

vidual criterion scores for both the operated and control rats involved in the 30-second delay problem. Again, the mean differences did not approach statistical significance. Inspection of Fig. 1 reveals that two operated animals (22 and 51) were virtually decorticated. The scores of these rats were not appreciably different from those made by rats with considerably less damage or by the controls. Thus, no relationship between the size of the lesion and the ability to learn a position habit is indicated.

It is intriguing that the decorticate rats were not inferior to the controls in learning the position habit involving delayed reinforcement. The interpolation of a delay between the response and the reward is known to retard the acquisition of a position habit (3). In this experiment, both the cortical and control rats required more than twice as many trials to reach the criterion with a 30-second delay than they did with no delay. This reduction in learning speed is generally attributable to the decaying memory trace of the response. Apparently the neocortex is not necessary to mediate this function in the rat. The corpus striatum, septal area, hippocampus, pyriform cortex, and amygdaloid nuclei also do not appear to be specifically involved in this function, for these structures were bilaterally damaged in one or more of the operated rats of this experiment. It cannot be concluded, however, that the neocortex and its adjacent structures fail to have any influence upon the formation of the memory trace. Studies concerned with the perseverative trace (4), delayed response (5), retroactive inhibition (6), and multiple-unit mazes (2), indicate that the neocortex of the rat does function to facilitate the estab-

lishment of the memory trace. It would seem, however, that the strength of the memory trace left by a single position response is undiminished by the removal of the cerebral cortex.

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#### References

1. R. B. Bromiley, *J. Comp. and Physiol. Psychol.* 41, 102 (1948); E. Girden *et al.*, *J. Comp. Psychol.* 21, 367 (1936).
2. K. S. Lashley, *Brain Mechanisms and Intelligence* (Univ. of Chicago Press, Chicago, 1929).
3. B. H. Pubols, Jr., *J. Exptl. Psychol.* 56, 32 (1958); J. B. Wolfe, *J. Comp. Psychol.* 17, 1 (1934).
4. R. Thompson, *Can. J. Psychol.* 12, 83 (1958).
5. R. B. Loucks, *J. Comp. Neurol.* 53, 511 (1931); C. T. Morgan and W. M. Wood, *J. Neurophysiol.* 6, 173 (1943).
6. C.-N. Hu, *J. Gen. Psychol.* 18, 267 (1938).

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### Electrophysiology of the Elasmobranch Stomach

*Abstract.* A consequence of the recent study of the mechanism of gastric secretion has been the presumption, implicit (1) if not explicit (2), that the distinctive gastric transmucosal potential has a fundamental role in the formation of hydrochloric acid. The following report indicates that the isolated surviving elasmobranch gastric mucosa does secrete acid but, unlike that of other vertebrates, does so without developing a significant epithelial potential difference. The gastric transmembrane potential is neither necessary for hydrogen ion secretion nor is its generation a fundamental feature of the mechanism that leads to the formation of hydrochloric acid.

The gastric mucosae from dogfish (*Squalus acanthias* L.) caught in Frenchman's Bay and subsequently maintained in a "live-car" were isolated and bathed by oxygenated saline within 10 min after the fish was removed from water. One square centimeter of mucosa separated two chambers containing saline solutions having volumes of 6 ml and the following compositions (in millimoles per liter): (i) Serosal solution; NaCl, 220; NaHCO<sub>3</sub>, 30; KCl, 10; CaCl<sub>2</sub>, 5; Na<sub>2</sub>HPO<sub>4</sub>, 1; MgCl<sub>2</sub>, 2; and glucose, 25. (ii) Mucosal solution; NaCl, 250; KCl, 10; CaCl<sub>2</sub>, 5; MgCl<sub>2</sub>, 2; and glucose, 25. Both solutions were gassed by 95 percent O<sub>2</sub> and 5 percent CO<sub>2</sub>. No urea was incorporated in the bathing solutions, nor was it found to be necessary for the satisfactory maintenance of the isolated epithelium. The transmucosal potential was measured by a pair of agar-saline bridges and calomel cells, combined resistance 0.15 megohms, which fed into a Hewlett-Packard 130B oscilloscope. The experiments were conducted at a mean ambient temperature of 22°C, which was probably supraoptimal.

In ten experiments, the mean rate of hydrogen ion secretion was  $0.65 \pm 0.6$  (standard deviation)  $\mu\text{eq cm}^{-2} \text{hr}^{-1}$ . The mucosae had a wet weight of  $177 \pm 33$  mg. The transmucosal potential difference at 40 and 160 min after isolation was  $-1.3 \pm 1.8$  and  $-0.9 \pm 0.9$  mv, considering the mucosal solution to be ground. The mean initial and final bridge junction potentials were  $-0.4$  mv. When the solution bathing the mucosal surface was replaced by one similar to that bathing the serosal surface, the potential difference became smaller by  $0.5 \pm 0.2$  mv. The d-c conductance was estimated from the potential change that occurred after the passage of current,  $100 \mu\text{a cm}^{-2}$ , for 1 min at 40 and 160 min after isolation. The conductance was  $4.1 \pm 1.8$  and  $5.6 \pm 1.7$  mmho  $\text{cm}^{-2}$ . A single experiment on a skate (*Raja erinacea*) yielded similar results. The mean potential difference developed across the entire thickness of the dogfish stomach was  $+0.5$  mv in two experiments, and the conductance was somewhat lower than that cited above for the mucosa when it was separated from the serosa and muscle coat. In three experiments the mucosa did not spontaneously secrete hydrogen ion, presumably because of the higher ambient temperature (26°C). The electrical characteristics were the same except for a more rapid deterioration of membrane resistance.

The isolated gastric mucosae of marine and fresh-water teleosts (*Pollachius virens*, *Microgadus tomcod*, *Myoxocephalus octodecimspinosus*, *Pseudopleuronectes americanus*, *Anguilla rostrata*, and *Ameiurus nebulosis*) were studied in the same fashion except for a reduction in concentration of NaCl of the bathing solutions by 50 mmole/lit. All of these mucosae spontaneously secreted acid and developed a potential difference of more than  $+15$  mv. This potential difference is similar to that developed by the isolated mucosae of amphibia (1), dog (2), and man (3). Microscopic slides did not disclose any striking histological difference between the elasmobranch and teleost gastric epithelia (4). The dogfish gastric mucosa was found to have a high content of carbonic anhydrase (5).

These results imply that elasmobranchs secrete H<sup>+</sup> without generating a significant transmucosal electrical potential difference. The absence of a potential difference cannot be attributed to a higher membrane conductance. Though the membrane conductance is somewhat larger than that of amphibia (1), so is the wet weight of a comparable area of mucosa. The absence of a potential is not necessarily attributable to excessive H<sup>+</sup> transport. At the prevailing temperature the rate of H<sup>+</sup> secretion was less than that of the isolated amphibian gastric mucosa. At lower temperatures the

Table 1. Individual criterion scores on the 30-second delay problem.

Rat No.	Cortical damage (%)	Trials	Errors
<i>Operated rats</i>			
9	80	12	4
51	99	18	10
50	81	24	15
22	97	24	10
4	51	30	13
8	71	48	24
20	88	54	28
Mean	81.0	30.0	14.9
<i>Controls</i>			
1		6	4
35		18	5
11		18	7
8		24	10
36		36	24
2		42	19
7		42	14
Mean		26.6	11.9

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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#### References and Notes

1. C. A. M. Hogben, *Am. J. Physiol.* 180, 641 (1955).
2. W. S. Rehm and W. H. Dennis, in *Metabolic Aspects of Transport across Cell Membranes*, Q. R. Murphy, Ed. (Univ. of Wisconsin Press, Madison, 1957).
3. N. C. Hightower, *Federation Proc.* 16, 58 (1957).
4. The slides were kindly prepared by W. L. Doyle.
5. The gastric mucosa were analyzed through the courtesy of T. H. Maren.
6. C. A. M. Hogben, in *Electrolytes in Biological Systems*, A. M. Shanes, Ed. (Am. Physiol. Soc., Washington, D.C., 1955); R. P. Durbin and E. Heinz, *J. Gen. Physiol.* 41, 1035 (1958).
7. E. Heinz and R. P. Durbin, *Biochim. et Biophys. Acta* 31, 246 (1959).
8. Anita Blanchard assisted in this work, which was supported by a grant from the American Institute of Biological Sciences.

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### Free $\beta$ -Hydroxy- $\gamma$ -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  $\beta$ -hydroxy- $\gamma$ -aminobutyric acid, we found that free  $\beta$ -hydroxy- $\gamma$ -aminobutyric acid exists in the brains of mice, rabbits, cattle, and human beings.

$\gamma$ -Aminobutyric acid ( $\gamma$ -ABA) has been shown to be converted to  $\beta$ -hydroxy- $\gamma$ -aminobutyric acid ( $\beta$ -OH- $\gamma$ -ABA) by beta oxidation and also to glutamic acid by transamination between  $\beta$ -OH- $\gamma$ -ABA and  $\alpha$ -ketoglutaric acid (1). On the other hand, hydroxyproline may be converted, in

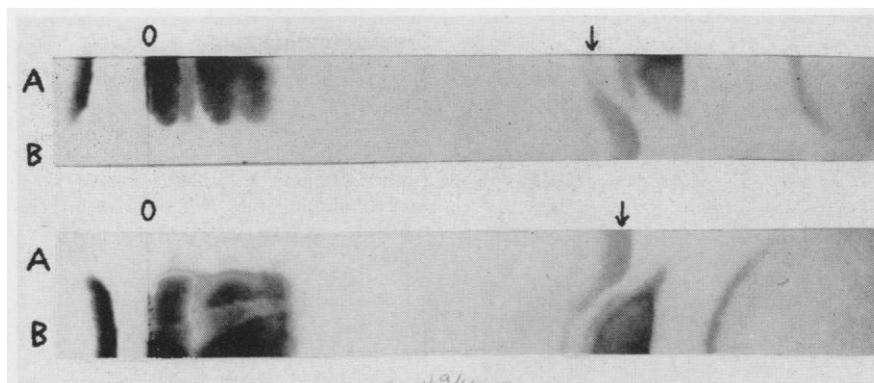


Fig. 1. Comparative electrophoresis of cattle brain (top) and human brain (bottom). O, starting line; A, prepared extract; B, synthesized sample; arrow, the juncture of the area of synthesized sample with the area of prepared extract. Condition of electrophoresis, 100 v/cm, 25 ma, 40 min; electrolyte,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

vitro, to  $\beta$ -OH- $\gamma$ -ABA via  $\Delta^1$ -pyrroline-4-hydroxy-2-carboxylic acid (2). Formation of  $\beta$ -OH- $\gamma$ -ABA through decarboxylation of allo- $\beta$ -hydroxyglutamic acid has also been shown with an enzyme sample obtained from various strains of *Escherichia coli* (3).

$\beta$ -Hydroxy- $\gamma$ -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.

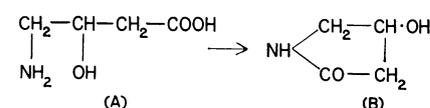
Comparative high-potential paper electrophoresis of the brain extract and of a synthesized sample (6) of  $\beta$ -OH- $\gamma$ -ABA was made. The extract and the synthesized sample were applied in juxtaposition on the starting line of the filter paper and gave the pattern shown in Fig. 1. Juncture, 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 constituent represented by the zone which is in juncture with the zone of the synthesized sample as  $\beta$ -OH- $\gamma$ -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.

The zone thus tentatively identified as  $\beta$ -OH- $\gamma$ -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  $\beta$ -OH- $\gamma$ -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 " $\beta$ -OH- $\gamma$ -ABA" thus obtained, however, was all too small compared with the original " $\beta$ -OH- $\gamma$ -ABA" content of the starting material, which was estimated by colorimetry—for example, 48.6 mg of  $\beta$ -OH- $\gamma$ -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  $\beta$ -OH- $\gamma$ -ABA: *l*- $\beta$ -OH- $\gamma$ -ABA and *d*- $\beta$ -OH- $\gamma$ -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.