

Fig. 2. M, eluted solution; S, synthesized sample; solvent, n-butanol-acetic acidwater (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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- Visiting scientist. Research Facility, Rockland State Hospital, Orangeburg, N.Y. We express appreciation to Dr. Yasuhiko Taketomo of the Research Facility, Rockland State Hospital, for his kind cooperation on this publication and to Dr. Masaji Tomita for his generous gift of syn-thesized & hodrown a comprehension thesized $\hat{\beta}$ -hydroxy- γ -aminobutyric acid.

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Blood-Brain Barrier for Adrenaline

found.

Abstract. The concentration of tritiumlabeled 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 H3-adrenaline were

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 -H³-adrenaline bitartrate, 1 mg = 267 μ c) 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 Immediately before the animal was killed by decapitation, blood samples were withdrawn from the femoral artery, heparin being used as an anticoagulant.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 0.1N 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 pH 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 0.2M sodium acetate and, after pH adjustment, was similarly passed over aluminum oxide and processed. Recoveries of added H3adrenaline 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 H³-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 H³-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 H3-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 H³-adrenaline, it was present in large amounts in plasma and in other tissues. This suggests that the failure to find significant quantities of H³-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 H³-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.

SCIENCE, VOL. 129

thalamic or peripheral receptors. Moreover, since adrenaline is present and, presumably, is metabolized in the brain, it must be formed there from precursors which cross the blood-brain barrier (10). HANS WEIL-MALHERBE

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Preliminary Identification of Crystalline Phases in a **Transparent Stalactite**

Abstract. Of two crystalline phases found in a cavern stalactite, the major phase is mirabilite, whereas the minor phase, according to preliminary data, is a new mineral, sodium hemicalcium sulfate dihydrate, which is unstable at temperatures above 25°C.

In 1955 W. T. Austin and J. J. Lehrberger explored and documented photographically a series of new passages in the Flint Ridge Cavern system of southcentral Kentucky. A number of spectacular, glasslike stalactites were found in one of the passages. When specimens collected from these deposits were taken above ground, they effloresced and melted. Hence, to obtain material for the study described in this report, two samples were transported to the laboratory under a cool atmosphere saturated with water vapor.

Both samples consisted chiefly of a transparent material that occurs in short prismatic crystals showing a vitreous luster and a conchoidal fracture. About 1 percent of each sample was made up of a second substance somewhat less transparent and occurring in monoclinic needles.

The major phase was extracted from 1 MAY 1959

the mineral sample with ice water. After the insoluble minor phase had been removed by filtering, the filtrate was found to contain only Na+ and SO4-- ions accompanied by traces of Ca, Si, Mg, and Al; these elements were detected with a spectroscope. The observed melting point of the major phase was 33° to 34°C (sealed capillary). Accordingly, the water-soluble phase present in the stalactite is unquestionably mirabilite $(Na_2SO_4 \cdot 10H_2O)$, which melts at 32.4°C; This finding was confirmed by measurements of the index of refraction and specific gravity. The observed value for the refractive index was 1.40 [literature value, 1.36 (1)], and the observed value for the specific gravity was 1.46 [literature value, 1.48 (1)].

A sample of the minor phase suitable for analysis was obtained by grinding the gross specimen to a fine powder, extracting the soluble major phase with an icecold alcohol-water mixture, and finally collecting the insoluble solid on a weighed, sintered glass filter. The weight of the minor phase indicated that it was originally present in the stalactite to the extent of about 1 percent. The weight loss obtained by heating the insoluble fraction to constant weight at 125°C (in a vacuum) indicated that the minor phase contains between 7.9 and 8.0 percent bound water. The anhydrous salt was found to be somewhat soluble in water and was readily soluble in dilute hydrochloric acid. The acid solution was found to contain only Ca++, Na+, and SO_4^{--} ions along with traces of Al, Mg, and Si (detected by spectroscope).

Two naturally occurring double sulfates of sodium and calcium are recorded in the mineralogical literature: These are glauberite $(Na_2SO_4 \cdot CaSO_4)$ and ciempozeulite $(3Na_2SO_4 \cdot CaSO_4)$, both of which are anhydrous salts. Therefore, a quantitative measurement of the Na : Ca ratio was necessary to ascertain whether the minor phase was a hydrated modification of those double salts or another mineral. A conventional determination of both elements gave a Na: Ca ratio of 3.821, which is not in agreement with the Na: Ca ratio of either glauberite (theoretically Na: Ca = 2.00) or ciempozeulite (theoretically, Na : Ca = 6.00).

Hill and Wills (2) have made a study of the ternary system CaSO₄-Na₂SO₄-H₂O at 25°, 35°, 50°, and 75°C. They obtained a labile sodium hemicalcium sulfate $(2Na_2SO_4 \cdot CaSO_4 \cdot 2H_2O)$ from the action of gypsum on an aqueous solution of sodium sulfate. Upon standing, the solution becomes filled with long, slender needles which are so closely knitted together that as little as 1 percent of the labile salt will hold the mass in suspension, so that no movement occurs when the reaction vessel is inverted. The calculated Na: Ca ratio for Hill and Wills' metastable salt is 4.00, which is in fairly good agreement with the value of 3.821 obtained for the minor phase of the stalactite. The measured refractive index of the minor phase is 1.518, which compares favorably with the value of 1.510 observed by Hill and Wills for their metastable salt.

On the basis of the observed Na: Ca ratio, water of hydration, refractive index, and the weight ratio between the major and minor phases, it is tentatively suggested that the minor phase is identical with the sodium hemicalcium sulfate dihydrate described by Hill and Wills. The somewhat low Na: Ca ratio and the slightly high refractive index can best be explained by the probable presence of a very slight excess of calcium sulfate in the mineral specimen taken for this preliminary study.

The labile salt described is metastable with glauberite within the temperature range of 25° to 75°C (2) and is, therefore, stable at the cavern temperature (12° to 15°C). At temperatures above 25°C, the reaction

$2Na_2SO_4 \cdot CaSO_4 \cdot 2H_2O \rightarrow$

 $CaSO_4 \cdot Na_2SO_4 + Na_2SO_4 + 2H_2O$

would account for the conversion of the minor phase to an equilibrium mixture of glauberite and thenardite (3).

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Occurrence and Morphology of a Phenotypic Male of a **Gynogenetic Fish**

Abstract. A phenotypic male of Mollienesia formosa, a gynogenetic fish, has been collected at Brownsville, Texas. The male and female fish are essentially similar, and their morphology supports a hypothesis that the species is of hybrid origin.

Many workers have demonstrated the occurrence of gynogenesis-activation of unfertilized eggs by sperms with the removal of paternal chromatin during early cleavage of the resulting offspring (1).