THE CHANGES IN THE PROPERTIES OF INTESTINAL JUICE ACCORDING TO THE METHOD OF PREPARATION

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A great deal of work has been published concerning gastric secretion induced by stimulation of the intestinal mucosa.

The stimuli have included balls of cotton wool, peas, wool [8], gauze tampons [16], and rubber balloons [22], but most frequently rubber tubes either with or without "windows" have been employed [1, 2, 6, 10, 11, 12, 13, 14, 17, 19, 20, 24, 26].

Many workers have used chemical stimuli, either alone, or combined with mechanical stimuli; the stimuli have included butyric acid [24], hydrochloric acid, glucose, and water [4, 5], but the commonest is calomel (Hg_2Cl_2) [24, 21, 9, 15, 27]. Calomel may be introduced into an isolated gastric pouch, either as a suspension in physiological saline or in gastric juice, or as a dry powder.

If calomel is introduced at the same time as mechanical stimulation is applied, drainage from the intestinal loop is discontinued during this time, and then resumed. Sometimes calomel is used as the only factor stimulating the secretion of juice, and there is then produced a 'pure' calomel secretion [15, 19].

In connection with modern concepts on the structure of the intestinal mucose membrane [7, 18] and evidence of its high sensitivity to stimuli [4, 5], the method of obtaining the juice has become important in the study of digestion and of the secretion of intestinal juice.

Here we have set ourselves the problem of following the effect of some of the more commonly employed stimuli on the structure of an isolated loop of intestine and on the composition of the juice secreted.

EXPERIMENTAL METHOD

The work was carried out on four dogs, in each of which two isolated loops made from a single length of intestine were established. In two of them, the juice secreted over a period was collected from fistuli of the anterior portions, while from the posterior portions of the isolated loops the juice secreted in response to mechanical stimulation was collected. As stimuli we used drainage with a rubber tube having an external diameter of 0.4 cm. The tube had an aperture with smoothed edges, and a rounded end. The tube entered freely into the lumen of the intestine, and remained there during the whole of the time that juice was collected. In the two other dogs, the juice secreted over a period of time was collected from fistulae in the posterior portions, the fistulae in the anterior part collected the juice secreted in response to a single injection into the lumen of a suspension of 0.3 g calomel in 20 ml of physiological saline. The calomel was first triturated in a mortar for 1-2 minutes while the required amount of physiological saline was slowly added. The suspension prepared in this way was introduced into the intestine by means of a 20-ml syringe connected through a tube of external diameter 0.3 cm, having apertures in it. The calomel was not washed out with physiological saline. In all the dogs, the intestinal juice was collected over a period of 5 hours.

The juice obtained in the different ways was estimated for enterokinase and phosphatase by methods worked out in the Laboratory of the Physiology of Digestion of the Institute of Nutrition, AMN SSSR; the pH of the juice was determined potentiometrically; and the reaction on blood was tested with pyramidone. In addition, the physical composition of the juice was determined. Without centrifuging, it was fixed in formalin, embedded in celloidin and gelatin, and stained with hematoxylin and thionine. To test for peroxidase with benzidine and for oxidase, Gold-

man's method was applied to frozen portions of juice embedded in gelatin. The dogs were killed at the end of the experiment. The isolated portions of the intestine were used for a histological study, as were also portions of the intestine adjacent to the loops. Portions of the intestine were fixed in formalin and stained in hematoxylin eosin, and in thionine. The frozen sections were tested for peroxidase and oxidase.

EXPERIMENTAL RESULTS

We found that samples of intestinal juice collected in the different ways differed both biochemically and morphologically. For example, with regard to pH, we found that although there is an individual variation, the pH of juice obtained by introducing irritants into the lumen (drainage, or calomel) was considerably displaced towards the alkaline side, as compared with the juice naturally secreted.

We also found that juice obtained in response to stimulating the mucosa was usually colored by blood, and that erythrocytes were present in it. These erythrocytes were seen in sections of portions of juice. In the biochemical test on the blood with pyramidone, after as little as 20-30 seconds quite a deep violet color developed. Juice secreted naturally gave a pale lilac color in the reaction with pyramidone, which did not appear immediately, but after 2-3 minutes or more, and was evidently due to the presence in the juice of peroxidase.

As our investigations have shown, there is a comparatively small amount of peroxidase in the intestinal juice of dogs collected by the methods described. The enzyme appears in the cytoplasm of the cells as either loosely scattered or closely packed granules . The number of cells containing the enzyme varies from one sample to another. In one dog, in juice obtained by drainage of an isolated loop we observed a regular increase in the number of cells rich in this enzyme. However, in another dog, no such tendency was found.

As regards the quantitative relationship of the fluid and the solid portions, it should be noted that the total amount of juice, of its fluid and solid constituents, increases when the mucosa of an isolated loop is stimulated. There is a particularly large increase in the secretion of the fluid of the juice in response to mechanical stimulation. The amount of the fluid also increases when the mucosa is stimulated with calomel, but the increase is not so great. When the mucosa is stimulated by drainage or by a calomel suspension, the secretion of the solid constitutents of the juice increases $1\frac{1}{2}$ to 2 times as compared with the natural secretion taken over the same 5 hour period.

The enzymes obtained in response to stimulation with a probe or with a calomel suspension were altered, differing in both cases from the naturally secreted juice. Thus, after mechanical stimulation, in 1 g of homogenate, the phosphatase activity was reduced, and the limits of variation fell from 6,700, 9,000-30,000, and 45,000, which are the values for the natural secretion, to 1,350, 1,800-6,000, and 9,000 in the juice obtained by drainage. The same was true for enterokinase, where the values fell from 200-2,000 to 33-268 units, and in another animal from 3,000-9,000 to 400-1,000 units. However, when referred to 1 g of the solid component, the activity of the enzyme in both dogs was noticeably increased, both for enterokinase, and for phosphatase, when compared with the activity of the same enzymes in the juice collected periodically.

On stimulating the mucosa of the isolated loops with a calomel suspension, we have not been able to observe any regular changes in phosphatase activity; sometimes there was a small increase, and in others it was reduced.

It was quite otherwise with enterokinase, and we demonstrated a regular reduction in the activity of this enzyme. It was shown particularly well in one dog in whom the activity of the enzyme per g of homogenate was reduced

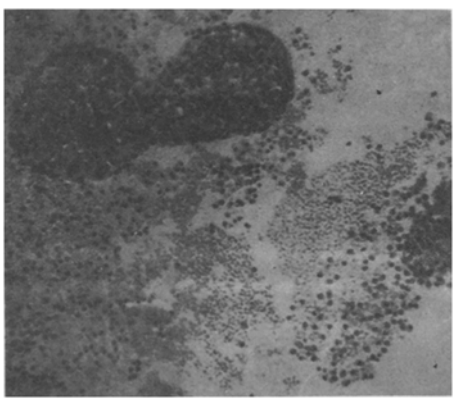

Fig. 1. Juice obtained in response to mechanical stimulation of the mucosa of isolated canine intestinal loops. In the dense part villi and erythrocytes can be seen. Stain hematoxylin-eosin. Magnification: ocular 10×, objective 20×.

2-10 times; when referred to 1 g of the solid part, the reduction was 2-5 times, as compared with the activity of enzyme in the juice collected periodically from the same dogs.

By examining the physical structure of the juice, we found that in all portions obtained by stimulation of the mucosa by drainage, longitudinal and cross sections of whole villi were encountered (Fig. 1). Their number in different portions of juice varied from 2 to 50, but most frequently there were about 8-10 of them in a section through a clump of the solid component. In the villi, it is easy to distinguish the stroma, and the structure of its constituent cells. The villi have a structure which depends upon the stage of the secretion of the epithelium. In some, the epithelium is continuous and closely covers the stroma, and in other villi the different stages of its engorgement and protrusion may be followed. In villi 'torn off' from the mucous membrane and lying in the mass of the solid portion of the juice, the cavities of lymphatic vessels may be clearly distinguished, together with the lumens of the blood vessels and their contained erythrocytes. Besides the villi, in different parts of the juice there were a large number of unbroken tubules, and fragments of the epithelial layers. In many samples of the juice obtained in response to stimulation of the mucosa by drainage, a large number of a few erythrocytes could be seen (see Fig. 1).

The presence of erythrocytes and even of whole villi in the juice secreted in response to mechanical stimulation is evidently related to the introduction of a drainage tube into the loop, and to its action on the mucosa for a comparatively long time. For this reason we are inclined to consider that the mechanical action of the tube on the mucosa cannot be considered to correspond to the mechanical action of the chyme. For this reason, drainage must be considered to be an unphysiological stimulus.

Calomel has rather a different action on the physical structure of the juice. In juice obtained by stimulating the mucosa with calomel, among the clumps and granules of the substrate formed by breakdown of the cytoplasmic

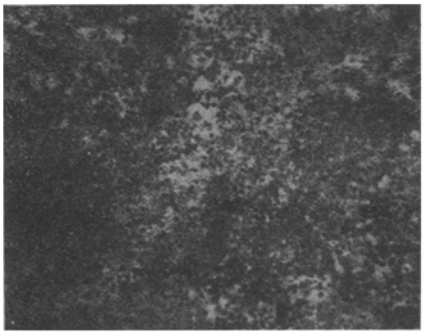

Fig. 2. Canine juice obtained by stimulating the mucosa of an isolated intestinal loop with calomel. There is quite a dense mass of nuclei derived from the disintegrated cells. Stain hematoxylin-eosin. Magnification: ocular 10x, objective 20x.

Fig. 3. Clump of calomel in the solid part of the juice. Stain hematoxylin-eosin. Magnification: ocular 10x, objective 20x.

portions of the cells, there are large number of nuclei, quite a dense mass (Fig. 2). In many of the nuclei, the structure is well preserved, and the distribution of the chromatin clearly visible. It is known that nuclei of a normal structure are always present in juice secreted and collected periodically, but here there are far more. Further, in the juice secreted in response to calomel stimulation, there are a large number of nuclei of lymphoid cells, and as a rule they are intensely stained, and stand out clearly against the mass of juice. Erythrocytes are present in the juice itself, and in places there are also whole villi with a well-preserved stroma and its constituent cells. However, the number of villi in the juice obtained in response to calomel stimulation is not great; no more than 1 or 2 are present, and they are found only in certain samples; when the tube used to introduce the calomel remained in the isolated loop for 1-1½ minutes their presence appeared to be related to its mechanical action for 1-1½ minutes on the mucosa.

Also, in the morphological studies, in the solid part of the juice, calomel could sometimes be found in the form of separate crystals, or in an aggregate of such crystals (Fig. 3). As a rule, the calomel crystals were surrounded by the solid constituents of the juice as though enshrouded in it. On this account, the separate calomel crystals or the small aggregates of crystals could not be separated from the juice mechanically, so that they formed part of the suspension of the solid portion of the juice.

Certain changes occurred also in the isolated loops stimulated with calomel. In the apical portions of the villi, the stroma was extensively infiltrated with lymphoid cells. The breaking away of the epithelium liberated the lymphoid cells into the lumen of the intestine, where they contributed to the solid component of the juice. Possibly the increase in the number of lymphoid cells in the juice brings about an enzymatic change whereby the enterokinase activity is reduced. However, the same change may be brought about by other factors.

In the mucosa, stimulated by calomel, in the stroma, the epithelium of the crypts was destroyed, the blood vessels were dilated, and there were hemorrhages.

There were no polynuclear forms in the mucosa of the isolated segments. They are however present in many samples of gastric juice, in different amounts according to the way it is collected, and apparently they enter the receiving vessel from the outer edges of the fistular aperture.

From the results of our own work on the qualitative changes of the intestinal juice, we have concluded that drainage and calomel, as used by us, represent unphysiological stimuli.

On the other hand, when collecting juice periodically, it must be remembered that the breaking away of the cells into the lumen of the intestine of an isolated segment, and the periodic liberation of the secretion from the fistula are not the same process. Apparently, during the periodic collection of the juice from the fistula, the epithelial cells have broken away sometime previously, and when they are collected they are at various stages of disintegration, as is well shown by studying the structure of the juice in preparations treated to reveal its structure.

SUMMARY

Mucosa of the isolated section of the small intestine was stimulated with a drain and calomel mixture. the pH index was found to change towards alkalinization with a marked positive blood pyramidone reaction. Intestinal juice obtained in response to the drain stimulation of mucosa contains detached villi and erythrocytes. An increased number of lymphoid cells and a reduction of enterokinase activity per gm of the solid portion was noted in the juice obtained in response to calomel stimulation. Therefore, the drain and calomel cannot be regarded as physiological stimulants in obtaining intestinal juice.

LITERATURE CITED

1. S. V. Andreev and S. I. Georgievskii, Zhurn. Éksper. Biol. i Med. Vol. 10, No. 25 (1928), p. 169.
2. S. V. Andreev and S. I. Georgievskii, Fiziol. Zhurn. SSSR, Vol. 17, No. 4 (1934), p. 810.
3. V. N. Boldyrev, The Periodic Work of the Digestive Apparatus when the Stomach is Empty. Dissertation [in Russian] (St. Petersburg, 1904).
4. N. E. Vasilevskaya, Bulletin of Leningrad University [in Russian] No. 6 (1948), p. 119.
5. N. E. Vasilevskaya, Byull.Éksper. Biol. i Med. No. 4 (1950), p. 266.
6. R. I. Gavrilov, Results on the Physiology and Pathology of the Excretory Function of the Small Intestine. Dissertation for Doctorate [in Russian] (Perm', 1941).
7. Z. N. Gadzhieva, Byull. Éksper. Biol. i Med. No. 11 (1957), p. 100.
8. D. L. Glinskii, The Physiology of the Intestine. Dissertation [in Russian] (St. Petersburg, 1891).
9. M. M. Gorvunova-Nikolaeva, Fiziol. Zhurn. SSSR, Vol. 18, No. 5 (1935), p. 824.
10. O. P. Dobromyslova, Byull. Éksper. Biol. i Med. No. 1 (1952), p. 3.
11. O. P. Dobromyslova, Byull. Éksper. Biol. i Med. No. 7 (1952), p. 8.
12. G. Kh. Leper, The Experimental Pathology of Intestinal Secretion. Dissertation [in Russian] (St. Petersburg, 1904).
13. A. N. Krestovnikov, Reports of the Lesgaft Petrograd Scientific Institute [in Russian] Vol. 3 (1921), p. 247.
14. L. G. Merkulov, Fiziol. Zhurn. SSSR, Vol. 19, No. 4 (1935), 871.
15. L. G. Merkulov, Fiziol. Zhurn. SSSR, Vol. 20, No. 1 (1936), pp. 116, 127.
16. L. A. Orbeli and V. V. Savich, Arkh. Biol. Nauk. Vol. 20, Nos. 1-2 (1916), p. 76.
17. N. F. Popov, E. I. Shmakova, and V. I. Kuznetsova, Fiziol. Zhurn. SSSR, Vol. 17, No. 1 (1934), p. 63.
18. M. I. Razumov, Vopr. Pitaniya, No. 4 (1952), p. 18.
19. V. V. Savich, The Liberation of Intestinal Juice. Dissertation [in Russian] (St. Petersburg, 1904).
20. V. V. Savich, Reports of the Lesgaft Petrograd Scientific Institute [in Russian] (1921), Vol. 3, p. 241.
21. V. V. Savich, Collection of Scientific Works to Celebrate the 50th Anniversary of A. A. Nechaev, Petrograd, Vol. 1 (1922), p. 15.
22. I. S. Samoilenko, Fiziol. Zhurn. SSSR, No. 2 (1950), p. 147.

23. L. S. Fomina, The Role of the Solid Portion of Intestinal Secretion in Liberating Enzymes of the Mucous Lining of the Intestine. Candidates Dissertation [in Russian] (Moscow, 1949).
24. N. P. Shepoval'nikov, The Physiology of the Digestive Juice. Dissertation [in Russian] (St. Petersubrg, 1899).
25. G. K. Shlygin, The Formation of Enterokinase in the Intestine. Dissertation for Doctorate [in Russian] (Moscow, 1948).
26. P. M. Khazen, Fiziol. Zhurn. SSSR, Vol. 28, No. 4 (1940), p. 345.
27. E. V. Cherkasova, Fiziol. Zhurn. SSSR, Vol. 29, No. 6 (1940), p. 566.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
