mental conditions, show that neither gonadal nor hypophyseal hormones play a primary rôle in the mouse.

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9743

Distribution of Chloride in the Gastric Mucous Membrane of the Dog.

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An improved histochemical method for the demonstration of chloride was employed in a study of the dog's gastric mucous membrane under different physiological conditions. It was hoped that with this sensitive method it would be possible to throw some light on the site and mode of formation of hydrochloric acid in the gastric juice. A recent reconsideration of the problem by Hoerr[1] confirmed the theory developed by Harvey and Bensley[2] 25 years ago. These workers believe that hydrochloric is formed in the outer portion of the lumen or in the foveolus of the gastric glands by the hydrolysis of some organic chloride formed, stored and secreted by the parietal cells. In support of this theory chloride in large amounts has been demonstrated in these cells by methods which, however, have been considered by some investigators to be of questionable value.[3] Opposed to this theory are the findings of Lopez-Suarez[4] and Lison[5] who claim that the parietal cells contain less chloride than the other types of cells in the fundic glands. These opposing views emphasize the need for a reinvestigation, with more reliable methods, of the distribution of chloride in the gastric mucous membrane.

The method used in this investigation for the histochemical identification of chloride[6] is believed to visualize this ion accurately

1 Hoerr, N. L., *Anat. Rec.*, 1936, **65**, 417.

2 Harvey, B. C. H., and Bensley, R. R., *Biol. Bull.*, 1912, **23**, 225.

3 Lison, L., *Histochemie Animale*, Paris, Ganthies-Villers, 1936.

4 Lopez-Suarez, J., *Biochem. Z.*, 1912, **47**, 490.

5 Lison, L., *Z. f. Zellforsch u. mikr. Anat.*, 1936, **25**, 143.

6 Gersh, I., *Anat. Rec.*, 1938, in press.

very nearly in the position which it occupies during life. Small pieces of gastric mucous membrane of dogs are carefully freed of the external coats of smooth muscle, and frozen in liquid nitrogen. They are dried in a vacuum chamber maintained at —60°C. or at —62°C. After warming to room temperature to avoid the condensation of water vapor, they are embedded in paraffin (m.p. 52°C.). Sections of this material are passed through petroleum ether, and are treated with a drop of acidified concentrated silver nitrate. The reagent is replaced with a drop of glycerine, and the section is reversed on a slide section-side down. The slide is exposed to carbon-arc radiation for a short time and observed directly thereafter. Depending on the amount present, chloride appears as a yellow to brown or black color which is resolved on greater magnifications into a lesser or greater number of particles of reduced silver. Properly carried out, this method is sensitive, specific and almost free from postmortem diffusion errors.

Sections were prepared in this way of gastric mucous membrane at "rest" and during activity. The "resting" phase was studied in the mucosa of dogs deprived of food for one day. The active phase was analyzed in the glands of dogs 40 minutes after feeding a meat diet, after the subcutaneous injection of histamine, or after both feeding and the administration of the drug. The animals were killed by bleeding under ether anesthesia.

The distribution of chloride in the resting gland was found to be very characteristic. Chloride was absent from the cytoplasm of all gland cells. Traces of this ion were identified, however, in the secretion granules of the zymogenic cells. Chloride was present in the lumen of the gland along the free border of the cells, it was present in greater amounts in the foveolus and on the surface epithelium. Chloride was evenly distributed throughout the connective tissue spaces of the lamina propria mucosa and the tela submucosa.

The distribution of chloride in the actively secreting mucous membrane did not differ essentially from that in the resting tissue. In the active glands chloride was demonstrable in the same sites which it occupied in the resting glands, the only difference being that it was present in greater amounts. More chloride was visible in the cytoplasmic inclusions of the zymogenic cells, on the free surface of the epithelial cells, and in the tissue fluid spaces of the connective tissue layers. The cytoplasm of all the glandular cells remained free of chloride.

The failure to visualize chloride in the cytoplasm of the glandular cells leads one to assign these cells to the same category as skeletal

muscle fibers which are also believed to be free of this ion. The presence of chloride in the secretion granules of the chief cells, if it is not caused by some unknown source of error, is unexplainable at the present time. The increase in the amount of chloride in the lumen and on the surface of the active mucous membranes may be attributed to increased amounts of hydrochloric acid secreted during digestion. In the same way, the prominent increase in the tissue fluid space may be correlated with greater amounts of chloride-containing tissue fluid and lymph that are formed during glandular activity.

It must be pointed out that there appears to be a very definite conflict betwen the conclusions derived from neutral red studies on the gastric mucosa and the more recent studies on the distribution of chloride in the epithelium. This conflict may be resolved, at least to some extent, by the possibility that the method employed cannot visualize a relatively non-reactive, not easily hydrolyzable protein chloride. Were this the case, it would be difficult to understand how the protein chloride could be hydrolyzed in the lumen or foveolus of the gastric glands by simple dilution in a manner advocated by Bensley, Harvey and Hoerr. A second possible way of removing this difficulty is that the protein chloride is extruded from the parietal cell almost as rapidly as it is formed, with no apparent storage of the compound in the cytoplasm. In this case, then, the resting and the active secretion phases differ only in their rate of the formation and extrusion of this protein chloride, all other processes remaining unaltered. However, it must be noted that if the distribution of chloride is really in fact that described in this paper, it does not implicate the parietal cell in the formation of hydrochloric acid any more than it involves any other cell type. By the same token, it does not exclude the parietal cell as the one whose specific activity results in the secretion of hydrochloric acid or its precursor.