RESEARCHES ON THE GASTRIC GLANDS.

I. THE GASTRIC MUCOSA OF THE CAT.

II. THE GASTRIC MUCOID CELLS OF FOETAL AND NEWBORN ANIMALS.

III. THE GASTRIC MUCOID CELLS IN MAN, DOG, RABBIT AND FROG.

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VII. GENERAL CONCLUSIONS REGARDING THE FUNCTIONS OF THE GASTRIC MUCOID CELLS.

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I. THE GASTRIC MUCOSA OF THE CAT.

Introduction.

The gastric mucous membrane has long been described as being composed of three regions, known as the cardia, fundus and pylorus. These regions, though individually distinct, merge so insiduously, the one into the other, that there is no well defined line of demarcation between them. The actual extent of each region varies in different animals - and to each is attributed a separate function. It has not been sufficiently recognised, however, that the cardiac and pyloric areas are very small, especially in the carnivora. For instance, in the cat, the microscopic pylorus is a narrow zone, extending for not more than 35 mm. from the pyloro-duodenal junction; it does not always correspond to the muscular pyloric antrum. In view of this fact, doubt is thrown on the exactness with which "pyloric pouches" have been isolated either by the Heidenhain (13) or Pavloff (21) technique.

Further, what is known regarding the function of the different regions of the stomach is not compatible with their differences in structure. And lastly, current detailed (textbook) description of the cells forming the gastric glands is by no means uniform. Much of the confusion has been due to the fact that the histological descriptions may vary considerably according to the method of fixation and staining employed; usually no qualification is made.

An attempt has therefore been made to re-investigate the histology of the gastric glands. The gastric mucosa of the cat will only be described in this paper as the observations made upon its stomach are the most complete, but other animals have also been investigated and are described later.

The cats were killed while fasting and also at various intervals after a meal/

meal. They were fed on boiled fish, milk and bread, but some were put on a meat and milk diet. In all, over twenty-five animals have been examined.

Histological Technique.

For microscopical purposes, the animals were killed either by carbon monoxide or chloroform. The stomach was then immediately examined or prepared for sections.

In the former case, a piece of mucous membrane was either scraped off and teased in Ringer or serum, or the fresh tissue was frozen at once in a little serum and sectioned on a microtome. The fresh sections, however, gave no more information than those obtained after fixation and were discontinued. In the latter case, the fixatives used were Zenker, Altmann's fluid, osmic acid 1% and formol (either neutral 20% or acid 10%). When Zenker or formol was employed, the stomach was slightly distended with the fixative and suspended in the same solution for twenty-four hours. It was then cut up into suitable pieces, placed in gum or carried through in the usual way into paraffin. On some occasions, pieces of stomach were first pinned out on a leaded cork and the whole immersed in the fixing reagent; this was chiefly the method employed when using osmic acid solutions, although a few pieces of mucosa were fixed in osmic without stretching. None of the above fixatives may be said to be ideal for use with the gastric mucous membrane. Zenker and formol both gave somewhat similar results, while the osmo-bichromate mixture is better than pure osmic acid. Osmic preparations give the best structural details, although they are the most difficult to stain.

The stains employed were alcoholic eosin and methylene blue (16), haematoxylin and eosin, van Gieson, iron haematoxylin (Heidenhain), Mallory (24) or polychrome methylene blue (2 minutes). Osmic acid was used in the manner mentioned/

mentioned above. It has not been thought necessary to give the details of the application of the above stains, as they may be found in the references indicated. In the description which follows, formol fixation is implied, although the observations recorded have been corroborated by the other methods of fixation. (This course has been adopted so as render the account of more practical value, especially as formol is so universally used.) Where a notable difference occurs, the special fixative concerned will be mentioned.

A definition of the terms used will obviate confusion. The terms cardia, fundus and pylorus are applied to the corresponding microscopic regions. The actual cardiac and pyloric orifices of the stomach are defined as the cardio-oesophageal and the pyloro-duodenal junctions respectively. For descriptive purposes a gastric gland tube is divided into a superficial half, which is the portion of the gland tube below the junction of the duct and the gland proper, i.e., the neck, and a deep half composed of the remaining portion of the gland.

The Mucous Membrane as a Whole.

It is not intended to describe the naked eye appearances; suffice to say that with a lens [Sprott Boyd (28)] differences may be noted between the duct orifices of the pylorus and of the remainder of the stomach. In the former region, the mucous membrane is thicker and the ducts wider, longer and more funnel-shaped than in the latter. The microscopical regions of the stomach will be dealt with afterwards.

The gastric glands consist of simple tubes, which branch slightly towards their blind ends. They run almost vertically from the surface, and several gland tubes are usually served by a common duct. Only in the duodenal half of the pyloric canal do the glands become markedly racemose, and this is to

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a much less extent, true of the glands adjacent to the oesophagus.

Between the glands, lies the supporting connective tissue (interglandular tissue) which contains plain muscle fibres arranged vertically, blood vessels, lymphatics and nerves. In addition to these, there are three kinds of cells which may be said to infiltrate the gastric mucosa.

(1) Finely granular branched connective tissue cells, which stain a deep magenta with polychrome methylene blue and a purplish blue with alcoholic eosin and methylene blue. They are by far the most numerous variety and occur principally in this portion of the digestive tract. This has also been noted by Cade (5).

(2) Finely granular oxyphil leucocytes; these are sometimes massed together in small areas: more usually they are scattered throughout the mucosa.

(3) Coarsely granular or globular eosinophil cells, (fig. 5, g) which are present in least numbers. They occur mainly near the surface, and may be found between the cells lining the duct of the gland or in the interglandular tissue. The eosinophil globules vary considerably, both in number and size, some being 2-3μ in diameter. They stain with iron haematoxylin while the oxyphil granules of leucocytes do not; they are thus not unlike the cells of Paneth.

All three types may be found in the interglandular tissue of other animals, e.g., dog, pig, and rabbit.

The interglandular tissue is more abundant at the cardiac and pyloric ends of the stomach, than in the middle of the fundus region. Here it is more plentiful immediately under the surface epithelium.

The mucosa rests on a thick condensation [membrane of Zeissl, stratum compactum of Oppel (fig. 4, a, sc)] of white fibrous tissue, immediately underneath which lies the muscularis mucosae. This membrane-like condensation/

condensation is of interest as it is not common to all animals, e.g., it is absent in the human, pig, and rabbit, but is present in the cat and rat. Further, it is non-elastic and separates the muscle fibres within the interglandular tissue from the muscularis mucosae. It is perforated by vessels, and the plain muscle fibres reach the mucosa mainly by the same communications.

With regard to the other coats of the stomach, there are no comments to offer. In the course of the description, due attention has been paid to the state of distension of the stomach.

The Surface Epithelium.

This epithelium includes the cells covering the surface and those lining ducts, as these cells are essentially of one type. Those on the surface are columnar, becoming shorter and more cubical as they are traced into the ducts. A corresponding change may also be noted in the nucleus, which is oval or rod-shaped on the surface, but almost rounded within the ducts (see fig. 5).

The cytoplasm is finely granular in the fresh and certain fixed (neutral formol, osmic) specimens and may be differentiated into two parts [Ellenberger and Scheunert (11)] by staining methods. An outer goblet-shaped area, which is clear but tinted red in haematoxylin and eosin preparations, stained blue by Mallory and a pale blue by polychrome methylene blue. An inner area consisting of the remainder of the cell, in which the nucleus is situated, and which is distinctly stained a reddish colour by Mallory. Surface cells show a larger goblet area than duct cells (see fig. 3 and 5).

During active digestion the goblet area diminishes in size, but in both fasting and feeding animals, cells in which this goblet area is defined but not stained, may be seen. This presumably indicates that the cells in question have discharged their contents and have not had time to supply the area with new material (granules).

With regard to the mode of attachment of the cells to one another, I have sometimes observed inter-cellular bridges. These, however, are only apparent when the cells themselves appear unduly vacuolated and show vagaries of staining. No bridges are to be seen in teased preparations and in tangential sections of the surface, there are no indications of them.

The surface epithelium is continous with the epithelium of the gland tubes, the transitional cells losing their goblet areas and staining a uniform bluish colour with Mallory. The transition, however, is short (see fig. 5, t).

The Cardia.

The junction of the oesophagus and stomach is well defined in the cat, the stratified epithelium of the former stopping abruptly, and giving rise to the columnar epithelium of the cardia. At this junction, a solitary lymph follicle may sometimes be seen and more frequently a large vesicle or cavity lined by one or two layers of cubical cells.

The cardia (when present) is an extremely narrow zone, measuring about 2-3 mm. from the cardio-oesophageal junction to the nearest group of parietal or oxyntic cells. It includes only cells of one type, unmixed with others. Beyond this, there is a boundary zone extending for another 3 mm., which consists of both oxyntic and cardiac cells. Frequently, there is no definable cardiac area; oxyntic cells are then found at the junction itself and only the "boundary zone" is present. Further than this, another type of cell is met with; this may be regarded as the cardiac limit of the fundus.

The glands of the cardia consist of relatively simple tubes, with short ducts and somewhat wide lumina. In most animals they are fairly numerous, in others only a few glands are to be found near the oesophagus. They are lined by a single layer of columnar or cubical epithelium, which appears granular in the fresh condition. In sections, however, granules are absent/

absent and a fine reticulum or precipitate is seen in its place. The reticulum is irregularly distributed throughout the cell and is stained blue by alcoholic eosin and methylene blue, pale magenta by polychrome mehtylene blue, and blue with Mallory (fig. 1). (In some cases a reticulum which stains reddish with Mallory is present in addition to the above finer reticulum which stains blue.) Haematoxylin hardly stains the "blue" reticulum at all, nor does it tint the spaces between the reticulum. In the case of the other stains just mentioned, the spaces are coloured in the same way as the reticulum - only more faintly.

The nucleus is irregularly rounded or ovoid and is invariably found towards the base of the cell. In the fasting animal, the cell is more columnar and the nucleus less flattened than in the animal which has been fed. On the whole, however, there is little change to be noted.

It will be noted that no compound tubular glands as have been noted by Ellenberger (10), Edelman (8), Schaffer (27) and others in various animals, are present in the cat, nor have any structures resembling crypts of Lieberkühn been met with; this also applies to other regions of the cat's stomach. The simple tubular glands of the cardia were first described by Schafer and Williams (26) in the Kangeroo, and with this arrangement the cardia of the cat agrees. It will be shown later that the cardiac cells do not constitute a special type, but form a variety of mucoid cells, a term which is explained elsewhere.

The Pylorus.

The pylorus is considerably larger than the cardia in area, though it is smaller than is generally supposed. It extends for about 15 mm. from the pyloro-duodenal junction along the greater curvature and about 12-15 mm. along the/

the lesser curvature. Beyond these limits small oxyntic cells make their appearance (pyloro-fundus) and about 20 mm. further up, full sized oxyntic and peptic cells are met with in large numbers. Here lies the pyloric limit of the fundus region.

With regard to the general features of the pyloric glands we may recall that they have long and wide ducts, and that they become more racemose and exhibit more interglandular tissue towards the intestine. Lymph follicles are most numerous in this region of the stomach, several being invariably present between the pylorus and the duodenum. At this junction, the pyloric glands may be observed to pass through the muscularis mucosae to become Brunner's glands of the duodenum. The lumen of the glands is frequently large, and this along with their more racemose character, serves to distinguish the pyloric glands from those of the cardia, which they otherwise resemble.

The glands of the pylorus are lined by a single layer of cells, which are columnar or cubical in shape and irregularly reticulated (granular) in sections (fig. 6). They are stained in the same way as the cardiac cell, the whole cytoplasm appearing blue with methylene blue combinations and with Mallory, pale magenta with polychrome methylene blue and colourless with haematoxylin. As was the case with the cardiac cells, the basal portion of the cell may in some animals be occupied by a second reticulum which stains red with Mallory. This may be seen in both fasting and fed animals, although more often in the latter condition. The nuclei are irregularly rounded and situated basally. During activty, the cells becomes shorter indicating a discharge of their granules and the nucleus appears more spherical, i.e., less compressed.

The similarity between the cardia and pylorus has been noted by many observers/

observers [Cobelli (6), Ebstein (7), Schaffer, Stohr (29) and others]. Bensley (2,3),however, compares the pyloric cells with the cells lining the "neck" of the fundus gland as well as with the cardiac cells. On the other hand, Heidenhain (13), Langley and Sewall (15), Kranenberg (23) and all later writers believe that they are fundamently the same as the "chief" cells of the fundus. It will be shown later, that there can be no doubt regarding their difference from one type of "chief" cells, and that their resemblance to the cardiac gland cell is too close not to regard them as identical in structure, if not in function as well.

The Fundus or Corpus.

Histologically, the portion of the stomach between the cardiac and pyloric regions just described, has a uniform structure. The glands of this intermediate area are generally known as the glands of the fundus, though they might be more appropriately termed the glands of the body. The general form and arrangement of the fundus glands have already been noted. They are more or less simple tubes, with short ducts and as the glands are closely packed together, there is little interglandular tissue.

Three kinds of cells occur in glands of this region though hitherto, with the exception of Bensley (1) and Cade (5), histologists have recognised only two, namely "central" or "chief" and parietal cells.

(1) Coarsely reticulated (granular) or peptic cells, which are more usually known as the "chief" cells; but which are quite distinct from the second type of central cell, with which they are mingled. Peptic cells occur throughout the lower or deep half of the gland tube, though it is comparatively uncommon to find this part of the tube lined wholly by such cells. They are somewhat columnar in shape in section, but when isolated are polyhedral.

The/

The cytoplasm contains granules in the fresh state (a fact already noted first by Langley and Sewall) which are irregular in size. On examination in saline, weak acids or alcohol, they tend to increase in size and to become less distinct. Finally they disappear apparently by passing into solution. A few of the granules always remain unaffected. Fresh preparations may be stained by a dilute solution of methylene blue (1/1000), after these alterations in the cytoplasm have begun.

In fixed preparations, whether formol, Zenker or osmic, the granules are replaced by a coarse but regular reticulum (fig. 2 and 3, p, XXI). Nevertheless with both formol and osmic, a few granules may be preserved, especially after osmic fixation (fig. 3, p, XX, III). The regularity of the reticulum suggests that the extra-granular cytoplasm had been coagulated in situ, while the granules themselves had been dissolved out. The reticulum may be therefore be taken as a rough index of the amount and size of the granules contained in the cell.

With regard to their reactions to various dyes, both the reticulum and the granules become intensely stained blue with alcoholic eosin and methylene blue, deep purplish blue with polychrome methylene blue and violet to orange with Mallory. They are only lightly stained by haematoxylin, but more strongly so by the iron (Heidenhain) method. The nucleus is irregularly ovoid or rounded, and varies in shape and position according to the activity of the cell.

Functional changes are easily noted in these cells. In the fasting condition, the nucleus is found towards the base of the cell and the cytoplasm is reticulated throughout. After a period of activity, i.e., during digestion, the cell gradually shrinks, and the nucleus becomes larger and occupies a more central position. Teased preparations seem to show that the granules are on the whole larger, while in fixed specimens the meshes of the reticulum are wider. Ergastoplasmic fibres appear at the base of the cell, while the reticulations/

reticulations (granules) diminish towards the lumen. In well-marked cases (5-6 hours after a large meal) half the cell may be occupied by fibres. These fibres stain in the same way (although more definitely) as the reticulum (fig. 3, p, III and fig. 5, p). Langley was the first to demonstrate the diminution of granules during activity and he also stated that the cells become clearer at their bases. Later Bensley, Zimmermann (30) and Theohari (23) showed that the basal clear zone was occupied by ergastoplasmic fibres [prozymogen of Macallum (19)]. These observations are fully confirmed in the cat. Apparently the swelling of the granules during digestion is a stage in the conversion of zymogen into soluble ferment and occurs more rapidly than the formation of new granules. Hence the diminished reticulated area, and the absence of any increase in the the size of the cell, contrary to Heidenhain's observation.

(2) <u>Finely reticulated (granular) or mucoid cells.</u> This other type of central cell has somewhat finer granules but when fixed, the granules are replaced by a fine irregular reticulum or perhaps a precipitate (fig. 3, m, XX, XXIb). No granules ever remain intact after fixation. In the fresh condition, these granules are more rapidly dissolved by reagents than those of the peptic cells, this perhaps, partly explains the entire absence of granules after fixation. Mucoid cells occur mainly in the superficial half of the gland, but are interspaced among the coarser reticulated peptic cells towards the deeper part, and may be found throughout the whole gland tube. In places, a portion of a gland may be lined entirely by these cells. In form, they are roughly globular, but variations in shape occur according to their position and "fit" in the tubule (fig. 5, m).

Their staining reactions render them distinctive. They are coloured a pale blue by alcoholic eosin and methylene blue, (a pale magenta by polychrome methylene blue and a deep blue by Mallory; as was the case with the fasting peptic/

peptic cells, they are unaffected by haematoxylin. When a definite reticulum is present, it stains blue with Mallory but in some of the cells the basal portion takes on a brownish or even a reddish tinge. When there is no reticulum, the precipitate-like material invariably stains blue.

The nucleus is small and assumes the shape of the base of the cell: it is generally densely stained. Changes during digestion consist in the cell becoming first larger and later smaller and staining less heavily with Mallory. The nucleus appears to be a little more prominent. Mucoid cells are most marked in the boundary zones, where they are continous with the cardiac cells on the one side and the pyloric cells on the other. They are no doubt similar to the cells described by Bensley and by Cade.

(3) Parietal or oxyntic cells. In the cat, these cells are mostly found wedged in between the central cells, with one corner abutting on the lumen, nevertheless they lie sufficiently far outwards to be termed parietal cells. They are most numerous in the superficial half of the gland, and may form the sole lining of this part of the gland tube. In shape, (judging from vertical and transverse sections) they are roughly pyramidal, but there are many variations from ovoid to crsecentic. In reality, however, they are polyhedral cells, obtaining their irregular form from their peculiar position. Unlike the peptic and mucoid cells, the granules of the oxyntic cells are very fine and are not readily attacked by reagents. They are always well fixed by all the methods employed, and with osmic those situated immediately underneath the membrane of the cell may be demonstrated to be lipoid in character. Similar observations have been made by Bohm and Davidoff (4) in the rat. The staining reactions of the oxyntic cells granules are as follows, - red with alcoholic esoin and methylene blue, haematoxylin and eosin or Mallory; pale blue with polychrome methylene blue, and dark brown with osmic acid.

The nucleus is spherical and usually central. Occasionally it is excentric, or/

or there may be two nuclei within the same cell.

A number of the cats examined showed the presence of parasitic spirochaetes [Lim (18)]. These organisms were sometimes found within oxyntic cells in what appeared to be a single dilated canaliculus, continuity with the lumen of the gland being demonstrated. Otherwise there was no histological disturbance. Vacuoles may often be seen within the oxyntic cells of all animals.

With regard to functional changes, oxyntic cells appear on the whole to become larger (Heidenhain) during digestion and their granules more easily distinguished, being less closely packed together and probably fewer in number. The difference , however, is not marked and may be partly due to the shrinking of the central cells.

It ought to be noted that oxyntic cells occur throughout the whole stomach, being absent only some 3 mm. from the oesophagus and about 15 mm. from the pyloro-duodenal junction. The oxyntic cells of the pyloric boundary zone are somewhat small and are situated mainly in the superficial portion of the gland; they are probably somewhat primitive in character. They have already been described in other animals [Stohr (29), Trinkler (23), Nussbaum (20)]. Nussbaum, however, does not consider these cells to be same as oxyntic cells.

General Considerations.

The observations which have just been described, show firstly, that the term "chief" or "central" cells is inadequate, since ther are two types differing widely from each other. Secondly, that the cells of the cardia and pylorus are similar in structure and are continous with the mucoid cell of the fundus. Thirdly, that the fundus or corpus is the all important region of the stomach, the other two regions being small by comparison.

Let/

Let us first consider the entity of the two types of central cells. We have seen that the peptic cell is granular (or reticulated) and that after a period of activity the granules diminish and are replaced at the base of the cell by ergastoplasmic fibres. In the case of the mucoid cell, the cytoplasm is also granular (when fresh) but functional changes do not cause any such alteration in its architecture. The nucleus of the peptic cell at rest is irregularly rounded or ovoid, and is applied against the basement membrane, but during digestion, appears more regular in outline, and frees itself from the base so far as to occupy a more central position. The mucoid cell nucleus on the other hand, is not markedly changed either in shape or position. There is also the difference in staining reactions. The peptic cell stains in an entirely different manner from that of the mucoid cell (compare m and p, fig. 3). This difference is manifested, not with one staining method alone, but with several, although Mallory's is the best for the purpose. Both types of central cells may be seen in man, dog, rabbit and pig, and also in the frog: they are probably common to all mammals.

There can thus be no doubt regarding the separate existence of these two types of cells. Edinger's theory that all the varieties of cells found in the stomach are functional modifications of one type, is untenable. It is impossible to reconcile this view with the facts regarding the differences in structure and reactions, in both fasting and feeding animals.

Heidenhain (13) long ago observed that some chief cells stain more readily with aniline blue than others - and referred this to functional changes. This was later confirmed by Greenwood (12) in the pig's stomach; she suggested that the "clear" cells were mucus cells, thus anticipating the results of two subsequent observers. Both Bensley and Cade have distinguished two types of central cells [older observers from Edinger (9) and Pilliet (22) downwards have found various "modifications" of the central cells but not separate types], which/

which appear to be similar to our peptic and mucoid varieties. Bensley was the first to note that the cells of the "neck" region of the fundus glands stain in the same manner as the mucus secreting cells, these cells he termed "indulinophilous mucus cells". Cade confirmed Bensley's finding and called them "cellules principales du col". I interpret this as the part of the gland tube immediately connected to the duct. In the cat, the neck region of the gland is lined almost completely by oxyntic cells and a few transitional cells, i.e., cells which show no division of the cytoplasm into two zones, and yet are not identical with the mucoid cells (see fig. 5, t). It is the portion of the gland below the neck, [redacted] therefore, that is lined chiefly by mucoid cells (see fig. 5). Bensley (2) does state , however, that an occasional "indulinophilous cell" may be found among the central (peptic) cells of the deeper part of the gland and from an examination of his figures (fig. 6), it is clear that the "neck region" he describes includes the superficial portion of the gland. To him credit is due for their discovery, although a more definite description and wider distribution of the mucoid cells must now be recognised.

"Mucoid" cells are described in only two textbooks in English, Schafer's Essentials of Histology (25) and the American edition of Bohm and Davidoff, translated by Huber (4). Of continental works, I can only find a mention in Prenant, Bouin and Maillard (23), who have an excellent diagram in their textbook of Histology, showing typical mucoid cells - which they hesitatingly label, "cellules principales muqueuses?" - to illustrate the mucus cells of Bensley. It is evident therefrom that hitherto, the distribution and even the existence of mucoid cells have scarcely been recognised.

The name "peptic" and "mucoid" have been chosen for obvious reasons. The structure of the peptic cell is characteristically that of a zymogen secreting cell and by the term "chief" or "central", this cell was meant, so/

so that there is no need to dispute its function. The term mucoid is applied because the cell resembles other mucus secreting cells but it is not identical with either the mucus secreting cells lining the surface or the goblet cells of the intestine [compare cells m and s in fig. 3; also compare Lim (17)].

We must next consider the relation between the cardiac, pyloric and mucoid cells. We have seen that there is little or no difference structurally between the two former (cardio-pyloric) cells and that the mucoid cells resemble them in most respects except position. They are stained in the same way and their structural characters are very similar both during rest and activity. The cardio-pyloric cells showed in some animals a reddish basal reticulum, this may or may not constitute a difference, although it is to be noted that the reticulum is more frequently absent than present. Lastly, they are continuous with each other, for the cardiac cells can be traced into the fundus in the form of mucoid cells: the same applies to the pyloric cells. Thus, while there seems to be the closest resemblance between these three types (they are all obviously "mucoid") there may ~~may~~ not be the same coincidence in their functions.

The striking difference in appearance between the peptic and the pyloric cells have been quite missed by all the workers on pyloric pouches and it ~~it~~ is possible that their histological examination was inadequate to ensure the purity of the pouches which they made. But apart from this, the smallness of the pylorus itself (even in the dog) renders the older work liable to criticism on the score of purity, i.e., the non-inclusion of peptic elements.

Summary.

Summary.

(1) The gastric mucous membrane is principally formed by relatively simple tubular glands, which become more complex near the orifices of the viscus, especially the pyloric. The glands are lined by one or more kinds of cells; the following types may be recognised.

(2) Surface mucus secreting cells, which include the cells lining the surface and ducts leading therefrom.

(3) Mucoid cells, of which there are two closely allied groups.
The cardio-pyloric cells which form the sole lining of the glands within about 0-2 mm. and 15 mm. of the oesophageal and pyloric orifices respectively.
The mucoid cells proper, which occur in the large intervening region (fundus) where they are intermingled with the peptic and oxyntic cells; they chiefly occupy the superficial or upper half of the gland tube.

(4) Peptic cells, which are found (often in conjunction with mucoid cells) within the deep half of the gland; both peptic and mucoid cells were formerly described as "chief" or "central" cells.

(5) Oxyntic cells chiefly occupy the superficial portion of the gland, but they take a parietal position throughout the remainder of the tube.

(6) The interglandular tissue contains numerous basiphil (connective tissue) cells, oxyphil leucocytes, and a few cells with large eosinophil globules.

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Illustrations.

Figure 1. A cross section of a gland tube from the cardiac end of the stomach along the lesser curvature, about 1 mm. from the oesophagus. Cat 20. Killed 10 a.m.; last meal 14 hrs. previously. Formol fixation; stained with Mallory. (Drawing).

Figure 2. A cross section of a gland tube from the cardiac end of the stomach along the lesser curvature, about 6 mm. from the oesophagus. Cat 20; 14 hrs.; formol; Mallory.
m, mucoid; p, peptic; o, oxyntic. These cells are in the resting condition. (Drawing).

Figure 3. Cells from the glands of the middle region of the stomach.
s, surface mucus secreting cells; m, p, as in fig. 2.
XX, Cat 20; 14 hrs.; Altmann's fluid; Mallory. The small reddish granules cannot be accounted for except as granules of the oxyntic cells which have been accidently dispersed while cutting the section. The appearance of the peptic cell on the left is somewhat homogeneous, but this is due to the granules being preserved in situ; the cell on the right shows the more usual reticulated appearance. Note the cytoplasm of the mucoid cell
I, Cat 1,; 1 hr.; alcoholic eosin and methylene blue.
The granules in the peptic cell are imperfectly preserved; the cytoplasm stains intensely in a blotchy manner. Note the almost homogeneous appearance of the mucoid cell.
III, Cat 3; 6 hrs.; formol; very dilute polychrome methylene blue.
The peptic cell here shows well marked ergastoplasmic fibres and zymogen granules, and is in marked contrast with the mucoid cell.
XXIa, Cat 21; 24 hrs.; formol; iron haematoxylin.

XXIb, same tissue; Mallory.

The peptic cells show the usual reticulated appearance. Note that the mucoid cells also show a reticulum. (Drawing).

Figure 4. The glands of the middle of the anterior surface of the stomach. Cat 10; 24 hrs.; formol; haematoxylin and eosin.

a, low power (x 75) ; b, high power (x 400).

sc, stratum compactum; mm, muscularis mucosae; p, gland lined with peptic cells; m, gland lined with mucoid cells; oxyntic cells occur in the parietal parts of both glands. (Photograph).

Figure 5. A longitudinal section of a gland tube from about the middle of the greater curvature. Cat 3; 6 hrs.; formol; Mallory.

s, m, p, o, as in previous figures; t, transitional cells; g, cell containing large eosinophil globules. This drawing gives an idea of the distribution of the various cells which compose a gastric gland tube in the fundus region. The cells are in an exhausted condition. Compare the mucoid cells with the peptic, and also this figure with fig. 2. (Drawing).

Figure 6. A cross section of a pyloric gland from the lesser curvature about 10 mm. from the pyloro-duodenal junction. Cat 21; 24 hrs.; formol; iron haematoxylin. The sparse reticulum which can be seen here/stains red with mallory. (Photograph).

II. The Gastric Mucoid Cells of Foetal and Newborn Animals.

Two litters of newborn and one of foetal cats as well as three still-born children and one human foetus have been examined. The method employed was formol fixation and after staining with either Mallory or Heidenhain's iron haematoxylin.

Cats.

In a foetus of about 6 weeks, the stomach exhibits a simple lining of columnar epithelium, which is entirely devoid of a superficial mucous area. The cytoplasm stains reddish with Mallory. Only a few invaginations represent the primitive gland tubes.

At birth; Short simple glands are now present. They are lined by small oxyntic and mucoid cells. Some of the latter are wholly mucoid and others are only partially so, having a portion of non-mucoid (red staining with Mallory) cytoplasm within the basal half of the cell. The surface cells are similar to those of the adult.

One week after birth, the glands enlarge, while the oxyntic cells become more prominent. Mucoid cells are present in large numbers but a few developing peptic cells (?) are visible. These have no mucoid reaction and are coloured principally by the red and brown dyes in Mallory's mixture. The pylorus is now becoming defined; it contains only mucoid cells.

Three weeks after birth, the peptic, mucoid and oxyntic cells are all plainly evident, the appearance of the mucous membrane now approximates that of the adult.

Human.

Human.

In a foetus of about 4 months, the stomach is lined by a mucous membrane of the simple type, bearing only short gland tubes. These are formed partly by mucoid and partly by red-staining non-mucoid cells; oxyntic cells are as yet absent. The junction between the stomach and the duodenum is sharply marked off by the pyloric sphincter, but the mucous membrane does not show a corresponding division. The pyloric portion of the stomach contains both goblet and columnar cells with striated borders, for some distance from the actual muscular junction. The glands are wholly mucoid.

At birth, peptic and oxyntic cells are fully developed and the glands are much longer and altogether more like the adult.

Conclusion.

It is quite clear that the gastric glands are in the first instance formed of non-mucoid, red-staining cells. Later, these cells become mucoid in character throughout the whole stomach. The next type to differentiate is the oxyntic and at a later stage still, comes the peptic.

Peptic cells are present at birth in the human, but do appear until between the second and third week after birth in the cat. This difference may give us an important clue to the function of the fundus mucoid cells, for it has been observed that the newborn stomach contains pepsin, while the stomach of the newborn cat contains none, and does not exhibit a ferment until the third week after birth [(Hammarsten 1874, Zweifel 1874, Morrigia 1876) quoted by Moore (2); Sewall (3)]. Obviously pepsin is not secreted by the mucoid cells.

These cells are essentially primitive or at least, less specialized than either the peptic or oxyntic. Cade arrives at a similar conclusion from an entirely/

entirely different point of view (1). He found that oxyntic cells disappear and peptic cells lose their granules in the vicinity of gastero-enterostomy openings, and all the cells appear mucoid in character. He thus inferred that the altered conditions had caused the specialized cells to revert to type, namely to assume the garb of the more primitive mucoid cells. I can confirm Cade's observations entirely (see paper VI).

Thus while the mucoid cells are undoubtedly a definite variety of the gastric gland cells, they are closely allied to the peptic cells, which they give rise to in early and perhaps later life.

Literature.

(1) Cade, Arch. d'anat. micr., 1901, IV, 1.

(2) Moore, Schafer's Textb. of Physiol., 1898, I, 330.

(3) Sewall, J. Physiol., 1878, I, 321.

III. The Gastric Mucoid Cells in Man, Dog, Rabbit and Frog.

The gastric mucous membrane of different animals have been examined in order to compare the histological features and the distribution of the mucoid reacting cells in each species. The technique employed is similar to that referred to in the first paper. The material was invariably obtained from the killed or from the living anaesthetised animal. Human material came partly from the operation table and partly from the post-mortem room. Formol fixation and Mallory's and Heidenhain's methods of staining were the routine procedures; the following description is taken from specimens thus prepared.

The Mucoid Cells of the Fundus.

Human:

In man, mucoid cells are abundantly present. They have the same characteristics as those of the cat except that their cytoplasm is more homogeneous and stains a lighter blue with Mallory. Their distribution is somewhat different; they form the entire central lining of slightly less than the superficial two-thirds of the tubule - hence their regular cubical outline. This portion of the tubule is thinner than the deeper portion which (with rare exceptions) contains typical peptic and oxyntic cells. A few tubes are lined throughout their whole extent by mucoid cells. There is not the same amount of intermingling between the mucoid and peptic cells as occurs in the cat and thus the mucoid portion of the tubule is more easily defined, especially as it is also thinner than the peptic portion.

Dog:

The mucoid cells of the dog are intermediary in appearance between those of man/

man and the cat. In some animals, the cytoplasm is almost homogeneous and stains lightly with Mallory; in others it is more reticular and stains heavily as in the case of the cat. Their distribution, however, shows fewer mucoid elements in each tubule, i.e., they line a little less than the superficial half and the mucoid and peptic cells do not intermingle to any extent. The widening of the calibre of the deep portion of the tubule occurs gradually as in the cat, but nevertheless the mucoid and peptic portions are sharply marked off from each other.

Rabbit:

The mucoid cells of the rabbit stain faintly blue with Mallory and are nearly homogeneous; they appear like the human. They are not easily made out as they are hidden by the numerous overlapping oxyntic cells. This seems to be a very characteristic feature of the rabbit and accounts for the shape of the cells being so irregular. They occupy the superficial three-fourths of the tubule, but there is a good deal of intermingling with the peptic cells. The deep portion of the tubule rarely shows mucoid cells. This is best determined in iron haematoxylin stained sections of the actively secreting stomach, as the presence of the overlapping oxyntic cells make it exceedingly difficult to examine the central lining. In the above preparations, only the peptic cells are clearly stained on account of the marked development of ergastoplasmic fibres - which occurs readily, (if not invariably present) in the rabbit. The mucoid cells are left unstained. The proportion of mucoid to peptic elements in each tube varies in different parts of the fundus; thus from two-thirds to four-fifths of the whole tubule may be mainly mucoid. The measurement given in the first instance is that of the middle of the greater curvature.

Frog (Rana Temporaria):

In/

In the frog's stomach , only oxyntic and mucoid cells are to be seen. The latter have a clear cytoplasm which stains a faint blue with Mallory. They are found in the superficial third of the gland tube and rarely extend to the deeper parts,

The Cardio-Pyloric Cells.

The cells forming the cardiac and pyloric glands are so similar in appearance and staining reactions that they may be grouped together as the cardio-pyloric mucoid cells. They differ from the mucoid cells proper of the fundus in their regular shape and in sometimes exhibiting a red-staining reticulum with Mallory. The average measurements of the cardia and pylorus are set forth below.

Animal	Cardiac Cells	Cardiac & Oxyntic C.	Pyloric Cells.	Pyloric & Oxyntic C.	Curvatures.
Cat.	0-4 mm.	3 mm.	15 mm.	20 mm.	Greater
	0-3 mm.	"	12-15 mm.	20-25 mm.	Lesser
Dog.	---	2 mm.	20 mm.	+ 40 mm.	Greater
	---	3 mm.	25 mm.	+ 45 mm.	Lesser
Rabbit.	0-1 mm.	2 mm.	35 mm.	2 mm.	Greater
	0-2 mm.	2-3 mm.	40 mm.	2-3 mm.	Lesser

+ Oxyntic cells small and primitive.

Human:

The pyloric cells of man resemble those of the cat in every respect except that they are taller and stain more lightly. Sufficient material was not available from which measurements of the cardia and pylorus could be made.

Dog:

There are no pure cardiac glands in the dog as oxyntic cells may be found at the cardio-oesophageal junction along both curvatures, while peptic cells are present within 2-3 mm. of the junction. In this small zone, the cells are taller/

taller but otherwise show the same feature as those of the cat. Salivary glands are very constantly present; they extend from the oesophagus into the cardia under the muscularis mucosae. Their acini are wholly mucous with a few serous crescents here and there. They are thus not to be considered as cardiac glands, but as part of the salivary apparatus which occurs abundantly in the submucosa of the oesophagus.

The pylorus extends for 40 and 45 mm. along the greater and lesser curvatures respectively. The boundary zone bearing full-sized oxyntic cells and pyloric cells occupies only about 2 mm., but small (primitive) oxyntic cells may be observed within 20-25 mm. of the pylorus, especially at the "neck" of the gland. The cells, like the cardiac group, resemble those of the cat - the red ~~staining~~ staining reticulum being more constantly present and best seen towards the duodenum.

<u>Rabbit</u>:

There are a few cardiac glands corresponding to those seen in the cat, present in the rabbit. These usually occur along the lesser curvature, occupying a small zone of about 2 mm. distal to the oesophagus. Along the greater curvature and sometimes along both curvatures, oxyntic cells may be found right up to the cardio-oesophageal junction. When the cardiac glands are present, the cells which form them are not typical. They only show a faint mucoid (blue) reaction near the surface, elsewhere the cytoplasm is both granular and reticular, and stains red with Mallory. The condition appears to be an exaggeration of the "red reticulum" seen in the cat. In addition to this peculiarity, glands of the salivary type are also met with under the muscularis mucosae. They extend(along the lesser curvature)for only a very short distance (about 3 mm.). The acini are mainly serous, a few being mucous; the cells lining the terminal ducts have granules arranged in striae and have centrally/

centrally placed nuclei; features which render a mistaken diagnosis impossible. True mucoid and peptic elements are present beyond the cardiac area described above, the former forming a boundary zone of about 3-4 mm. with the oxyntic cells before the latter are added.

The pyloric region is somewhat larger than that of the cat as oxyntic cells are only seen about 35-40 mm. and 40 mm. from the duodenum, along the greater and lesser curvatures respectively. There is almost no boundary zone as the peptic cells appear a few millimetres after the oxyntic. The gland cells are more mucoid than those of the cardia, but like them show a well-marked non-mucoid basal area.

Langley (3) described the cells of the rabbit's fundus along the greater curvature as being finely granular and similar in appearance to the pyloric cells. While the cells of the remainder of the fundus were coarsely granular. I have not been able to make this distinction but perhaps Langley mistook the superficial mucoid cells to be the sole central cells, and failed to see the peptic (coarsely granular) cells in the deepest part of the mucous membrane.

Frog:

There are no cardiac glands in the frog, the peptic cells merely stop short and mucoid and oxyntic cells make their appearance. The pylorus extends about 3-4 mm. from the duodenum and its gland cells are not different from the mucoid cells of the fundus.

Conclusions.

As has been noted by Bensley (1) and more especially by Cade (2), who has examined all the species dealt with here, mucoid cells (or "les cellules principales muqueues") are plainly evident in all the higher animals one may care to investigate. There can thus be no doubt as to their existence as a normal/

normal type of the central cells of the fundus.

Only in man can the portion of the tube lined by mucoid cells be called the neck (Bensley), for in the other cases, the calibre of the gland shows no marked alteration or differentiation as compared with the deeper part. It is true that there is a slight enlargement towards the pit of each gland tube, but the intermingling of mucoid and peptic cells, especially well marked in the cat and rabbit, indicates that the whole composes the gland. This is as true of the frog as of mammals. Another point which supports this view, is the close relationship which exists between the mucoid and peptic cells (see paper II); the whole tubule containing these two varieties must form the gland proper. Attention has been called to this question, not for the purpose of disproving Bensley's nomenclature but because it is too narrow and apt to be misleading.

With regard to the cardiac and pyloric cells, they are on the whole indistinguishable from each other in each of the species examined. In man and the dog, they are tall and columnar in shape, while in the cat and rabbit they are shorter and often cubical. The basal redstaining substance (with Mallory) which is so well developed in the rabbit is of interest as it undoubtedly represents the precursor of the secretion of the cell. It has been observed in every species (not including the frog) investigated, but has not been noted in the fundus mucoid cell, unless the occasional occurrence of a reticulum which stains brownish or reddish in the cat, be accounted similar. For this reason, I would classify the mucoid cell of the fundus separately; the cardio-pyloric cells as a slightly more advanced group, showing signs of a precursor, (prozymogen ?); and the peptic and oxyntic as the most specialized types of gastric cells.

Literature.

(1) Bensley, Quart. J. Micr. Sci., 1898, XLI, 361.
(2) Cade, Arch. d'anat. micr., 1907, IV, 1.
(3) Langley and Sewall, J. Physiol., 1879, II, 281.

IV. The Question of a Gastric Hormone.

Historical.

Pavloff and his pupils have shown in a conclusive manner that appetite is responsible for the initial flow of gastric juice but not for the sustained flow which continues for several hours afterwards (25). Their experiments tended to show that the later secretion was due to a nervous mechanism. For example, Lobasoff (14) found that a reflex secretion of the stomach could be obtained by introducing various chemical substances (secretagogues) either into the duodenum or the stomach: introduction of the same substances into the rectum, although absorbed, failed to evoke any secretion. Popielski (25) demonstrated that secretion occurred even after all the extrinsic nerves to the stomach had been divided. He concluded that the reflex was carried out through the nerve plexuses situated in the stomach wall. Edkins (4), however, showed that the reflex might be a chemical one. He found that decoctions of the pyloric mucous membrane, especially when made with dextrin or HCl, caused a flow of gastric juice when injected into the blood stream. Starling (28) has elaborated this view, suggesting that the secretagogues act by stimulating the pyloric mucosa to secrete a "hormone" into the circulation, whence it is transported to the fundus and there excites the formation of juice. Gross (8) working in Pavloff's laboratory, also demonstrated the existence of a pyloric mechanism; he succeeded in provoking gastric secretion by introducing meat extract into the pylorus, after the introduction of the extract into the fundus had failed. Edkins found extracts of the cardia to be slightly active. Later observers have extended the list of substances, mainly organ extracts, which are capable of exciting gastric secretion.

Popielski/

Popielski especially, stated that small doses of de Witte's peptone, extracts of fundus, pyloric, intestinal and rectal mucous membranes, extracts of other organs, e.g., brain, pancreas, and even defibrinated blood, when injected intravenously into a dog with a gastric fistula, are all capable of causing gastric secretion. (16, 17, 20, 22, 23). He now believed that the secretion was due to a substance "vaso-dilatin" which he conceived to be present in all extracts having a secretory effect on the stomach. He argued that as a lowering of blood pressure and diminished coagulability of the blood followed every successful injection of an organic extract, these were the direct effect of vaso-dilatin, in virtue of which, secretion was evoked(19, 23). The secretion was thus part of a general disturbance, for he obtained intestinal (16, 23) and even salivary secretion (18) when these changes were brought about in the blood. In addition, such states as anaphylactic shock, blood transfusion, morphine narcosis (19) which exhibit these two conditions of the blood markedly are accompanied by secretion of the stomach among other glands. As to the nature of vaso-dilatin, Popielski described it as containing C.H.O.N. but no S or P (23); it is a hydrolytic product of the proteins and contains no choline; it does not give the biuret reaction (20). Dale and Laidlaw (3) have since compared vaso-dilatin with histamine; and in his last paper Popielski (24) admits the identity.

Ehrmann (5), Emsmann (7), Tomaszewski (29) and Keeton and Koch (10) all confirm Popielski in the finding of a "hormone" in both fundus and pyloric extracts and in extracts of many other parts of the alimentary mucosa and its connected glands. They used dogs (Keeton and Koch used cats as well) with accessory stomachs (pouches) or with chronic fistulae.

On the other hand Eisenhardt (6) found that injection of gastric juice from the fundus was quite inactive, while the pyloric juice was active. Maydell (15) also obtained positive results with pyloric extracts only; pancreatic and duodenal/

duodenal extracts and neutral gastric juice were all negative. He employed dogs with chronic fistulae. Keeton and Koch, however, do not agree with Popielski regading the mechanism of secretion - they believe after Edkins and Starling in a specific hormone "gastrin", occurring throughout the stomach, to a less extent in the duodenum and remaining intestine.

The aim of the present investigation is to determine whether there is a specific hormone mechanism, or whether the secretion is part of a general phenomenon associated with low blood pressure and diminished coagulability, or lastly, whether a reflex through the intrinsic plexuses accounts for the secretion.

Technique Employed.

The experiments were all carried out in normal cats and a few rabbits under an anaesthetic. Edkins' method was not employed as the saline which he introduces into the stomach in order to receive the juice which is secreted, is liable to stimulate secretion in itself (Khigine (13)). The main objection to his method, however, was that it could not demonstrate the commencement and duration of the flow of juice excited.

The method which was devised and employed in every case is as follows. The animal (cat) receives no food on the day of the experiment, and on the previous day the only precaution necessary is to see that it is lightly dieted. The experiment is best performed at once shortly after the time of the usual morning meal. This procedure ensures that the stomach is empty and that the glands are in a normal state of repletion.

The anaesthetic used was always chloroform. As soon as the animal is under it is properly secured to the operation table, which is provided with a warming apparatus. The trachea is then opened and a two-way cannula tied in.

The/

The anaesthetic may be given by saturating a continuous blast of air (at low pressure) and introducing this through one limb of the tracheal cannula or by connecting the cannula with a small bottle containing chloroform. In either case, the animal respires itself and can be kept perfectedly controlled with no subsequent trouble to the operator. A constant state of complete anaesthesia is essential to the success of the experiment. A vein tube is next tied into the external jugular vein.

The abdomen is opened by a median incision going through the linea alba. The fundus of the stomach is gently pulled out so as to expose the cardio-oesophageal junction around which a ligature is applied. If desired, the vagi can be excluded from the ligature by a little dissection. The necessity of tying the oesophagus arises on account of swallowing movements which sometimes occur and which carry down saliva and mucus into the stomach. The pyloroduodenal junction is next sought for; a ligature placed round it, care being taken to avoid the pancreas and the neighbouring vessels. Another ligature is placed round the duodenum half an inch from the first ligature with the same precaution and tied tightly. The duodenum between is opened and a curved perforated glass cannula (see fig. 1) with six inches of rubber tubing attached is carried into the stomach through the pylorus, so that the curve of the cannula is adapted to the greater curvature. The pyloric ligature is tightly secured round the cannula and the rubber tube. The tube is connected by means of a short piece of glass tubing with another rubber tube which leads to a drop recorder, placed about six inches below the level of the animal. Before actually joining the stomach cannula with the recorder, the cannula and system of tubes are filled with saline or water (about 10 c.c.); a certain amount of fluid runs into the stomach but as soon as the connection is made it escapes, the flow stopping when the stomach is drained, leaving only the tubes filled with fluid. The abdominal wound is closed and the animal is ready.

If/

If the movements of the stomach are to be simultaneously recorded, a purse-string suture going through both the muscle and mucous coats is inserted in the anterior wall of the pyloric antrum; an opening is made in the centre and a small rubber balloon, introduced, and the purse-string suture tied on the glass holder of the balloon. A rubber tube is previously attached to the holder and connection is made with a U-tube filled with water and this with a piston recorder. A glass T-tube connection between balloon and water-trap will allow the balloon to be distended to any desired extent. The blood pressure and respiration may also be recorded in the same animal.

Reference to the succeeding figures will give an indication of the tracings obtained. As much as 30 c.c. of juice containg free HCl and pepsin can be collected in two and a half hours. Muscular movement may cause a few drops to flow out, but this cannot be mistaken for a true secretion which is sustained for many minutes. The balloon serves as a control of this factor.

The mucous membrane of the stomach and intestine was used both in the fresh condition and after drying. Small pieces from different areas were removed and ground with sand (sometimes not ground at all) before extracting in boiling water or 0.2% HCl for 10 minutes. The strength employed was either a 5 or 10 per cent. extract; full details are given in the protocols of the experiments. The material was taken from freshly killed cats.

Experimental Results.

Twenty cats, three rabbits and one dog have been investigated up to date. The work on cats only is recorded.

Pyloric Extracts.

No. of Drops

Before:After

The number of drops secreted from the end of the latent period to the cessation of a secretory effect (i.e., secreted within the period called "duration of secretion") is placed under the heading "After". The drops placed under the heading "Before", were secreted in the interval immediately preceding the end of the latent period and equivalent in duration to the secretory period "After".

Pyloric Extracts.

Animal	Weight grm.	Sex	Dose and Source of Extracts.	Latent Period	Duration of Secretion	No. of Drops Before	No. of Drops After	Remarks
				in minutes.	in minutes.			
Cat 31	3000	M	5c.c. 5% (F) water.	3	25	11	23	P
			(followed 6 minutes after dose of Adrenalin)					
			1c.c. "	2	15	2	8	P?
Cat 32	2270	F	3c.c. 5% (F) water.	$3\frac{1}{2}$	10 } 31	1(?)	5 / 10 } 15	P?
			6c.c. "		21 }		10 }	
			(14 minutes afterwards)					
			2c.c. 5% (F) acid.	2	17 } 36	0	5 } 14	P ?
			5c.c. "		19 }		9 }	
			(21 minutes afterwards)					
Cat 33	1850	M	4c.c. 5% (F) water.	3	15	9	24	P
			4c.c. "	3	20	1	13	
Cat 34	2200	M	3c.c. 5% (F) acid.	6 3(?)	13	5	12	P
Cat 35	3000	F	3c.c. 5% (F) acid.	$3\frac{1}{2}$	11	0	14	P
			3c.c. "	7	15	0	18	P
			3c.c. "	$5\frac{1}{2}$	11	1	8	P
Cat 36	2500	F	3c.c. "	3	20	0	23	P
Cat 37	2600	M	3c.c.10% (F) acid. D.	14(?)	6(?)	0	3	?
			3c.c.10% (F) acid. S.	9	30	3	48	P
			(15 minutes afterwards)					
Cat 40	3400	M	3c.c. 5% (F) acid	3	$14\frac{1}{2}$	2	19	P

F; fresh mucosa: P, positive: in the case of Cat 37, D, deep portion of mucosa; S, superficial half of the same piece of mucous membrane.

From the above protocol, it will be noted that extracts of the pylorus, whether/

whether made with water only or with HCl, invariably provoke secretion. That the secretion obtained is not abnormal, has been ascertained by testing for pepsin and free HCl, both of which are present. The latent period is usually between 2-3 minutes, although it is sometimes longer. The duration of the flow depends upon the dose - this is only roughly so, for different extracts show varying effects. Taking a 3 c.c. dose, the average duration is about 15 minutes, counting from the the time the drops appear or come faster to the time when they become slow again or cease. A few residual drops continue to flow but these are best excluded otherwise they extend the secretion period unduly. Subsequent doses will always furnish (within limits) a fresh response. Where no note is made in the above table the succeeding doses were injected ~~with intervals of~~ about 15 minutes after the previous dose had ceased to be effective.

There is always a depressor effect on the blood pressure, whether the extract is acid or not. The effect on the respiration is entirely due to the acid and is not constant, and there is no effect on the muscular coat of the stomach (see figs.).

The effect produced by 3 c.c. of a 5% Pyloric extract has been taken as a standard - no secretion being admitted unless the drops flow at a rate of at least 8 drops per period of 10 minutes. Results which approach this standard are labelled either doubtful positive or negative.

Cardiac Extracts.

Animal	Weight grm.	Sex	Dose and Source of Extracts	Latent Period in minutes.	Duration of Secretion in minutes.	No. of Drops Before	No. of Drops After	Remarks
Cat 30	2600	?	2c.c.10% (D)water	3	14	0	11	Vagi intact P
			3c.c. "	5½(?)	5(?)	0	2	N
			4c.c. "	6(?)	7(?)	2	3	N

Animal	Weight grm.	Sex	Dose and Source of Extracts	Latent Period (in minutes)	Duration of Secretion (in minutes)	No. of Drops Before	No. of Drops After	Remarks
Cat 31	3000	M	3c.c. 5% (F) acid	2(?)	4(?)	2	5	~~P?~~ N
Cat 32	2270	F	3½cc.10% (D) water	2(?)	14(?)	5	3	N
			(17 minutes after dose of Adrenalin)					
Cat 36	2500	F	3c.c. "	4	15	3	14	P
Cat 42	2300	F	3c.c. 5% (F) acid	8	7 } 29	0	12 } 38	P
			3c.c. "		22		26	
			(15 minutes afterwards)					

Cardiac extracts have a secretory effect which is much less certain than that of the pylorus. From Cat 30 it will be seen that dried material do not provide more potent extracts than fresh material (Cat 42). Figure 4.

Fundus Extracts.

Animal	Weight grm.	Sex	Dose and Source of Extracts	Latent Period (in minutes)	Duration of Secretion (in minutes)	No. of Drops Before	No. of Drops After	Remarks
Cat 30	2600	?	4c.c.10% (D) water	5½(?)	4½(?)	1	2	Vagi intact N
Cat 36	2500	F	3c.c. "	2	15	11	11	N
			(19 minutes after dose of Cardiac ext.)					
Cat 42	2300	F	3c.c. 5% (F) acid	1½(?)	3½(?)	0	3	N
			3c.c. "	2(?)	8(?)	3	3	P?
			(6½ minutes afterwards)					

Fundus extracts have no effect when given in the same doses which are effective with pyloric extracts, but with larger doses a doubtful secretion is obtained. Figure 5.

<u>Duodenal Extracts.</u>

Duodenal Extracts.

Animal	Weight	Sex	Dose and Source of Extracts	Latent Period (in minutes)	Duration of Secretion (in minutes)	No. of Drops Before	No. of Drops After	Remarks
Cat 34	2200	M	3c.c.10% (F) acid	10(?)	13(?)	0	4	N
Cat 35	3000	F	3c.c. "	5(?)	6(?)	0	2	N
Cat 40	3400	M	3c.c. 5% (F) acid	2	20	2	11	P?
Rabbit	2350	F	1c.c.20% (F) acid (autonomous extract)	$2\frac{1}{2}$	10	– 6 in four m.	17	P?

Duodenal extracts are like the fundus extracts, inactive; Cat 40 and the Rabbit showed a possible secretory effect.

Jejunal Extracts.

These were invariably negative, no drops followed upon the injections. One example will suffice (see fig. 8).

Cat 41	3500	M	3c.c. 5% (F) acid	–	–	0	0	N

Miscellaneous Extracts.

Intravenous injection of 0.2% HCl never produced any secretion, nor did any follow the injection of the animals own gastric juice.

Transfusion Experiments.

Four experiments were carried out to determine whether the blood of fed animals contained a gastric hormone in the circulation. The recipient whose secretion/

secretion was recorded, was on a normal fast of about 18 hours, while the donor was fed 2-3 hours before the actual transfusion. Int

In the first pair, blood was withdrawn from the Coronary Vein of the stomach (donor) and immediately injected into the Right Gastric Artery of the recipient. Rather small amounts were transferred at each time (3-10 c.c.), the results were quite negative.

In the next, direct transfusion of blood from the Carotid Artery to the External Jugular Vein was employed. The duration of each transfusion was five minutes; this was done on three occasions.

Cat 46 2650 M Recipient : Cat 47 1600 M Donor.		
Blood transfused for 5 minutes - Carotid to Jugular.		
Drops	Time	
2	10 minutes before transfusion	2.18 p.m.
2	5 minutes during transfusion	2.14 "
5	15 minutes after transfusion	2.24 "

Two subsequent transfusions did not show this ~~this~~ slight effect.

In the third, 8 and even 15 minutes transfusion failed to evoke secretion. The tubing and cannulae connecting the two animals were examined after each transfusion and found to be patent; further the vein was watched for pulsation and the red colour of arterial blood.

In the fourth pair, the transfusion was from Carotid to Femoral; there was was a great tendency to clot but even the injection of 10 c.c. of blood withdrawn from the recipient by means of a syringe was ineffective.

Further experiments, allowing for a longer duration of transfusion, are being made. But at present, the indications are that the blood of a fed animal does not contain any gastric exciting substance.

Adrenalin.

Adrenalin.

Animal	Weight grm.	Sex	Dose and Source of Extracts	Latent Period	Duration of Secretion in minutes.	No. of Drops Before	No. of Drops After	Remarks
Cat 30	2600	?	1 c.c. 0.0001%	9	10	2	10	Vagi intact P
			2 c.c. "	10	20	6	21	P
			1 c.c. "	7	8	1	5	P
Cat 31	3000	M	2 c.c. 0.0001%	5	12	6	10	P
			1 c.c. "	5	10	2	4	N
Cat 32	2270	F	2 c.c. 0.0001%	6½	14½	5	8	P?
			(20 minutes after dose of Pyloric ext.)					
			2 c.c. "	8	8	0	2	N
			1 c.c. 0.00005%	6	9	2	5	P?
Cat 33	1850	M	1 c.c. 0.00005%	5(?)	12½(?)	13	9	N
			(16 minutes after dose of Pyloric ext.)					
			2 c.c. "	2(?)	8(?)	0	2	N
Cat 43	3000	M	2 c.c. 0.00005%	2(?)	13(?)	3	7	P?
			3c.c. Pyloric ext. acid + 1c.c. Adren. 0.00005%	2	16	7	15	P
			(15 minutes afterwads)					
			3c.c. Cardiac ext. acid + 1c.c. Adren. 0.00005%	2½	3½	0	4	P?
			2 c.c. 0.00005%	7	-	0	1	N

The Adrenalin solution used was Parke, Davis & Co., Adrenalin Chloride, 1-1000.

The/

The effect of adrenalin has been rather conflicting, but it has undoubtedly caused a flow of juice in two animals (Cats 30, 31). In the other animals doubtful or definitely negative results were obtained. The latent period is more prolonged (5 minutes or more) on account of the inhibition of the muscular coat of the stomach, and the drops, if any are present, may cease for a few minutes. These results agree partly with those of Yakawa (30) who found that Adrenalin provoked gastric secretion in man. They are also partly contradictory to those of Hess and Grundlach (9) and Rothlin (26), who found that it inhibited gastric secretion in dogs with accessory stomachs.

It is notorious, however, that in cats the distribution of sympathetic fibres to any organ vary in different animals, take for example those going to the pulmonary blood vessels (Schafer and Lim 27). It would seem that the sympathetic secretory fibres to the stomach are very poorly developed, and have little or no influence in the majority of animals.

Adrenalin does not appear to inhibit secretion though it may delay its flow out of the stomach. In Hess'and Grundlach's experiments, the secretion was diminished for a definite period (from about a quarter of an hour to two hours) but a"compensatory"secretion followed which made the total volume secreted the same in all cases. It will be seen from Cat 43 that when Pyloric extract and adrenalin are injected together the secretion evoked is about normal; note also that the depressor effect is not abolished (fig. 4, Cardiac ext. and Adrenalin).

Parallel results have been obtained by Keeton, Koch and Luckhardt (11) with Tyramine on cats (with accessory stomachs); they found that it caused secretion inconstantly.

Conclusions.

Conclusions.

Our results confirm those of Edkins, Eisenhardt and Maydell in particular, and conflict with those of Popielski, Keeton and Koch, etc., in regard to the activity of fundus extracts. Popielski's objection to Edkins' work was that the fundus extracts used by the latter contained large amounts of vaso-dilatin thus overdosing the animal at each injection, with the result that the secretion was inhibited. These objections apply equally to the present work, but they can be shown to be invalid. Firstly, there is no ground for the criticism at all, as the fundus does not contain more depressor substance than the pylorus. Secondly, judging from the blood pressure effect, secretion is not dependant upon a low blood pressure (vide adrenalin and adrenalin and pyloric injections). In our experiments, pyloric extracts are by far the most potent, but according to Keeton and Koch, fundus extracts are the most powerful. They worked with dogs with Pavloff pouches, and carried out their injections without an anaesthetic. This may explain the discrepancy in the results, but Eisenhardt and Maydell worked under similar conditions and failed to obtain a positive effect with the fundus. Under the circumstances, the question cannot be considered as closed. Nevertheless, it must be recognised that the stomach is probably not different from the other alimentary glands - that it can be stimulated by a number of substances which are not its normal excitants.

The real question is whether there is a hormone mechanism at all. If by the term "hormone", any chemical messenger is meant, such as CO_2 - then there may be such a gastric hormone. On the other hand, a gastric endocrine system requires that there should be an anatomical ductless arrangement, and that the autacoid be demonstrated in the blood stream. Neither of these fundamental conditions have been established. My transfusion experiments have/

have produced negative results, possibly because sufficient blood was not transfused and also because it may be necessary to obtain blood in every case from the gastric venous return; but even if an exciting substance was to be found in the blood, the histological structure of the stomach does not admit of an endocrine mechanism. Our ideas on the chemical stimulus to gastric digestion must remain tentative. What other explanations are available ?

Leaving aside a local reflex mechanism which has already been discussed, there are two general possibilities.

(a) A direct stimulation of the gastric cells within the stomach cavity. The ease with which the gastric cells discharge their granules (Langley) makes this even probable. This would not explain a secretion obtained by stimulating the duodenum.

(b) The absorption of the stimulating substances through the pyloric mucous membrane into the circulation. These may be altered during their passage through the mucosa. This would allow certain of the products of gastric digestion to act as "hormones".

In relation to this point of view, the possibility of Histamine in the pyloric (and other) extracts must be considered. Barger and Dale (2) have isolated crystals similar to histamine from the ox small intestine, under conditions which avoided putrefaction. Only recently, Abel and Kubota (1) state that they have isolated histamine picrate from the gastric mucosa. Popielski (24), Rothlin and Grundlach (26) and Keeton, Koch and Luckhardt (12) have shown histamine to be a potent gastric stimulant; but according to these observers histamine is more toxic than an extract of the stomach mucosa. It may be that histidine is formed during gastric digestion and undergoes decarboxylation through the agency of bacteria (the stomach is never sterile) or during its passage through the pylorus.

These possibilities are yet to be tested, but so far, our experiments/

experiments show that the specific secretion of a gastric hormone is doubtful.

Summary.

(1) Extracts of the pyloric mucous membrane are the most powerful excitants of gastric secretion; cardiac extracts have a slight secretory effect.

(2) Fundus , duodenal and jejunal extracts have no effect or at the most caus a doubtful secretion.

(3) Adrenalin produced distinct secretion in two animals, but in the majority it caused a temporary cessation of the flow, due to motor inhibition.

(4) Transfusion of blood from a fed animal to a fasting one failed to evoke secretion. The question of a gastric hormone is discussed.

(5) The method of determining the gastric secretion is new, and permits observations on the latent period and the duration of flow.

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Illustrations.

Figure 1. A diagrammatic representation of the stomach with the ligatures, stomach cannula and balloon in position. O, oesophageal ligature; P, Pyloric ligature securing both rubber tube abd cannula; D, ligature closing duodenum; GV, gastric vessels. The length of the stomach cannula is 7 cm., while the diameter is 4 mm.

Figures 2-9, are tracings of the experimental records which are desired to be kept intact. The figures are exact as far as the time relations are concerned; the variations in the blood pressure and respiration curves are as accurate as they could be drawn. Each tracing is separately labelled and the corresponding data will be found in the protocols.

Figure 1.

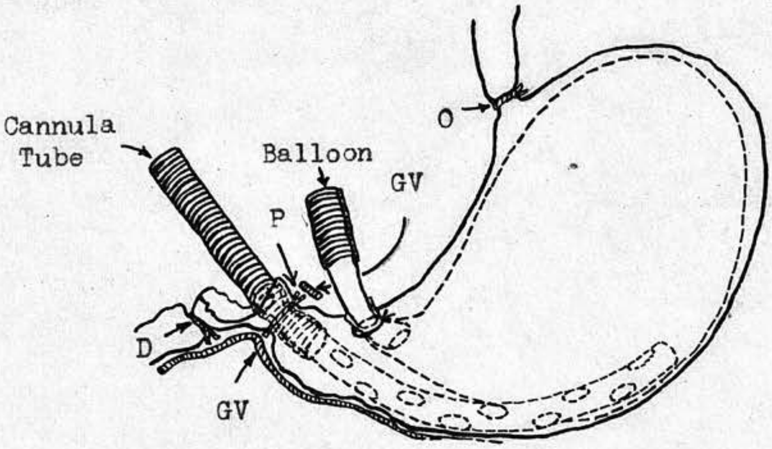


Figure 2.

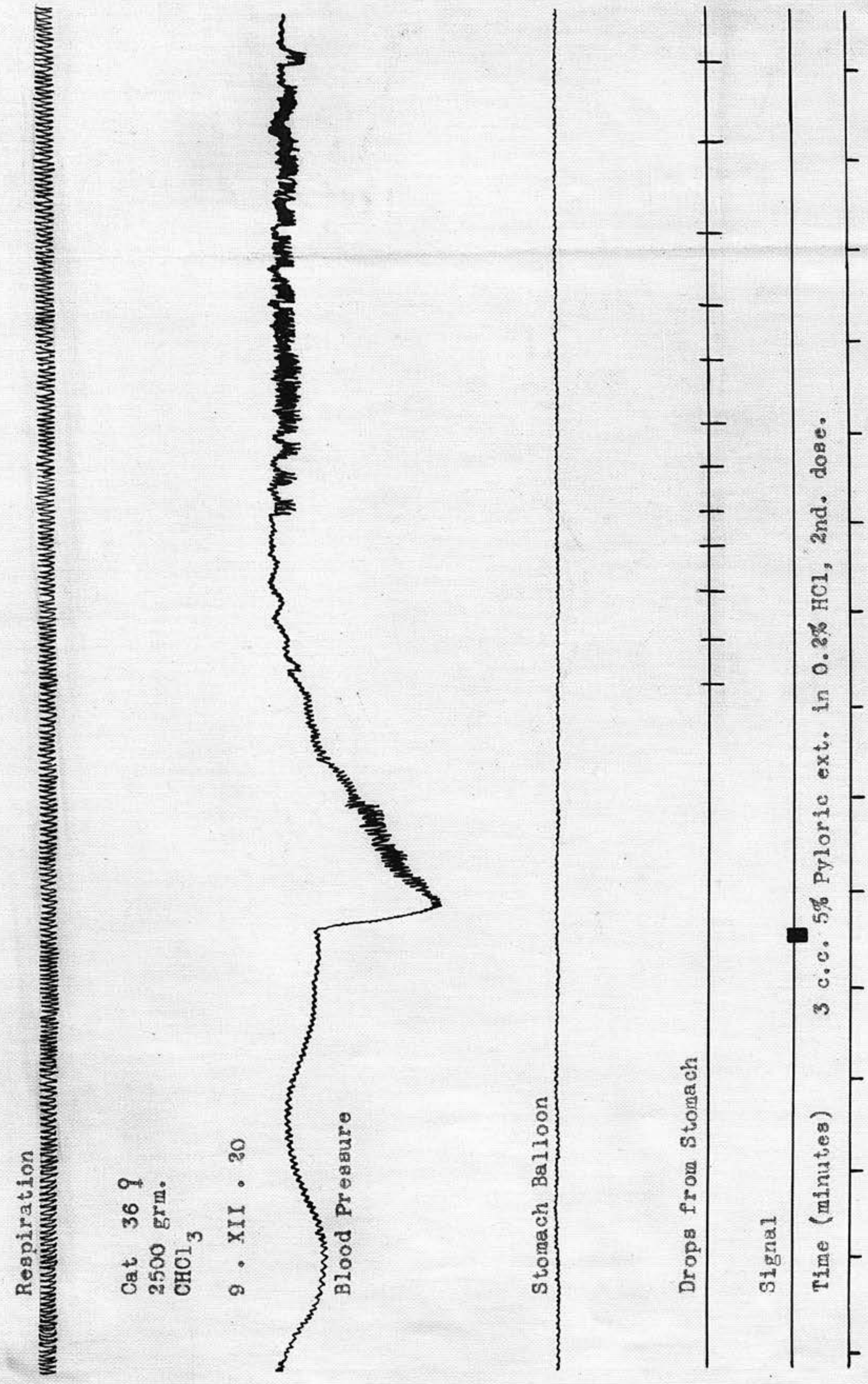
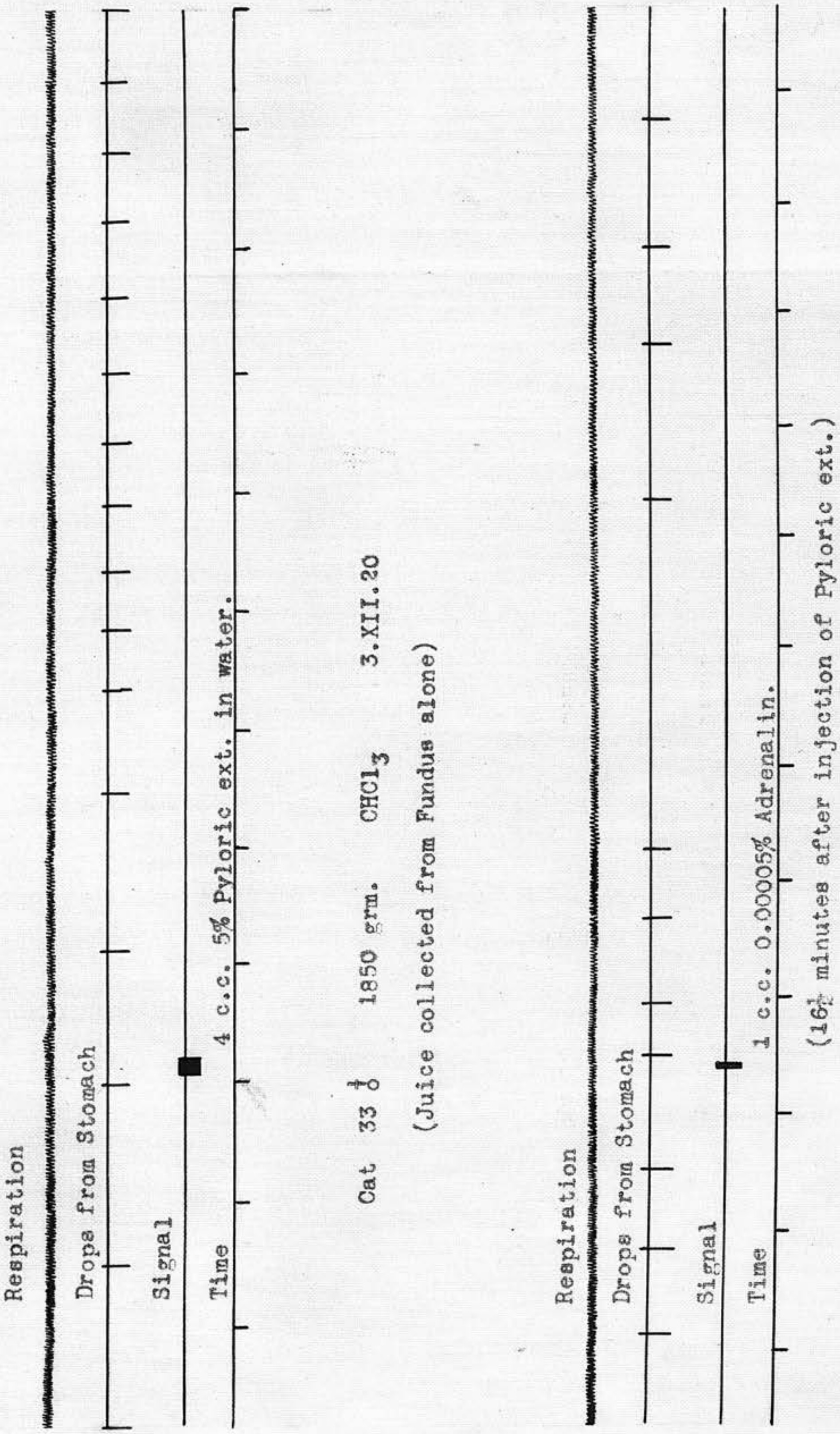

Respiration
Cat 36 ♀
2500 grm.
$CHCl_3$
9 . XII . 20
Blood Pressure
Stomach Balloon
Drops from Stomach
Signal
Time (minutes)
3 c.c. 5% Pyloric ext. in 0.2% HCl, 2nd. dose.

Figure 3.



Respiration
Drops from Stomach
Signal
Time
4 c.c. 5% Pyloric ext. in water.
Cat 33 ♂ 1850 grm. $CHCl_3$ 3.XII.20
(Juice collected from Fundus alone)
Respiration
Drops from Stomach
Signal
Time
1 c.c. 0.00005% Adrenalin.
(16½ minutes after injection of Pyloric ext.)

Figure 4.

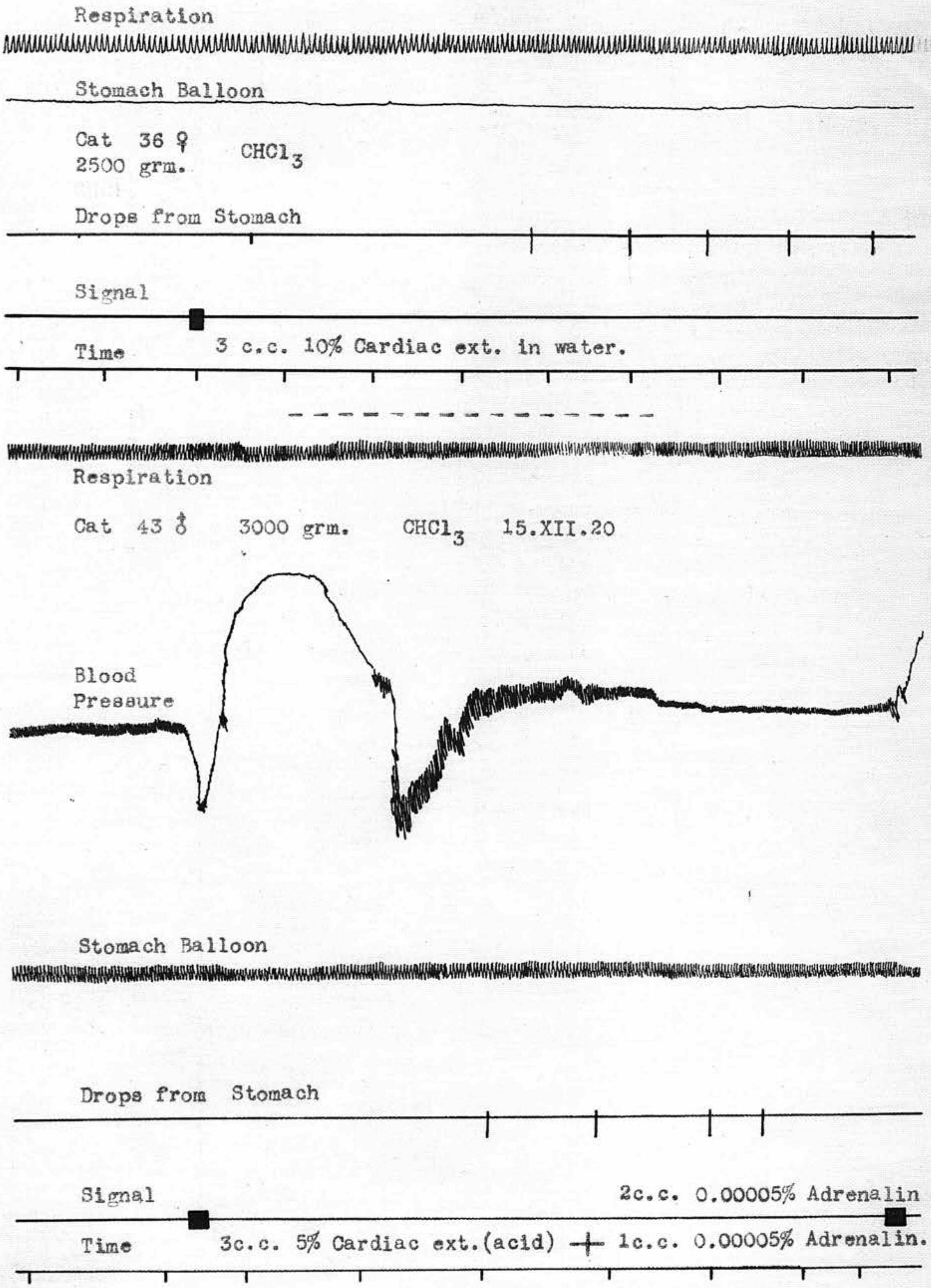

Respiration
Stomach Balloon
Cat 36 ♀
2500 grm.
$CHCl_3$
Drops from Stomach
Signal
Time
3 c.c. 10% Cardiac ext. in water.
Respiration
Cat 43 ♂ 3000 grm. $CHCl_3$ 15.XII.20
Blood Pressure
Stomach Balloon
Drops from Stomach
Signal
2c.c. 0.00005% Adrenalin
Time
3c.c. 5% Cardiac ext.(acid) + 1c.c. 0.00005% Adrenalin.

Figure 5.

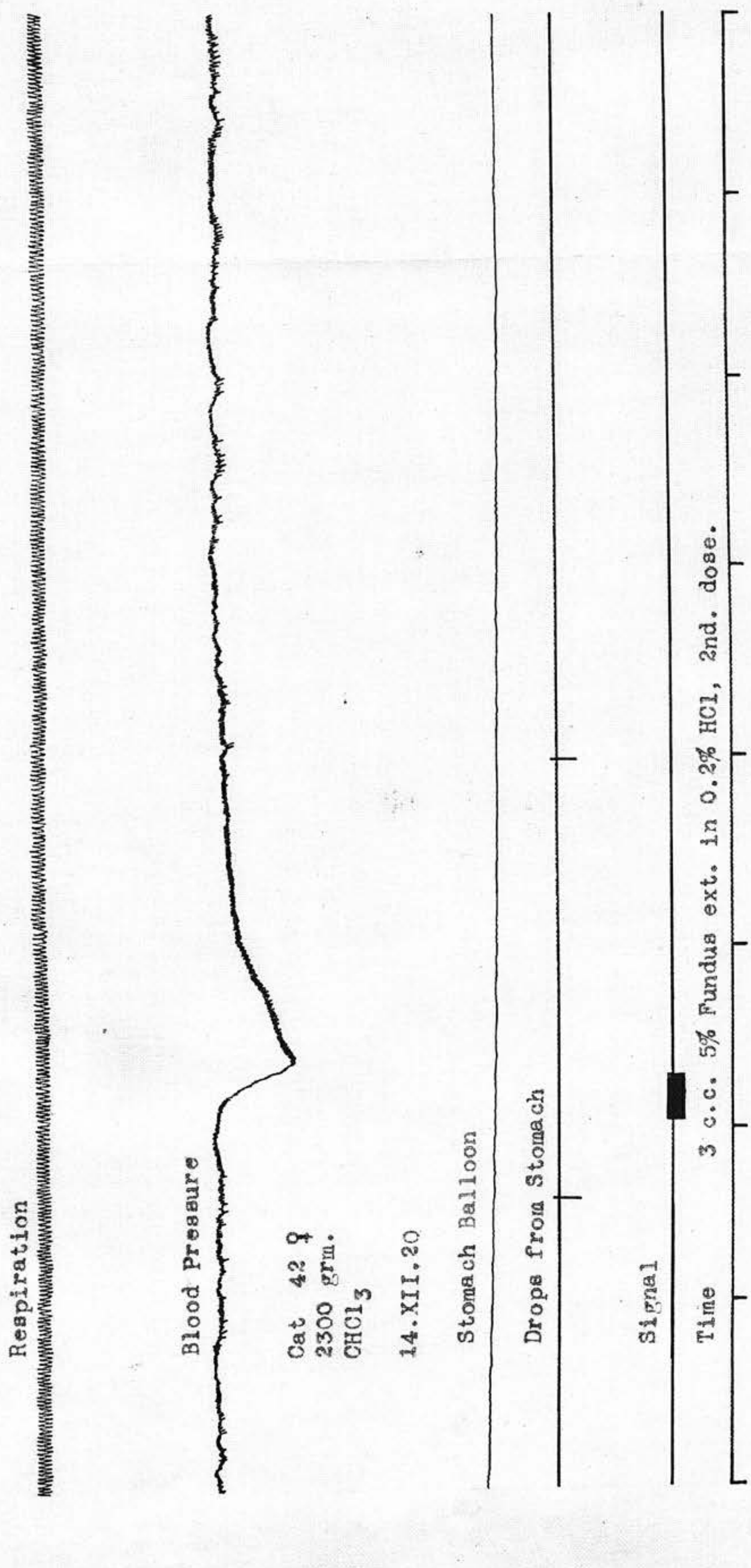
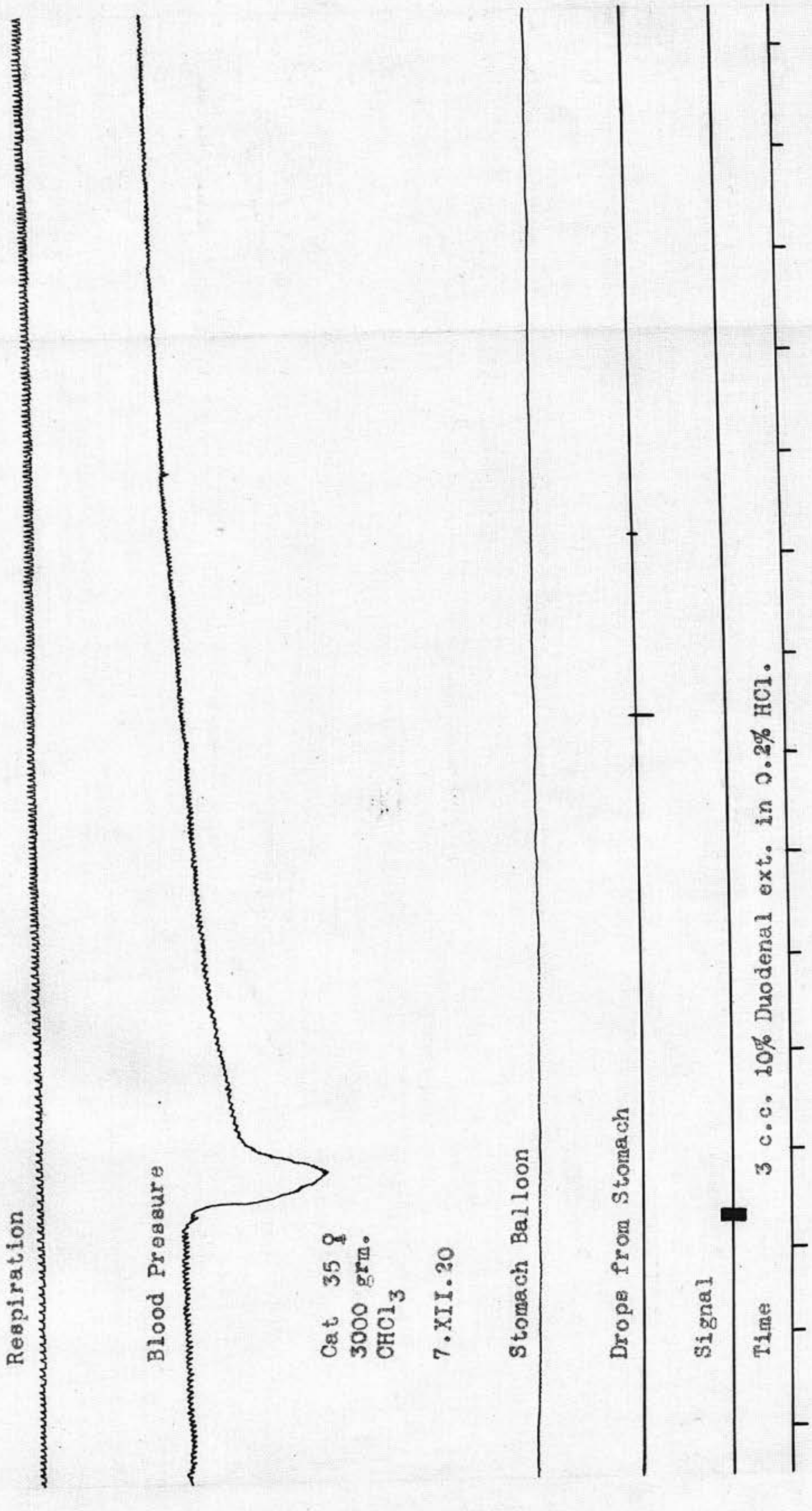

Respiration
Blood Pressure
Cat 42 ♀
2300 grm.
$CHCl_3$
14.XII.20
Stomach Balloon
Drops from Stomach
Signal
Time
3 c.c. 5% Fundus ext. in 0.2% HCl, 2nd. dose.

Figure 6.



Respiration
Blood Pressure
Cat 35 ♀
3000 grm.
$CHCl_3$
7.XII.20
Stomach Balloon
Drops from Stomach
Signal
Time
3 c.c. 10% Duodenal ext. in 0.2% HCl.

Figure 7.

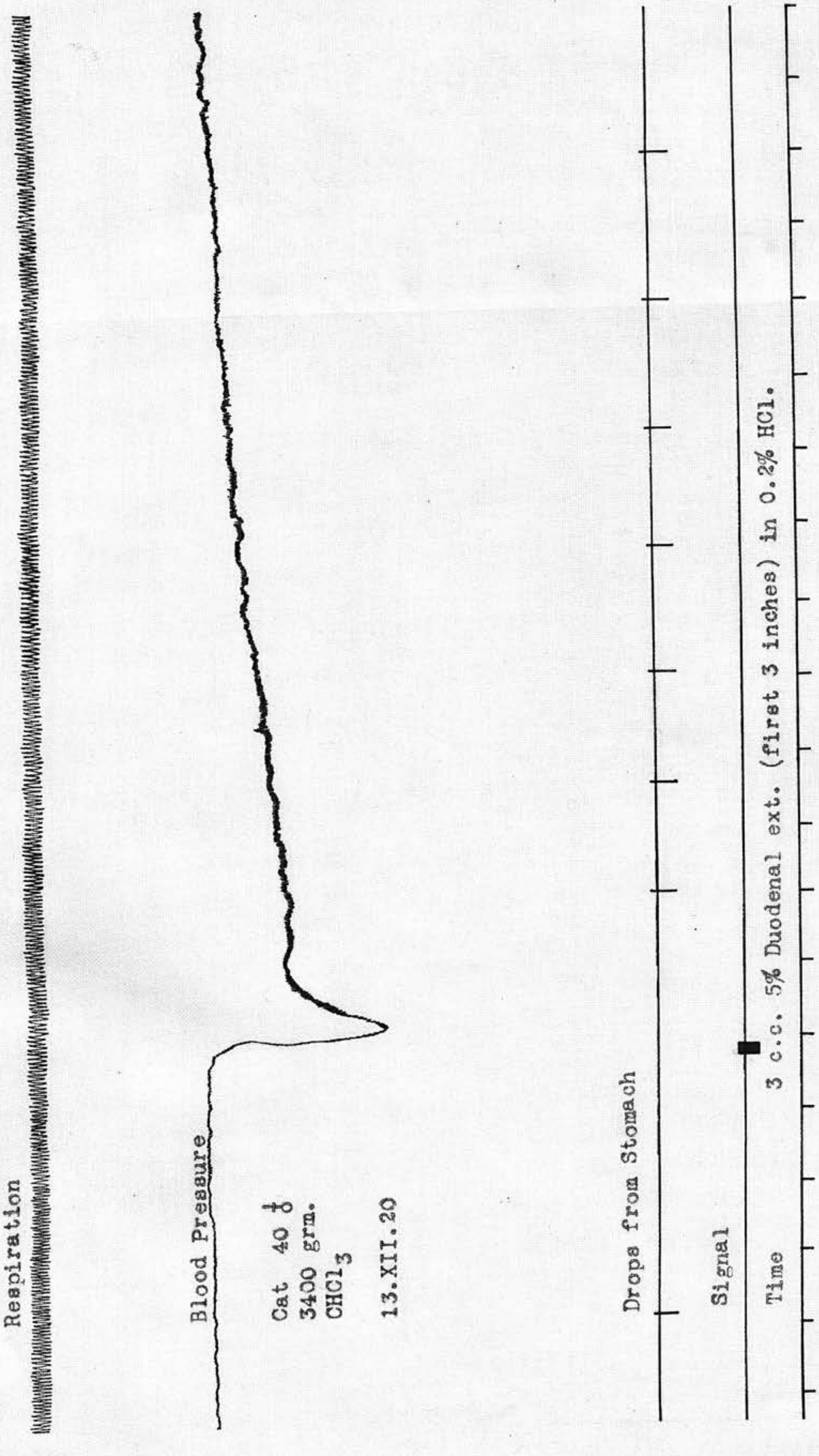
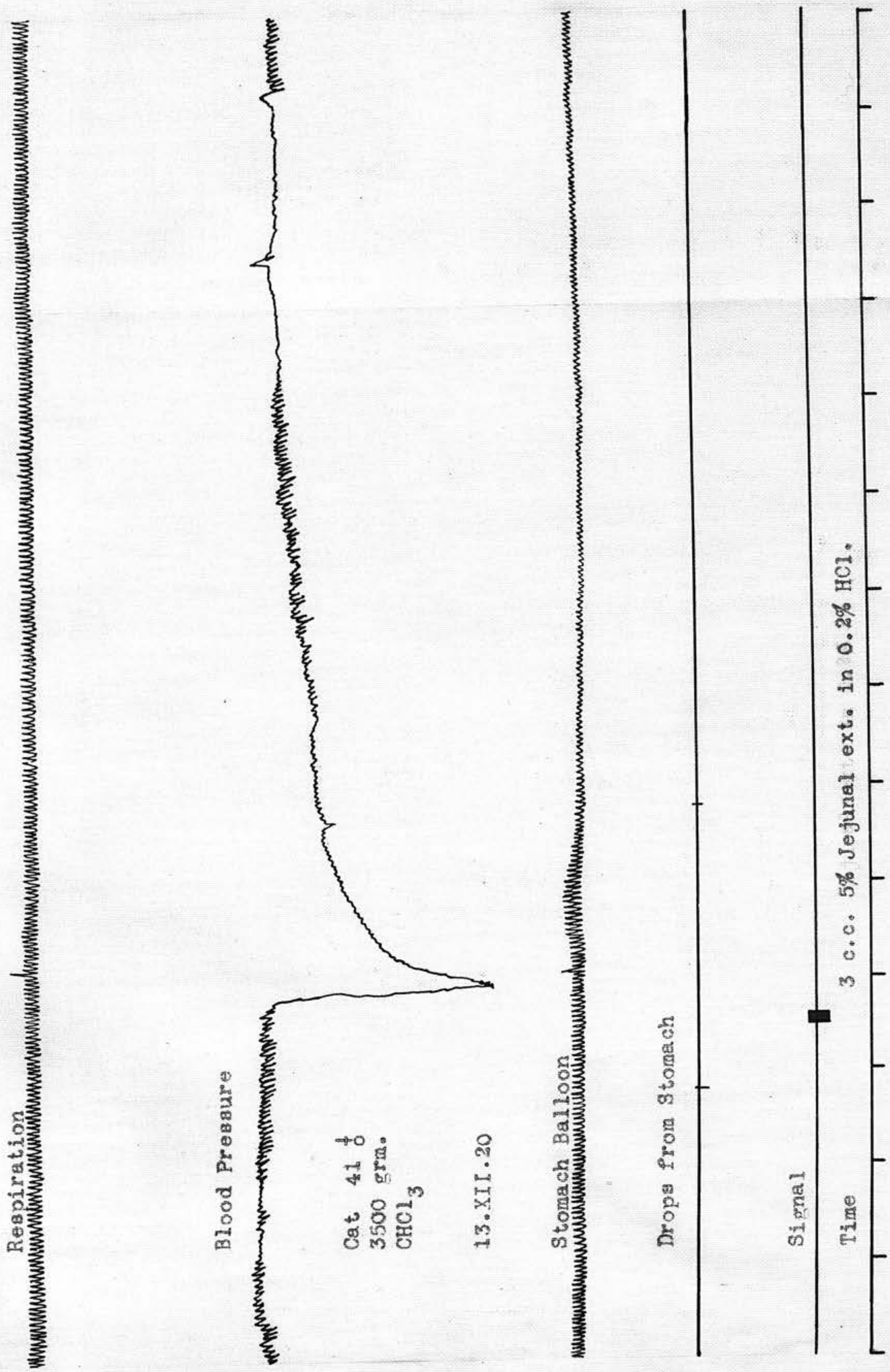
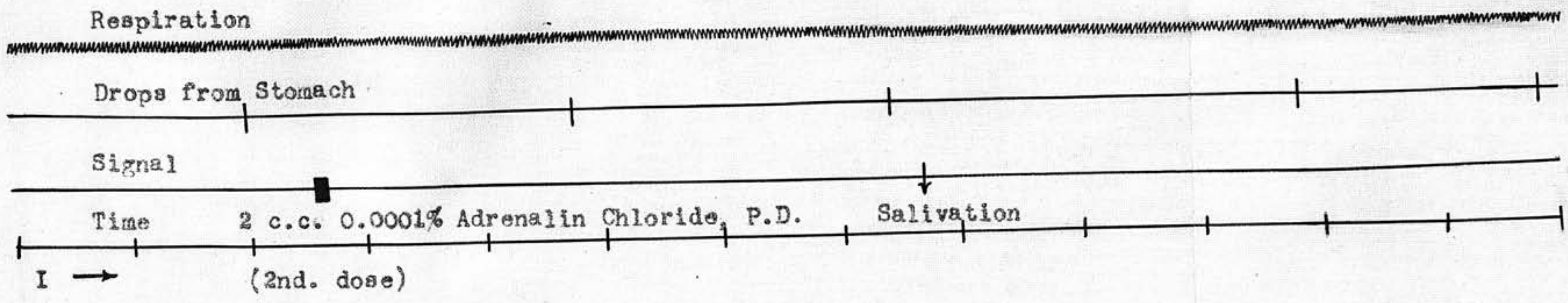

Respiration
Blood Pressure
Cat 40 ♂
3400 grm.
$CHCl_3$
13.XII.20
Drops from Stomach
Signal
Time
3 c.c. 5% Duodenal ext. (first 3 inches) in 0.2% HCl.

Figure 8.


Respiration
Blood Pressure
Cat 41 ♂
3500 grm.
$CHCl_3$
13.XII.20
Stomach Balloon
Drops from Stomach
Signal
Time
3 c.c. 5% Jejunal ext. in 0.2% HCl.



Cat 30 ♂ 2600 grm. $CHCl_3$ Vagi intact.

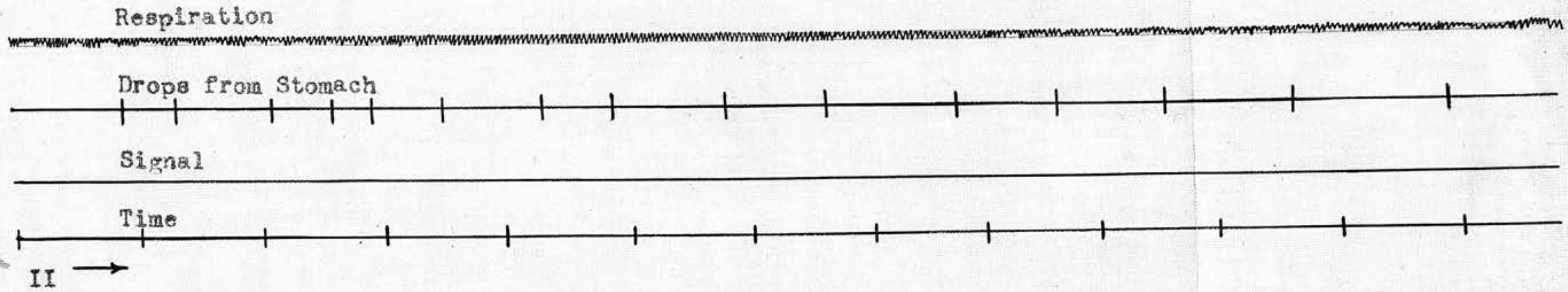


Figure 9.

V. The Source of the Proteolytic Enzyme in Extracts of the Pyloric Mucous Membrane.

Historical.

It has been the experience of many observers that extracts of the pyloric mucous membrane invariably yield a pepsin-like ferment [Wassmann (16), Ebstein (3), Grützner (6), Wittich (17) and others]. The activity of such extracts is far below that made from the fundus. Thus taking the activity of the resting fundus as unity, the activity of the pyloric extracts have been found to be as follows.

Author.	Animal.	Activity of Pylorus.
Grützner (6)	Dog	1/15-25
Ebstein (3a)	"	1/2-3
Glaessner (4)	"	1/21
"	Rabbit	1/17
"	Calf	1/20
Langley (12)	Mole	1/73
Greenwood (5)	Pig	1/40

Further, Grützner has noted that unlike the fundus, the pylorus showed an increase of activity as digestion advanced. For example, if the activity of the pylorus before feeding was 1/20, five hours after a meal it would be 1/2.2 and nine hours later the maximum would be reached with an activity of 1/2. Another difference between the fundus and pylorus was observed by Klug (10). He found that if the pyloric mucosa was extracted with hydrochloric acid for 24 hours, no activity could be detected in the extract. A second set

A second extraction for a similar period proved active, while a third extract showed a still greater activity. Extracts of the fundus made in the same manner did not show this difference. Ebstein and Grützner found that the "pepsin" in the pyloric mucous membrane was removed only very slowly by water alone; this is contradictory to an earlier observation of Wassmann.

Several explanations are available for the presence of the ferment in the pylorus. Wassmann long ago held that the fundus had infiltrated the pyloric mucous membrane. The coup de grâce was given to this view by the experiments of Klemensiewicz (8) and Heidenhain (7) [see below]. These observers isolated the pylorus and found that the secretion was invariably alkaline and proteolytic. As no infiltration was possible, the ferment must have been secreted. While this must be admitted (providing the pouch made contained no fundus elements; see paper I) the question of the identity of the ferment has still to be answered. Langley (12) suggested that the pyloric cells contained a primitive precursor of pepsin (mesostate or propepsinogen) which was slowly activated into pepsin itself by the acid from the fundus. Glaessner (4), however, could not identify the proferment as a propepsin; he found that the extracts were active in either an acid or an alkaline medium and concluded that the ferment was not pepsin but one (pseudo-pepsin) which resembled erepsin very closely. His observations are confirmed by Reach (14) and Pekelharing (13) although the existence of pseudo-pepsin is disputed by Klug (10). Bergmann (1) considered the ferment to be erepsin itself. #

The object of the present investigation was to repeat the older experiments with pieces of pyloric mucous membrane which have been determined histologically to be pure.

<u>Methods.</u>

Part I ...

(3)

Methods.

The stomach was opened as soon as the animal was killed, its interior throughly washed and then dried as much as possible with a cloth. Small pieces of the mucosa from the fundus, pylorus, duodenum and jejunum were dissected or scraped off, separately weighed and then ground with sand. Each portion was subsequently extracted for 24 hours with ten times (volumes) its weight of 33% glycerine in water. The extracts were ultimately filtered and centrifuged, the final product being used for estimation. As the extracts were somewhat opalescent, in spite of the above treatment, dried fibrin (0.02 grm.) was used as the substrate in a medium of 0.2% HCl at 37°C. This was the procedure followed in the first series (I) of four animals, all of whom were in the fasting state.

In the later series (II, III) exactly 0.2 grm. of the fresh mucous membrane of each part was taken; in the case of the pylorus this included the mucous membrane within about 10 mm. from the duodenum. The material was ground without sand and shaken up with 1 c.c. of water for 15 minutes. The whole extract containing the debris was employed. Acid and fibrin were added as before and the tubes incubated. Controls of the acid alone and also of the extract without the addition of acid were utilized.

Results.

Series I

4 Cats Fundus 1-3 hrs.+ Pylorus 8-20 hrs.+

Duodenum 30-48 hrs.+ Jejunum and controls negative 48 hrs.

The fundus extract was invariably the most active, and dissolved the fibrin in the case of three of the animals within 2-3 hours and in the other animal between/

between 1-2 hours. The pyloric extracts in all four cases did not dissolve the fibrin in 8 hours - the next observation was made at the 20th. hour when the reaction was complete. The duodenal extracts were negative during the 20 hours period but were found to be positive after 30-48 hours, while the jejunal extracts and controls were still negative. The pyloric extracts without HCl were quite inactive during this period: the reaction was neutral to litmus and probably slightly on the alkaline side of neutrality.

Series II.

Cats.	Fundus	Pylorus	Hours since last meal.
26	27 minutes +	1 hr.55 min. +	5 hours
23	45 " +	4 hrs.(negative)	1 "
25	45 " +	7 " +	18½ "
20	50 " +	3 " (negative)	14 "
22	1 hour +	4 " "	19 "
24	1½ hours +	5 " "	1 "

The strength of the extracts in this series was double that in the first series so that a shorter reaction time was expected. Careful observations were made every 5 minutes for the first hour and evry 15 minutes later in order to determine the exact end point, but only in Cats 25 and 26 were the observations sufficiently prolonged to complete the digestion of the fibrin. Taking the fundus time in each animal to be unity, the activity of the pylorus (calculated according to the Schütz-Borisoff law) in Cat 25 is about 1/87 and in Cat 26, about 1/18. In the case of the other animals the activity of the pylorus was certainly less than 1/18, with the possible (though improbable) exception of Cat 24. The results show that the pylorus (containing only the pyloric cells) has a "pepsin" content which is considerably lower than that of the fundus. When digestion/

digestion is in progress, both parts of the stomach appear to increase their ferment, but this is only apparent some five hours after the meal. One hour after, there may be no change (Cat 23) or there may be a marked fall in the activity of the fundus (Cat 24). This last result is in conformity with Grützner's observations, while the increase in fundus activity in Cat 26 may be a normal variation. This is extremely likely since the histological appearance of both the peptic and pyloric cells vary considerably in animals apparently in the same stage of fasting or digestion. (we would have passed this over as being within the experimental error had I not found the same change in the case of the frog: see below).

Series III.

Frogs	Oesophagus (includes muscle coat)	Stomach	Remarks
R. Esculenta	+ 45 minutes	+ 1 hr. 40 m.	Fed 5 hrs. ago
"	+ 55 "	− 2 " 30 "	Unfed
"	+ 55 "	+ 5 "	"
"	+ 1 hr. 15 min.	+ 5 "	"
R. Temporaria	+ 1 hour	+ 4 "	"
"	+ 55 minutes	+ 1 " 35 "	Dead parasite in stomach.

The above results entirely confirm those of Swiecicki (15), Contejean (2) and Langley (12), and show that during digestion there is an increase in the activity of the stomach extracts. It is well known that the frog's stomach contains no peptic cells; these results indicate either infiltration or the secretion of a small amount of pepsin (or a pseudo-pepsin) by the mucoid cells (see paper III).

Part I; Conclusions.

Discussion.

Discussion.

The results are on the whole in agreement with previous work. The doubt which I had regarding the histological purity of the pyloric extracts made by the older observers have thus been dispelled; the repetition was essential. While we must accept the presence of a proteolytic ferment in the pyloric extracts as a constant feature of such preparations, it is very probable that a portion is due to infiltration of pepsin from the fundus. This seems inevitable when the triturating action of the pylorus is considered. Further, infiltration would account for the enormous rise in pyloric activity during digestion. I have on two occasions found the gastric mucus (frog) to be strongly proteolytic - and it is obvious that the ferment only becomes activated in the presence of the acid secreted by the stomach. On the other hand infiltration cannot account for"pepsin" in the stomach of winter frogs (R. Temporaria) who had not fed(and therefore could not secrete any juice) for at least a week. So that secretion must also occur. As to the nature of the ferment, I cannot agree with Glaessner that it can act in an alkaline medium. It certainly does not digest fibrin in a neutral medium and therefore could not do so in an alkaline one. The optimum activity is situated on the acid side of neutrality and does not embrace a wide range like erepsin. The ferment differs from pepsin in its weakness; it may be as Langley suggested a primitive pepsin.

Its exact nature and how its is secreted can only be properly determined by observing the secretion of the isolated pylorus.

Summary.

(1) A proteolytic ferment, which is much weaker than pepsin in its action, is invariably present in pyloric extracts. It does not act in a neutral/

neutral or faintly alkaline medium.

(2) Infiltration of pepsin from the fundus accounts for part of the activity observed, especially the increase in activity during digestion, but a secretion of ferment must also be admitted.

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VI. A New Method for Obtaining a Pure Pyloric Secretion.

The necessity for the formation of a histollogically pure pouch for observing the pyloric secretion has already pointed out. The objection to the pouches made by previous workers has been expressed on several occasions throughout these papers, it is that they may have included fundus (peptic) elements.

The first to institute these experiments was Klemsiewicz (3). He made his pouch after the Thiry method, i.e., he resected the pylorus and joined the fundus to the duodenum behind the isolated pylorus, which was connected with the exterior by a fistula. His measurements for the resection are of interest from the point of view of my criticism. He divided the stomach 5 cm. from the duodenum along the lesser curvature and 2 cm. along the greater curvature; in larger dogs, he allowed another inch on each side. If the measurements given in Paper III, be compared it will be noted that they exceed the limits of the pylorus along the lesser curvature. (The dogs which I examined were about 10-12 kilos and were slightly above the terrier size.)

Heidenhain (2) improved the technique but followed the method in principle. Akermann (1) employed a similar method, while Schemiakine (6) used both the Thiry and the Pavloff (5) procedures. The last method has the advantage of having both the nervous and vascular connections intact, but these are outweighed by the extreme narrowing of the pylorus which gives rise to gastric obstruction. Even with Heidenhain's method this is liable to occur for Heidenhain lost two/

two out of three animals, which had escaped sepsis, from this cause. Kresteff (4) has also investigated the pyloric secretion in the above manner. #

All these observers have found that the secretion is alkaline and proteolytic.

Part II.

Technique.

The method which I have employed differs from both the procedures already described in several ways, and has not the the disadvantages which are attached to them. The essential steps of the operation are as follows.

The abdomen is opened in the mid-line for about 4 inches. The stomach is obtained and the large omentum opened at its right border. The duodenum is next sought for and a portion (3 inches from the pylorus) is clamped in readiness for a gastero-enterostomy with the posterior surface of the stomach. This is carried out about 2 inches (roughly 5 cm.) from the pyloric sphincter. A small oblique incision is next made in the anterior surface of the pylorus, through the muscular coat only, along a line joining the incisura angularis and a point 15 mm. from the pyloro-duodenal junction, along the greater curvature. The incision does not extend quite to either curvature and is about 15 mm. long. It is carried down to the submucous layer and then by blunt dissection, the mucous membrane is completely separated from the muscular coat along a circular strip, about 10 mm. broad, right round the bowel. The freed mucosa is doubly clamped and divided, leaving not more than 20 mm. of the pylorus along the lesser curvature and a little less on the greater. The ends are sewed up, the fundus being inverted but the pylorus not. A little omentum, a portion of the right border, is freed and inserted into the muscular pocket, and the wound in the stomach wall sewed up lightly. The duodenum next to the pylorus is next divided between clamps, the duodenal stump being carefully closed and inverted, while the pyloric sphincter is brought out through a stab wound/

wound in the right rectus muscle and anchored to the abdominal wall. The main wound is closed and the animal recovers completely from the operation in three or four days. The danger of this operation is surgical shock, but this can be in a measure combated by giving the animal dextrose subcutaneously during the course of the operation. Of course the strictest asepsis has to be observed. (I have had the assistance and collaboration of Dr. Norman M. Dott in the performance of these operations and their complete surgical success is largely due to his care on the last point I have mentioned.)

At the moment of writing, there are two cats and one dog, which have been operated in the above manner over a month ago; they had an uninterrupted course of recovery. In each case the histological examination (made from the parings of the clamps holding the pylorus) showed that the ~~pylorus~~ pouch left was purely pyloric.

The advantages of the method are that the nerves are not interfered with to such an extent as in the Pavloff operation, the blood supply is almost intact, the muscular coat is little altered in its relations, and lastly the fistula has the powerful pyloric sphincter to prevent undue leakage.

The accompanying figure will give an idea as to the extent of the operation.

Report.

This is necessarily brief as the animals have not yet been subjected to a thorough investigation.

Cat 50 Female 2300 grm.

Operation lasted 1½ hours: recovered from the anaesthetic almost immediately Lived about 30 hours. Post mortem showed no signs of abdominal sepsis. Death from shock. Pyloric pouch contained a viscid secretion which was alkaline and actively proteolytic.

Cat 51

Cat 51 Male 2900 grm.

Operation lasted 1½ hours: this animal never properly recovered its appetite.

4th. day: Pouch secretion alkaline and proteolytic.

8th. day: " " " "

11th. day: Killed on account of severe diarrhoea (Distemper?)

Post mortem showed perfect healing of all the sutures: no signs of sepsis or pneumonia. The intestines were very congested and full of fluid chyme. The pyloric mucous membrane was extracted and found to contain no free HCl but a proteolytic ferment.

Cat 52 Female 2900 grm.

Operation lasted 1¼ hours. Animal recovered perfectly on third day: took milk and fish broth.

20th. day: Pyloric secretion contains a ferment but no acid. Wound perfectly healed except at lower end, but the pouch is everted and it is difficult to collect juice. (This animal had its pyloric mucous membrane stump inverted, hence the prolapse.)

30th. day: Juice again examined with the same result.

40th. day: Animal well but has liquid stools; this is probably due to the gastero-enterostomy.

Cat 54 Male 2700 grm.

Operation lasted 1⅓ hours: recovered perfectedly after the third day.

20th. day: Wound healed. Juice collected is scanty and viscid. It contains a ferment and is alkaline. Animal is keeping well.

Dog 3

Dog 3 Female 10 kilos Terrier.

Operation lasted 1½ hours: recovered itself four days afterwards.

Takes milk or water and biscuits in the morning, and mince in the afternoon.

6th. day: Cuff of pyloric mucous membrane sloughed off. Wound is not quite healed.

12th. day: Animal very lively: pyloric pouch healing fast -tendency to close.

15th. day: Secretion tested and found to be proteolytic and alkaline.

20th. day: " " " " " "

30th. day: Animal keeping well.

The above observations though scanty show that the pyloric secretion is undoubtedly proteolytic. This confirms all previous work and shows that the same result would have been obtained (qualitatively) even if a portion of the fundus had been included in the pouch.

This part of the work was only begun in the beginning of February this year and I have not had sufficient time to carry out a minute investigation into the nature of the pyloric secretion as yet. However, one result is already achieved, namely that the conclusions derived from the experiments on pyloric extracts is confirmed and all doubts have been removed.

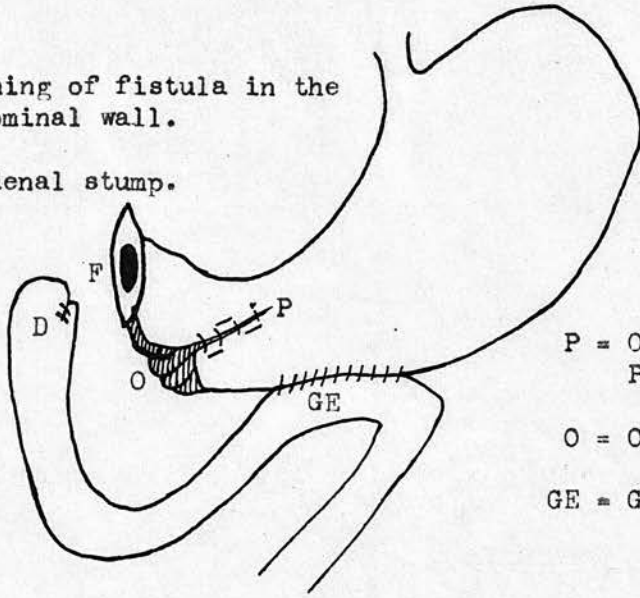
Literature.

(1) Akermann, Skand. Arch. f. Physiol., 1895, V, 134.
(2) Heidenhain, Pflüger's Arch., 1878, XVIII, 169.
(3) Klemensiewicz, Jahresb. ü. d. fort. d. Tierchem., 1875, V, 162.
(4) Kresteff, Rev. méd. d. l. Suisse Romande (quoted from Schemiakine)
(5) Schemiakine, Arch.d. sci. biol., St. Petersbourg, 1903-4, X, 87.
(6) Pavloff, The Work of the Digestive Glands, 1910, 2nd. edition, transl. by W. H. Thompson, London.

Figure to show the method for isolating the Pylorus.

F = Opening of fistula in the abdominal wall.

D = Duodenal stump.



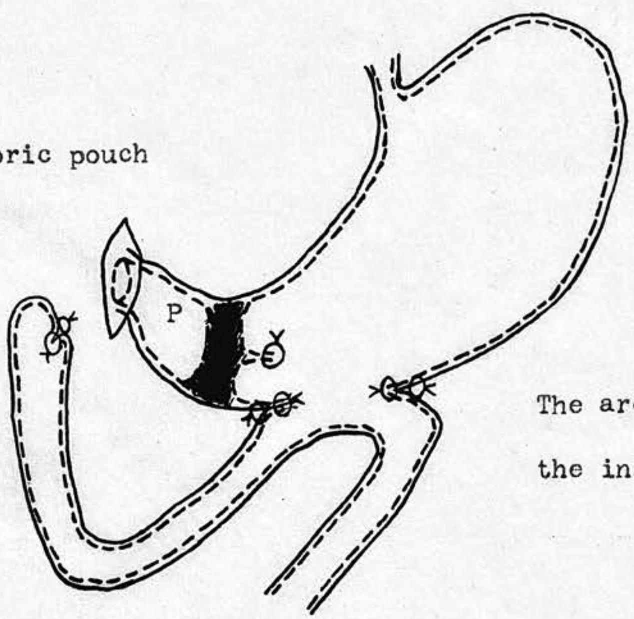
P = Oblique incision in Pyloric muscle coat.

O = Omental insertion.

GE = Gastero-enterostomy.

Diagram showing the external appearance of the complete operation.

P = Pyloric pouch



The area in black represents the inserted omentum.

Diagram showing the relations of the mucous membrane after the Operation.

VII. General Conclusions Regarding the Functions of the Gastric Mucoid Cells.

(1) The mucoid cells of the fundus do not secrete pepsin in foetal or newborn animals: whether they secrete a ferment in later life remains to be determined. Histologically there is no indication of zymogen formation.

(2) The pyloric cells (and possibly the cardiac) secrete a pepsin-like ferment and probably mucus as well. Besides, they have an absorptive function. There is no conclusive evidence of an internally secreting function of these cells.

The expenses of this work have been defrayed by a grant from the Earl of Moray Fund for the Promotion of Original Research in the University of Edinburgh.
