. Juncture, in such a pattern, of the area of a synthesized sample with the area of a certain constituent of an extract of biological materials has always been demonstrated when the two are identical; we may, therefore, tentatively identify the constitutent represented by the zone which is in juncture with the zone of the synthesized sample as β-OH-γ-ABA. Similar results were obtained with the brains of mice, cattle, rabbits, human beings, and also with the blood of rabbits and of human beings.

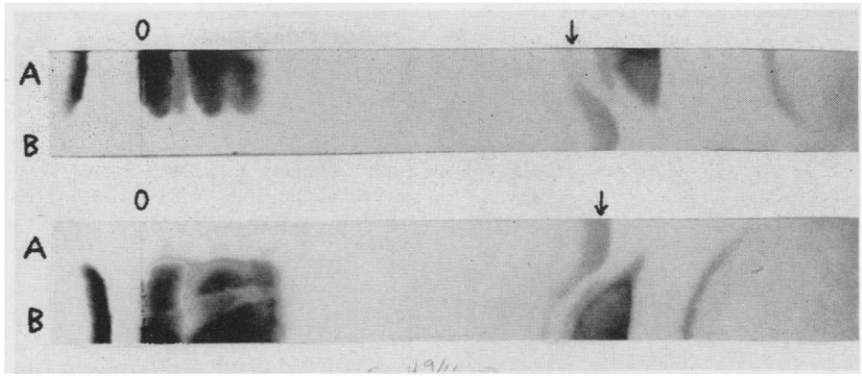
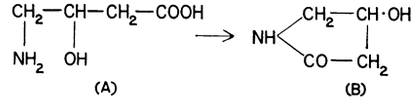


Fig. 1. Comparative electrophoresis of cattle brain (top) and human brain (bottom). *O,* starting line; *A,* prepared extract; *B,* synthesized sample; arrow, the juncture of the area of synthesized sample with the area of prepared extract. Condition of electrophoresis, 100 v/cm, 25 ma, 40 min; electrolyte, pyridine-acetic acid-water (1:10:89), *p*H 3.6; filter paper, Toyo-Roshi No. 131, 20 by 2 in.; stain, acetone solution of 0.5 percent ninhydrin.

The zone thus tentatively identified as β-OH-γ-ABA was cut from the paper and subjected to water elution. The eluate was applied on Toyo-Roshi No. 131 filter paper for one-dimensional paper chromatography. *n*-Butanol-acetic acid-water (4:1:1) was used as solvent. The R_f value of the constituent in the eluate coincided perfectly with that of the synthesized β-OH-γ-ABA at 0.38 (Fig. 2).

For further identification, a small amount (0.2 mg) of purified crystalline sample of R_f identical with that of the synthesized sample was obtained from the homogenate of 1.6 kg of cattle brain after deproteinization, removal of the lipids, and treatment with charcoal and ion-exchange resin and fractionation by means of paper chromatography and high-potential paper electrophoresis.

The yield of "β-OH-γ-ABA" thus obtained, however, was all too small compared with the original "β-OH-γ-ABA" content of the starting material, which was estimated by colorimetry—for example, 48.6 mg of β-OH-γ-ABA was found in 100 g of wet temporal lobe of cattle (*7*). This was assumed to be due to the fact that there are four isomers of β-OH-γ-ABA: *l*-β-OH-γ-ABA and *d*-β-OH-γ-ABA, both in chain and cycloid forms; the chain form may be easily converted to the cycloid form, particularly in an alkaline medium (*8*).



The chain form (*A*) gives a violet color with Ninhydrin and a yellow color with KIO_4-Nessler's reagent, and the cycloid form (*B*) gives a bright yellow color with Ninhydrin and remains colorless with KIO_4-Nessler's reagent on the filter paper.

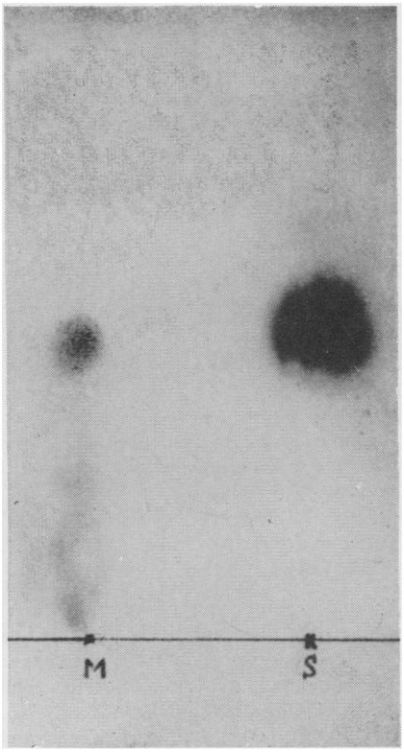


Fig. 2. *M*, eluted solution; *S*, synthesized sample; solvent, *n*-butanol-acetic acid-water (4:1:1); developed for 12 hours at 17°C; stain, acetone solution of 0.5 percent ninhydrin. Because the filter paper was too large for our photocopying instrument, the upper part of the paper had to be omitted from the photographic reproduction.

The synthesized sample, when it was developed by means of paper chromatography and high-potential paper electrophoresis, gave a second area of faint color (Fig. 1). The area showed the above-stated characteristic reactions of the cycloid form.

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Blood-Brain Barrier for Adrenaline

Abstract. The concentration of tritium-labeled adrenaline was determined in various areas of cat brain after intravenous infusion. It did not exceed that expected from the blood content of the tissue except in the hypothalamus, where small but significant amounts of H^3-adrenaline were found.

Since adrenaline, after peripheral administration, produces mental and central-neurophysiological effects, it has been assumed that it acts directly on the central nervous system. However, there is no experimental evidence whether or to what extent it crosses the blood-brain barrier.

The availability of tritium-labeled adrenaline of high specific activity (*dl*-β-H^3-adrenaline bitartrate, 1 mg = 267 μc) made it possible to study the passage of adrenaline from blood to various regions of cat brain. Tritium-labeled adrenaline was infused into the femoral vein of cats anesthetized with Nembutal at rates varying in different experiments from 2.7 to 20.7 μg/kg per minute over a period of 30 minutes. Immediately before the animal was killed by decapitation, blood samples were withdrawn from the femoral artery, heparin being used as an anticoagulant. Samples of brain and other tissues were homogenized in 9 volumes of 0.1*N* HCl. The extracts, after the addition of 0.1 volume of 1 percent (wt./vol.) disodium ethylenediaminetetraacetate and 0.05 volume of 2 percent (wt./vol.) ascorbic acid, were adjusted to *p*H 8.4, centrifuged, and passed over columns of aluminum oxide (*1*). After washing and eluting (*1*), the eluate was evaporated in a vacuum and taken up in methanol, and a portion was added to a mixture of 3 ml of ethanol and 10 ml of 0.4 percent (wt./vol.) 2,5-diphenyloxazole and 0.01 percent (wt./vol.) β-bis [2-(phenyloxazolyl)] benzene in toluene for counting in a liquid scintillation spectrometer. Plasma was diluted with 1 volume of 0.2*M* sodium acetate and, after *p*H adjustment, was similarly passed over aluminum oxide and processed. Recoveries of added H^3-adrenaline were about 80 percent.

The specificity of the procedure was tested by paper chromatography: extracts of plasma and tissues were treated as described, and the resulting methanol solutions were applied to Whatman No. 1 paper and run in butanol:acetic acid:water (*2*) or in phenol:water:SO_2 (*3*). Scanning of the chromatograms revealed only a single peak of radioactivity having the same R_F-value as adrenaline.

Since brain contains on the average 0.024 ml of blood per gram (*4*), some radioactivity, amounting to about 2 percent of that of plasma, is contributed by the blood content of the tissue. Table 1 shows that the H^3-adrenaline of brain significantly exceeds 2 percent of the radioactivity of plasma in only one area, the hypothalamus, indicating that this is the only area where transfer occurs (*5a*).

This particular property of the hypothalamus is of special interest in view of the localization of sympathetic centers and the high concentration of noradrenaline (*5*) in this region. It should be noted, however, that the quantities of adrenaline transferred are relatively small. Even at the highest rate of infusion, which was probably twice as high as the maximum rate of adrenaline secretion (*6*), the concentration of H^3-adrenaline in the hypothalamus did not exceed 0.025 μg/g. Correspondingly smaller amounts were taken up at lower rates of infusion. It is known that the periventricular gray substance which is located in the region of the hypothalamus bordering the third ventricle and backward along the cerebral aqueduct is more accessible to vital stains circulating in the blood than the rest of the brain (*7*). We cannot say at present whether the increased permeability to circulating adrenaline is related to these elements.

In marked contrast to brain, large concentrations of H^3-adrenaline were found in all other tissues examined; those in heart, spleen, and pituitary and adrenal glands exceed the concentration in plasma several fold (*8*).

Negligible amounts of radioactive metanephrine (3-O-methyladrenaline), the principal metabolite of adrenaline (*9*), were found in brain though, like H^3-adrenaline, it was present in large amounts in plasma and in other tissues. This suggests that the failure to find significant quantities of H^3-adrenaline in brain was not the result of an unusually rapid metabolism.

It may be concluded from these experiments that adrenaline is unable to cross the blood-brain barrier except to a small extent in the hypothalamus. Any central effects of adrenaline after peripheral administration may therefore be the result of its interaction with hypo-

Table 1. Transfer of H^3-adrenaline into various areas of cat brain.

Area	Brain/plasma ratio*
Medulla oblongata	0.049 ± 0.0209
Cerebellum	0.029 ± 0.0093
Pons	0.023 ± 0.0054
Hypothalamus	0.106 ± 0.0177
Midbrain	0.027 ± 0.0074
Thalamus and corpus striatum	0.025 ± 0.0122
Cerebral cortex	0.017 ± 0.0070
Cerebrospinal fluid†	0.005

* Means of five experiments ± standard error.
† Single experiment.