Publications referred to.

1. Period of Survival of the Shore Crab in Distilled Water.
2. Experiments on the Respiratory Mechanism of the Shore Crab.
3. An Alcoholic Eosin and Methylene Blue Staining Method.
4. (With Sir Edward Sharpey Schafer), The Effects of Adrenaline on the Pulmonary Circulation.
5. The Effects of Thyroid Extract on Tadpoles.
7. A Parasitic Spiral Organism in the Stomach of the Cat.
8. The Histology of Tadpoles fed with Thyroid.

The above pamphlets constitute a thesis for Ph.D. presented by R. K. S. Lim, M.B.
AN ALCOHOLIC EOSIN AND METHYLENE-BLUE STAINING METHOD.

By R. K. S. Lim,
From the Department of Physiology, Edinburgh University.

An Alcoholic Eosin and Methylene-Blue Staining Method.

By

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Aqueous or alcoholic solutions of eosin and aqueous solutions of methylene blue have long been individually and successively employed for the double staining of sections. (It is not intended here to deal with the mixed or combined staining method of Romanowsky (8), Ehrlich (1), and others.) The various methods now in use (Schafer (9), Sims Woodhead (10), Miller (5), etc.) may be generally described as follows:

Sections are stained with a water-soluble eosin solution for periods of 5–20 minutes or longer. They are then washed with water and brought into contact with a methylene-blue solution for a usually shorter time. After being again washed, they are differentiated and dehydrated in absolute alcohol, and subsequently cleared and mounted. Mallory and Wright (4) employ Unna’s methylene-blue, which contains 1 per cent. of potassium carbonate. Richard Muir (6) uses a saturated solution of alcoholic eosin in rectified spirit, but drives off the alcohol by heat during the process of staining, leaving the eosin in watery solution. He then rinses in water and places in saturated potash alum for 3 minutes, subsequently decolorising with alcohol containing a trace of ammonia, and, after washing with water, stains with methylene-blue.

For blood-films the general method given above for sections
has been advantageously used after adequate fixation, e.g. with methyl-alcohol or with formol-alcohol (Gulland (2)), with the exception that the film is dried and mounted after the methylene-blue has been washed off. Türk stains with 0.5 per cent. eosin in 60-70 per cent. alcohol; he both dries and heats the film before applying methylene-blue solution.

Films of pus or of other exudates are also stained in a somewhat similar way (Muir and Ritchie (7)).

The results obtained by the above methods mainly depend upon the experience of the histologist. A successful preparation demonstrates well the nucleus, cytoplasm, and cell-granules, the latter especially if Richard Muir's method is used. The failure to obtain constant results is due to the difficulty of obtaining good differentiation. This difficulty is largely overcome with formalin-fixed tissues by the use of the following solutions, viz. 1 per cent. solution of alcohol-soluble eosin in rectified spirit, and 1 per cent. solution of methylene-blue in distilled water. In employing these for sections the latter are treated as follows:

1. Remove paraffin with xylol or benzol, then wash with absolute alcohol.
2. Pour on alcoholic eosin solution and leave for one minute.
3. Wash with water (distilled or tap).
4. Pour on methylene-blue solution and leave for one minute.
5. Wash with water; the sections should appear purplish.
6. Wipe slide dry with a fine cloth, leaving only the section moist.
7. Pour on absolute alcohol liberally to differentiate the staining, and immediately carry out the next step.
8. Pour on xylol or benzol to stop the differentiation and to clear the section for mounting.

During the manipulations the slide should be held obliquely in order that the reagents may run off.

The section may now be examined, and if found to be insufficiently differentiated, steps 7 and 8 may be rapidly
repeated; as a rule this is not necessary. When correctly differentiated and cleared, mount in dammar.

It is sometimes more convenient to have the methylene-blue and xylol in vessels large enough to accommodate a slide. In this case the slide should be agitated within the xylol until the section is cleared, when it may be mounted in dammar.

For blood or exudate films the same technique, if carried out implicitly, will give good results. The film is allowed to dry as slowly as possible in a cool place—the slower the better. When quite dry, staining may be commenced. Fixation is accomplished by the alcohol of the eosin solution, although for rapid work the film may be inundated with absolute alcohol for from 1 to 3 minutes prior to staining.

The above-described method has been used successfully upon sections for some time in this laboratory. It is rapid, simple, and certain, and is well suited as a routine procedure for most tissues. The preparations do not readily fade; some made in 1916 are as yet quite unchanged. It is particularly useful for the central nervous system and for the peripheral ganglia. It stains axis cylinders a deep red, Nissl's granules blue. Connective tissue is also stained an intense blue. It is valuable for glands, especially the pituitary, pancreas, and suprarenal. The oxyphil and basiphil granules of the anterior lobe of the pituitary are clearly differentiated; while in the pars nervosa (in man), free, coarse, greenish (basiphil?) granular masses, seemingly not identical with those described by Herring (3), are shown.

The results with blood-films are equal to, but are not claimed to be better than, a good Leishman (8) stained film. Nuclei of white blood-corpuscles are stained blue, granules according to their affinities, while the red blood-corpuscles come out bright red. In films of pus, etc., the bacteria are also stained blue.

In connection with this part of the subject, I wish to thank Dr. A. K. Towers for providing me with clinical material.
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THE HISTOLOGY OF TADPOLES FED WITH THYROID. By R. K. S. Lim. (From the Department of Physiology, Edinburgh University.) (With nine figures in the text.)
THE HISTOLOGY OF TADPOLES FED WITH THYROID. By R. K. S. Lim. (From the Department of Physiology, Edinburgh University.) (With nine figures in the text.)

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PRELIMINARY REMARKS.

The influence of thyroid and other endocrine organs on larval amphibians was first studied by Gudernatsch (7), who found that thyroid "caused a precocious differentiation of the body, but suppressed further growth." Since 1912 his results with regard to thyroid have been amply confirmed by Romeis (19), Cotronei (5), Giacomini (6), Lenhart (11), Marine and Rogoff (14), Kahn (9), and other investigators. The effects of removal of the thyroid (by cauterisation, Smith (20), Allen (1), and by excision, Hoskins and Morris (8), Allen (1)) have also been observed, and were found to be the converse of those obtained by feeding. The results of the present experiments agree with Gudernatsch with regard to the general effects, but deal mainly with the histological changes.

The technique employed was somewhat similar to that of previous investigators. Tadpoles of Rana temporaria were fed at various periods after hatching. Series of experiments were performed as far as possible with larvæ from the same set of spawn. In some of the experiments many individuals were grouped together in glass jars holding about a litre of tap-water, sometimes less; later, only one individual was placed in each vessel. This was found necessary as the tadpoles had cannibalistic tendencies.

In a few of the experiments the tadpoles were given pieces of fresh glands from the sheep, although in the majority water-extracts of both fresh and desiccated (sheep) materials were used along with some pond weed. The extracts were made by boiling with water and subsequently filtering; those made from fresh material were all of 10 per cent. strength, while those from dried glands were 2 per cent. Extracts of muscle, pituitary, suprarenal, and thymus were fed to other animals in each series.

For microscopic examination the tadpoles were fixed in 10 per cent. formol, carried through alcohol and xylol, and embedded in paraffin. Serial sections, 5–7 μ thick, of each animal were cut. The stains used were either haematoxylin and aqueous eosin or alcoholic eosin and methylene blue (12, a).
RESULTS OF EXPERIMENTS.

In all, eight series of experiments were carried out. A summary of the results is given below:

Series A.—Five eggs were placed in each of six 300-c.c. jars with 10 c.c. of each of the above extracts but no weed. On the third day all the eggs had hatched out. Observations on this series were terminated after the tenth day, no notable differences having been observed.

Series B. (Tadpoles 10-12.5 mm.)—Twelve tadpoles were placed in each one-litre jar with a little green weed. The experiment lasted twelve days, at the end of which period all the thyroid tadpoles were dead, and appeared very small and shrivelled.

Series C. (Tadpoles 12.5-17.5 mm.)—A similar experiment to the last, except that the doses of the extracts were halved (5 c.c.). On the nineteenth day all the thyroid animals were small and inert; one tadpole from each jar was removed for microscopic examination.

Series D. (Tadpoles 15-20 mm.)—A continuation of the above. On the twenty-second day all the thyroid tadpoles were found lying at the bottom of the jar. They were inert and small, with shrunken tails and bodies, the foremost part of which presented clear swellings. When touched they could only wriggle their tails; they were unable to swim. These were all placed in formol. On the twenty-sixth day the largest tadpole from each of the other jars was placed in the thyroid jar, which on this occasion received 1 c.c. of the 2 per cent. extract. No change occurred, except that several of the tadpoles showed deviation of the tail. On the fortieth day (fourteen days after treatment) all the thyroid tadpoles were small, shrunken, and inert; one was dead. (The daily dose of thyroid had been increased to 20 c.c. a week previously.) Among the rest, the anterior pituitary and pituitary groups included both the largest and the smallest individuals. The experiment was then terminated.

Series E. (Tadpoles 25-35 mm.)—This was a repetition of former experiments, but with only a single tadpole in each jar. The animals were weighed and measured from the outset. The results, along with those of series F, are set out in graphs in fig. 1. On the seventh day the thyroid, pituitary, and anterior pituitary tadpoles had begun to bud out hind limbs. The thyroid was smaller and was continually “gasping.” Both thyroid and muscle tadpoles had lost weight, but the others had gained. On the ninth day the thyroid tadpole showed a marked decrease both in weight and length, and had developed all four limbs; it died in the afternoon. Feeding of the rest was continued till the thirty-sixth day. The pituitary and anterior pituitary tadpoles were found dead on the twenty-sixth and twenty-eighth day respectively. The differences in size and weight of the tadpoles may be seen in the graphs. The muscle and suprarenal tadpoles were least developed.

Series F. (Tadpoles about 30 mm.)—A similar experiment to the
last, except that the thyroid extract was increased to 50 c.c.; a "no extract" control was introduced. The thyroid tadpole was found dead on the ninth day. Of the remainder, the suprarenal tadpole was least developed. This experiment was terminated on the thirty-fourth day.

Series E.

Series F.

Series G. (Tadpoles 20–25 mm.)—Two series of experiments were carried out by feeding with pieces of the fresh gland, one gramme of each being used. The experiments were terminated on the eleventh day; all the rest were larger than the thyroid tadpoles, which were small and shrunken.

Series H. (Tadpoles 20–25 mm.)—This experiment was carried out to compare the effects of extract and gland substance in the case of thyroid,
**Fig. 2.**—Muscle-fed tadpole. Series C. x 60. Photograph.
L, liver; P, pancreas; S, stomach; I, intestine.
The interior of the intestine is occupied by vegetable débris and pigment.
A portion of the pronephros (HK) is also included in the section.

**Fig. 3.**—Thyroid-fed tadpole. Series B. x 60. Photograph.
The intestine is small, but has a thick muscle coat; pigment masses may be seen rupturing the mucosa and lying within the lumen. The liver is deeply pigmented. (Compare with fig. 2.)
Thymus, and suprarenal. Half a gramme of each gland, 20 c.c. of the thyroid and thymus extracts and 2 c.c. of adrenalin chloride (1–1000, Parke, Davis & Co.), were given. On the seventeenth day all the gland-fed tadpoles were larger than the “extract” animals, except the thyroid, which were both small; the gland-fed (thyroid) group were found dead, and were removed. On the twenty-fourth day the differences previously noted were still present: all the groups were now placed in formol.

Thyroid thus causes the tadpoles to be small and inert. The head region appears swollen and the tail is absorbed, and, further, the limbs are rapidly developed; in short, the animals are “miniature frogs.” These changes are in accord with all previous observations. The “gasping” exhibited by the thyroid tadpole in series E may be a functional indication of the involution of the gills.

From series A it seems that tadpoles are not obviously influenced by feeding until some days after hatching, but histologically the thyroid tadpoles of this series appear to be affected (see below). Series G and H show that the only difference between feeding with extract and with gland substance is that animals grow larger under the latter treatment. Differences in size and weight met with during the experiments are largely attributable to variations in feeding. The thymus-fed animals were in no way different from the other “non-thyroids,” contrary to Gudernatsch’s observation; suprarenal-fed animals were less developed. The graphs on p. 505 indicate the relation between length and weight; they also show the variations encountered. A noteworthy point is the sudden decrease in length and weight consequent on metamorphosis; if this process be incomplete, the tail remains long, but nevertheless the weight falls. The deviation of the tail observed in some tadpoles has also been noted by Allen, who attributes it to the quality of tap-water used.

Microscopical Appearances of Thyroid-fed Tadpoles.

Histological changes may be found as early as series A, but are not so marked as in series B and those following. Here the appearance of the alimentary tract differs entirely from controls, the difference being more evident the older the tadpole. The stomach and intestines are small but have well-developed glands in the mucous membrane, and a muscular coat which is even better differentiated, whereas the same organs in controls are large (distended) and are lined only by a single layer of columnar epithelium, the muscle coat being absent or at least inconspicuous (cf. figs. 2 and 3). Further, the amount of pigment in the thyroid-fed mucosa and submucosa is enormously increased. In the mucosa pigment masses lie between the epithelial cells in rounded spaces, which may be so large that the cells overlying them are flattened and the mass, which by this time has become vesicular, projects into the lumen. These vesicles appear to discharge their contents by rupturing, since the lumen is
**Fig. 4.**—Thyroid-fed tadpole. Series D. ×300. Photograph.
Transverse section through the intestine, showing pigment masses breaking through the mucosa. Note how the surface cells are projected into the lumen.

**Fig. 5.**—Thyroid-fed tadpole. Series G. About ×1500. Drawing.

a, an involuntary muscle fibre; b and c, cells from the deep layer of the mucosa.

Mitoses seen in a tangential section of the stomach. (All these mitoses were seen in one field.) Note also the size of the resting nuclei.
in places completely filled with cell-débris and pigment granules (see figs. 3 and 4) and the excreta are invariably pigmented. In series E the pigment is less evident, but its place is taken by eosinophil cells which may be seen passing through the mucosa into the gut lumen. Mitotic figures abound in all coats (see fig. 5), while the resting nuclei are large and reticular.

Among the other tadpoles, the condition of the suprarenal-fed intestine is worthy of note. The whole gut has a wide lumen; the mucous membrane of the stomach and the upper part of the intestines are only moderately developed, and the muscular layer is very thin. In the later series the suprarenal animals continue to exhibit this condition, while in other tadpoles these organs are considerably better developed.

Both liver and pancreas appear to be well developed. The liver cells are larger than those of controls, while the sinusoids are correspondingly narrower. The numerous mitoses seen in this organ are almost wholly in red-blood corpuscles. The pancreatic acini are broken up into individual cells; this, however, also occurs in some of the other tadpoles; a few mitotic figures may be seen.

What remains of the tail in the thyroid-fed tadpole (series D, E, and F) is also markedly different from that of the other animals. The muscle
fibres in this region are broad, and are composed of well-formed striated fibrils. They are not straight as in the normal condition, but wrinkled, the distorted parts being homogeneous and devoid of fibrillar structure. Vacuolation is also seen within both the hyaline and the fibrillated areas (fig. 7). Near the tip of the tail the muscle is fragmented and disintegrated, the fibrils being broken up and isolated into short fragments. Some of the fibres and all the fragments stain deeply with eosin (see figs. 6 and 7). In this neighbourhood there are also many eosinophil cells, of which two distinct types may be distinguished: the one containing rounded or spheroidal granules, the other rod-shaped granules (figs. 8 and 9). In type I. the nucleus is simple or undivided, thus differing from the typical oxyphil leucocyte of the blood, which is also present. The cells of type II. have similar shaped nuclei; some of the rods which they contain are so long that they merit the description of eosinophil filaments. Both types occur elsewhere in the tadpole. Besides eosinophils, a few large and small mononucleated cells can be seen; the connective tissue appears to have increased, at any rate it is more prominent. The muscles of the eye were also found to be wrinkled, but were otherwise unchanged.

The heart and blood-vessels are more developed than in controls; they are larger and have thicker walls. This is not apparent, however,
The Histology of Tadpoles fed with Thyroid

Fig. 8.—Thyroid-fed tadpole. Series E. About ×1500. Drawing. Distal part of tail. Muscle fibres fragmented and disintegrated into short fragments. 

a, neutrophil polymorph leucocyte; b', eosinophil leucocytes; c', eosinophil of type II; e, connective tissue cells.

Fig. 9.—Thyroid tadpole. Series E. About ×1500. Drawing. 

c, leucocyte from a blood-vessel in the tail containing a few eosinophil granules; b, eosinophil cells of type II. from the heart; c, similar cells from the tail; d, portion of degenerating muscle showing nucleus and sarcoplasm containing a few muscle fibrils.
until series D. With regard to the blood, there is an increase in the number of red and to a less extent of white corpuscles. In addition, there are many more red cells with distorted nuclei. Corpuscles of all kinds may be found in the blood-vessels of the abdominal viscera, especially in the kidneys and the alimentary tract. Some of these cells are eosinophil (oxyphil) leucocytes, while the remainder are largely eosinophils of type I; a few eosinophils of the second type are also to be found. The latter, however, are more often seen in the heart, but here they have rosette-like nuclei.

The skin is heavily pigmented in series B and D, both with pigment granules in the epithelium and branching pigment cells in the cutis vera. Mitoses are much in evidence. In series E and F the skin, on the contrary, is lightly pigmented, the pigment having apparently been withdrawn to the underlying tissues. This is best seen in the tail, the skin of which still contains masses of pigment in the intercellular spaces, while under the epithelium numerous cells may be seen loaded with pigment. The integument of series E and F is as well developed as that of any of the tadpoles which had been treated with other extracts for a considerably longer period.

With regard to the fore and hind kidneys, there is only great congestion of blood-vessels to be recorded. A large number of the cells are eosinophils, some of which, as well as some of the red corpuscles, may be seen in mitosis. (It is noted here that normally the cells of the metanephroi are devoid of pigment granules, which are abundantly present in the pronephroi.)

The effect on the endocrine organs is slight; the thyroid gland retains its early pigmentation, and is composed of few but large vesicles.

To recapitulate. Mitotic figures may be seen nearly everywhere in thyroid-fed tadpoles. They are especially evident in the blood and the intestinal mucosa, but are also easily discernible in the skin, connective tissues, voluntary and involuntary muscles, liver, pancreas, brain, retina near ora serrata, and nasal glands. There is an undoubted increase as compared with controls.

**DISCUSSION.**

The effect of thyroid increases in intensity the nearer the animal is towards metamorphosis, since the changes in the older animals are more marked than those in the younger ones. The effect appears to vary directly with the dose given, hence tadpoles have been utilised as a means of assaying thyroid (Rogoff (18)). The first change to occur is seen in the alimentary tract, the large thin-walled tube becoming small and thick, and at the same time developing a more complex mucous membrane. This agrees well with Swingle (21), who has described the naked-eye appearances of the thyroid-fed intestine. Mitoses are abundant in most tissues, while the nuclei of many of the resting cells are large and
The Histology of Tadpoles fed with Thyroid

reticular, indicating that they have been only recently formed. With longer treatment the tail becomes absorbed, both fore and hind limbs are budded out, and all the other organs throughout the body are more developed, so that a thyroid-fed tadpole which died after nine days appeared more adult (histologically) than tadpoles of the same series (E) which had lived for twenty days longer. Thyroid-feeding may thus be said to cause an "age-accelerating" effect.

The effect of thyroid on the corpuscular elements of the blood is simply an example of its general action. Mitosis of red cells within blood-vessels is normal in larval amphibians (see Bryce (4)), and merely exaggerated by thyroid. This results in the production of numerous cells, which accumulate in the sinuses of the abdominal organs. The number of red cells having irregularly shaped nuclei is also increased. A careful examination of these cells shows that the nuclear substance has budded or flowed outwards at various points; thus the simplest irregularity appears like a yeast torula, the more complex like a rosette. The latter form in some cases seems not unlike the monaster stage of cell division. On the supposition, therefore, that the extensive mitoses caused by the thyroid hormone result in the rapid formation of red cells, it is conceivable that these unusually formed cells are less stable than those formed more leisurely, and that an imperfect nuclear membrane has given rise to these irregularities.

Of the white cells, considerable interest is attached to the oxyphil variety, apart from the occurrence of mitosis in them, on account of their resemblance and perhaps relationship to certain other eosinophil cells which are not blood-leucocytes. Two types of such eosinophils have been recognised, the one containing spheroidal and the other rod-like granules. The origin of the rod-shaped granules seems to be intimately associated with the absorption of the caudal muscles. In the distal region of the tail numerous cells may be seen containing rod-like eosinophil structures which appear to be fragments of muscle fibrils, for many of these rods are striated and some of them of considerable length. A few of these cells are branched, but the majority are rounded; they have oval or circular nuclei. All are probably amoeboid connective-tissue cells, which are mainly responsible for the phagocytosis of muscle-débris. When loaded with muscle-rods they become eosinophils of type II. The gradual disintegration of the engulfed rods must follow, whether by a process of digestion or autolysis, and as a result the rods will become globules, thus transforming eosinophil II. into eosinophil I. Further, the occurrence of large numbers of eosinophils of both types at the same site suggests the transformation of the one into the other. Eosinophils of type I. may usually be distinguished from the oxyphil leucocytes of the blood by the large size and simplicity of their nuclei. Nevertheless, it must be admitted that intermediate-looking forms exist, and it is by no means easy to determine the character of these cells. The possible derivation of
eosinophil granules from ingested muscle has been suggested by Badertscher (2), who states that in Amphibia eosinophils may be derived by the ingestion of muscle-fragments by lymphocytes. In the case of man the eosinophilia of trichinosis has been associated with the muscle degeneration consequent on the presence of the parasite by Brown (3) (see also Opie (16)). Brown noticed the accumulation of eosinophil cells in the neighbourhood of the encysted worm, and concluded that they were formed by the phagocytosis of muscle-débris. It is to be noted that the above authors have claimed the blood leucocyte as the muscle phagocyte; it is here suggested that the amœboid connective-tissue cells sustain this rôle. For there is no evidence of invasion of leucocytes from the blood-stream, the work of muscle absorption being carried out entirely by cells already present in the connective tissue. The final destination of these eosinophil cells from the tail is perhaps to be found in the large accumulation of them in the intestines of the older thyroid-fed tadpoles. Here they may be seen wandering through the mucous membrane into the lumen. It thus seems that eosinophils I. and II. should be regarded as carriers of the disintegrated muscular tissue to the intestinal mucosa. This may serve a more useful purpose than is at first apparent, i.e. than the mere riddance of muscle débris, for it may be that the materials derived from the tail are utilised for nutriment, especially as the tadpole does not feed towards the end of metamorphosis (Marshall (15)).

There remains to be discussed the effect on the pigmentary system. Almost every organ in the tadpole contains pigment granules, and these, along with special pigment cells (chromatophores), constitute the pigmentary system. These chromatophores are branched, although under certain circumstances they may “appear” rounded. They occur mainly in the connective tissue of the peritoneum and skin, where they form imperfect sheets, which invest the abdominal viscera and body. Their pigment granules respond rapidly to various stimuli, e.g. light, pineal extract (McCord and Allen (13)), and thus differ from the pigment contained in other cells. The widespread distribution of pigment granules in cells indicates that such granules are formed intrinsically, and points to their being products of cell metabolism. This is the more probable since Tornier (22) has shown that under-feeding may produce albinism, and over-feeding melanosis; moreover, changes in temperature also affect pigmentation (Kammerer (10)). On this assumption it is supposed that the granules are withdrawn from the skin, muscles, and elsewhere, and are carried to the pronephroi. These, however, are not the only channels of excretion, since the intestine may also offer a passage for the outflow of pigment. The increase in growth, and therefore in cell activity, caused by the influence of thyroid necessarily results in augmenting these processes of excretion, so that masses of pigment may be seen accumulated between and below the cells of the intestinal mucosa. So extensive are these masses that the cells on the surface form a flattened layer protruding into
The Histology of Tadpoles fed with Thyroid

Finally, this layer becomes so thin that it ruptures, and thus allows the pigment masses to escape. As already noted, tadpoles are so inert during the height of metamorphosis that they do not feed, yet they may be observed to pass pigmented feces. The "fine-pigment" granules seem peculiar to the metabolism of the young tadpole, since they are not marked in the adult organs, and the mesonephroi are completely devoid of them. The mode of transference of the granules appears to be by means of ameboid wander-cells.

**Summary.**

1. The effect of thyroid is to "accelerate the ageing" of tadpoles, whereby the number of mitoses and development is increased. All organs thus appear more "adult" in character. Metamorphosis is induced and hastened.

2. The alimentary tract is the first to be affected by thyroid treatment, the large and thin-walled larval type of intestine being replaced by a small thick-walled type. Masses of pigment are accumulated in the mucosa, and may be seen breaking through the epithelium.

3. Thyroid-induced metamorphosis results in rapid absorption of the tail, the caudal muscles being disintegrated partly by phagocytes and partly by autolysis. The phagocytes loaded with muscle-débris simulate eosinophil leucocytes. They wander into the circulation and are eventually extruded through the intestinal mucosa.

4. The effect of thyroid on blood corpuscles is chiefly that of increasing the numbers undergoing mitosis; the red cells frequently exhibit irregularly shaped nuclei, perhaps a further indication of rapid division.

5. The "fine-pigment" granules throughout the body are regarded as excretion products. They are well developed in thyroid-fed tadpoles, and appear to be peculiar to the metabolism of young tadpoles.

The effects of thyroid feeding may be summarised in general terms as normal processes which have been hastened and exaggerated by excess of thyroid hormone.

This study was commenced in 1917 by Sir Edward Sharpey Schafer, to whom the author is grateful for being permitted to continue it, and for help and advice during its progress. Acknowledgment is due to Messrs C. Siung and A. Dower for their assistance in the preparation of some of the microscopic specimens. A short note has already been published in the Proceedings of the Physiological Society (12, b).

The expenses of the research have been assisted by grants from the Earl of Moray Fund of the University of Edinburgh and from the Carnegie Trust.
The Histology of Tadpoles fed with Thyroid

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Contributors are requested to place their bibliographical references at the end of their articles, drawing attention to each by a bracketed number in the text, and avoiding footnotes as much as possible. Each reference should appear as follows:

1. Number.
2. Name of author.
3. Title of book (or article if wanted).
4. Title of journal.
5. Year of publication.
6. Number of volume.
7. Number of page (if wanted).

Capital letters should not be used in the titles of papers except at the commencement and for German substantives.


Examples:—


(33) CARNOT and CHASSEVANT, Compt. rend. soc. biol., 1905, lix. 106.
CONTENTS

LIM, R. K. S., The Histology of Tadpoles fed with Thyroid. 303

KILBORN, L. G., and J. J. R. MACLEOD, Observations on the Glycogen Content of certain Invertebrates and Fishes. 317

LANG, R. S., and J. J. R. MACLEOD, Observations on the Reducing Substance in the Circulating Fluids of Certain Invertebrates and Fishes. 331

BURRIDGE, W., Researches on the Perfused Heart: Its Mode of Working. 339

HEWITT, JAMES ARTHUR, The Effect of Administration of Small Amounts of Thyroid Gland on the Size and Weight of certain Organs in the Male White Rat. 347

BURRIDGE, W., A Survey of some Elements of Cardiac Excitability. 355

SCHAFER, E. SHARPEY, Note on Cats with Double Vagotomy. 367

WALLER, A. D., and M. KOJIMA, Measurements of Emotivity in a Japanese Subject. 369

SCHAFER, E. SHARPEY, The Influence of the Depressor Nerve on the Pulmonary Circulation. 373

SCHAFER, E. SHARPEY, The Influence of the Respiratory Movements upon the Blood-Pressure in the Pulmonary System. 395

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A PARASITIC SPIRAL ORGANISM IN THE STOMACH OF THE CAT

BY

R. K. S. LIM

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A PARASITIC SPIRAL ORGANISM IN THE STOMACH OF THE CAT.

By R. K. S. LIM.

(From the Department of Physiology, University of Edinburgh.)

(With Plate VII).

While examining sections of the apparently normal cat's stomach, clusters of organisms were found within the lumina of numerous ducts and glands—especially of the pyloric region. Further examination, employing various methods of staining, revealed the spiral nature of the organisms. The presence of large numbers suggested that they were actively growing and in the absence of obvious gastric disturbance in the cats in which they were found, it was concluded that they were non-pathogenic parasites.

The author has been unable to find any reference in bacteriological literature to such an organism in the cat, although Noguchi (1915–16) refers to the finding by Bell and Ruquet of a similar form of organism in the stomach of the dog. Spiral organisms have also been described by Lucet (1910) in a case of gastro-enteritis in the dog.

DISTRIBUTION.

Eight animals were affected: they had all been in the laboratory for some months. Cats which were killed immediately on admission or which had been isolated, were unaffected. Rabbits which had been kept in adjacent cages were not infected.

The stomach was the only organ in which the organisms (hereinafter referred to as "spirochaetes") were found, except a few in the duodenum, close to the pyloric sphincter. Preparations from the liver, spleen and bone marrow were negative. Within the stomach, the spirochaetes were found throughout the whole fundus, including the cardia, and in the pyloric antrum and canal. They were most numerous in the latter situation. It should be noted that in the cat, oxyntic cells are only absent within a narrow area, half to three-quarters of an inch, proximal to the pyloric sphincter, and that therefore the fundus and the major portion of the antrum are histologically, and probably functionally, similar. The term "fundus" in the following description is applied to the whole area of the stomach bearing oxyntic cells, and "pylorus" to the narrow area devoid of them.
In the fundus region, groups of spirochaetes may usually be seen within the lumen of the tubules in the middle zone of the mucosa, where the oxyntic cells occur most abundantly and where they frequently abut directly on the lumen. In sections stained with alcoholic eosin and methylene blue \((\text{vide} \text{ Lim, 1919})\) and in those stained with polychrome methylene blue alone, spirochaetes can be shown within the oxyntic cells\(^1\), lying either in large clear spaces, enclosed by a membrane or amongst the granules in the interior of the cells (see Plate VII, figs. 2 and 3). Deeper down in the mucosa, where there are few oxyntic cells, only isolated organisms are present. They are not seen in the interior of the central or peptic cells.

In the pylorus proper, dense masses of spirochaetes occur at all levels of the mucosa, but only within the lumina of the ducts and secretory tubules (Plate VII, fig. 1), which are extremely wide in this region. No spirochaetes are visible outside the glands, either in the mucous, submucous or muscular layers.

The presence of organisms does not alter the histological appearance of the stomach further than has been described above.

**MORPHOLOGY.**

*Measurements.* The spirochaetes have been found to average in length from 4 to 8\(\mu\) in preparations taken direct from the fresh stomach and examined immediately. In stained films, somewhat longer forms are sometimes met with. The breadth varies from 0.75 to 1\(\mu\) and the thickness of the spiral, which is cylindrical, from 0.25 to 0.5\(\mu\). Some of the smaller spirochaetes are extremely slender and have a spiral thickness of less than 0.2\(\mu\). The spirals are regular, and closely set together, there being usually about 7 to 8 spirals in each spirochaete of from 5 to 6\(\mu\). Occasionally as many as 14 spirals may be present, especially in the large forms. The spirochaetes are generally straight, but may exhibit one or more waves or curves (see Figs. 1, 3 and 5). Both extremities are tapered. When compared with the organism found in the dog\(^2\) (PL VII, fig. 4), the cat spirochaete appears to be nearly of the same size (perhaps a little shorter), but differs in having more numerous and more regular spirals.

*Reaction to stains.* The spirochaetes stain slightly with Gram, i.e. they are not perfectly alcohol-fast. They are readily stained by most aniline dyes, especially when any of the Romanowsky combinations are used. With Giemsa, they appear bluish, with alcoholic eosin and methylene blue, blue, and with polychrome methylene blue, violet or purple. For clearness of staining, polychrome methylene blue is undoubtedly the best, both for smears and sections; it should be applied for 2 to 3 minutes. The Levaditi method has given poor results in the author’s hands. Stained specimens of

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\(^1\) I have received an unpublished diagram from Dr Murray, drawn in 1907, showing spirochaetes within the oxyntic cells of the dog’s stomach.

\(^2\) I am indebted to Prof. Ashworth for a smear preparation (and a drawing) made by Dr J. Murray in 1907, from which the above comparison was made and a photograph taken.
the spirochaetes often exhibit granules and vacuoles. The granules stain darkly with the basic dye and are undoubtedly chromidia; they are inconstant in number (see Pl. VII, fig. 5). Vacuoles are not nearly so common, and may be artefacts resulting from overheating. Very broad forms of the spirochaetes are probably also due to this cause.

Behaviour of living spirochaetes. If a little of the gastric mucosa is scraped off and transferred to a slide, the spirochaetes may be easily studied without the use of the dark ground illumination. They are highly refractile and quickly catch the eye, by the distinctness and regularity of their spirals. One or two more highly refractile spots may be seen at one or both ends of some of the organisms, but, otherwise, they appear homogeneous and structureless. When not in progression, the spirochaetes are seen in various attitudes, either straight, curved like a semicircle, or more completely, like a loop, and show one or other of the following movements which may be very active:

1. An intermittent corkscrew-like motion of their spirals, sometimes in one direction, and sometimes in the reverse direction. Occasionally, one end appears to turn more slowly than the other. 2. Large spiral movements, superadded to the smaller movements. 3. A circular sweeping movement of one extremity. 4. Lateral oscillation of the whole organism. 5. A rotary disturbance of the fluid medium at one end of the organism, although the visible extremity of the spirochaete is not in motion. This seems to suggest the presence of a fine terminal filament (flagellum).

The behaviour of some spirochaetes in the proximity of cells is interesting. They ram the cell and spiral or corkscrew furiously, occasionally giving large circular sweeps with their free ends. Suddenly they disengage themselves, turn a "somersault" and repeat the performance with the former free end. No spirochaetes, however, have been actually observed to penetrate a cell by this means. The method of progression is difficult to analyse, but it appears to consist of a combination of corkscrew and lateral movements.

CULTIVATION.

All attempts at cultivation have hitherto failed. Deep agar, cooked-meat, and gastric digests, with or without pepsin, HCl or serum, have been used with no success. Partial anaerobiosis was maintained in all cases, except when agar was employed, by means of a supernatant layer of liquid paraffin. The only result obtained, worthy of note, was that the spirochaetes may survive at least four days in an acid medium (0.02 per cent. HCl).

CONCLUSIONS.

From the incomplete data accumulated, it is not possible to classify the organism just described, but sufficient is known to regard it as a new species of the Spirochaetoidae. Morphologically, it resembles both the genus Spiro- nema and the genus Treponema (as defined by Noguchi, 1918). It reacts to
Giemsa in the same way as a Spironema (stains bluish), but on the other hand, it is about the size of a Treponema and, like it, has regular spirals. Its exact position in the group must be left undetermined until more is known regarding its cultural reactions.

Of the mode of infection, nothing definite can be stated. The organism was not found below the pyloric sphincter and is certainly not passed in the faeces in a free living condition. Fleas were often found in the gastric content, but when examined microscopically (living specimens were also procured direct from the body surface), there was nothing to indicate that they were the carriers. This part of the investigation was however not sufficiently pursued to negative the "carrier" possibility. The food (fish, bread and milk) does not appear to be responsible for the infection, since animals which were recently admitted to the laboratory were fed on the same diet without being infected. Taking into consideration the length of time the infected animals had been kept in the laboratory, it seems most likely that the infection was introduced by a single animal, many months ago, and that this animal infected the others. The mode of spread from one animal to another is still to be explained.

I have to thank Professors Ashworth and Ritchie for kindly confirming my observations and for helpful suggestions. I am indebted to Professor Ritchie for the supply of some of the culture media.

The expenses of the research were defrayed by a grant from the Earl of Moray Fund of the University of Edinburgh.

**SUMMARY.**

1. A parasitic spiral organism averaging 4 to 8μ long, with regular, closely set spirals about 0.75μ broad, has been found in the stomach in eight cats, none of which showed any obvious signs of gastric disturbance. The organisms occurred in the lumina of ducts and glands throughout the stomach, and also within the oxyntic cells. They were not seen in any part of the intestines except the very beginning of the duodenum, or in any other organ.

2. They are extremely active, and are readily stained by aniline dyes.

3. The mode of passage from one animal to another is not known, but food and faeces may be eliminated as possible sources of infection.

**REFERENCES.**


EXPLANATION OF PLATE VII.

Fig. 1. Cat III. × 1000. Polychrome methylene blue. Photograph. Section of pylorus, showing spirochaetes in the lumen of a duct.

Fig. 2. Cat I. × 1000. Drawing of selected oxyntic cells. (a) Oxyntic cell showing spirochaetes lying among the granules; (b) spirochaetes lying within a dilated canaliculus; (c) spirochaetes lying partly in the canaliculus and partly in the lumen of the gland.

Fig. 3. Cat IV. × 1000. Smear preparation. Alc. eosin and methylene blue. Photograph. This shows the variations in size.

Fig. 4. Smear preparation from the Dog's stomach. × 1000. Leishman. Photograph. Compare with preceding figure. Note that the spirochaetes here show larger spirals.

Fig. 5. Cat VII. × 2000. Smear preparation. Alc. eosin and methylene blue. Photograph. Spirochaetes showing chromidial granules.
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The effects of thyroid extract on tadpoles. By R. K. S.Lim.

A number of tadpoles of *Rana temporaria* were shown which had been kept in water to which a small amount of extract of dry sheep’s thyroid had been added (20 c.c. of a 2 p.c. solution to each litre): as well as others of the same spawning kept under ordinary conditions and used as controls.

The effect of thyroid extract in causing precocious metamorphosis—which has been described by Gudernatsch and others as the result of feeding with thyroid—is well seen; and microscopic sections which were also exhibited show the concomitant changes which occur in the viscera, as well as a remarkable number of mitoses indicative of rapid cell proliferation.
Neutral formol as a fixative for mucous membranes. By R. K. S. Lim.

Formol, made from commercial formaldehyde, is almost invariably acid in reaction, from the presence of formic acid. With most tissues, the acid impurity is of no consequence. In the case of mucous membranes bearing goblet cells, however, the acid formol causes a discharge of the mucinogen (see Fig. a), and thus alters the histological appearance. This effect is apparently due to the acid alone, since a neutral formol solution preserves the granules intact (Fig. b). It thus appears that formaldehyde does not fix the goblet cells rapidly enough to prevent the action of acid on mucinogen. A 20 per cent. formol neutralized by caustic soda, has been found to be the most satisfactory, and has been successfully employed in the fixation of all parts of the intestine. Tissues thus fixed, stain readily with hematoxylin and with any of the aniline dyes.

The accompanying figures are photographs of two sections, the one (a) prepared by acid formol, and the other (b) by neutral formol fixation. They represent the two halves of a segment of the same intestine (cat). Both sections were stained by alcoholic eosin and methylene blue.
Experiments on the Respiratory Mechanism of the Shore Crab (Carcinus mænas).

By Robert K. S. Lim.
WHILE carrying out some immersion experiments on *Carcinus mænas*, I had occasion to investigate its respiratory mechanism. Comparing my results with those recorded in the literature of the subject, I found that I could add a few new observations.

**TECHNIQUE.**

The materials used consisted of a large glass basin, containing 2½ inches of fine sand and enough sea-water (Millport) to cover the sand to a depth of about an inch, this being found sufficient to completely immerse the crabs. By means of a small syringe a fine suspension of Indian ink in sea-water was then injected into the fluid surrounding the animals, whereby the presence and direction of the respiratory currents could be detected.

Lastly, it may be of importance to note that the habitat of the crabs used was a rocky patch in the middle of a large beach of fine sand.

**EXPERIMENTS WITH CRABS LYING ON THEIR BACKS ON THE SURFACE OF THE SAND BUT WHOLLY IMMERSED IN SEA-WATER.**

**Exp. I.**—When Indian ink was injected near the prostomial chamber it was swept outwards by a strong exhalent current proceeding from the whole breadth of the chamber. The direction of the current was forward and generally parallel to the median line of the crab, although sometimes it deviated to one or other side.

Occasionally an inhalent current was noticed between the anterior borders of the third maxillipeds and the adjacent carapace. This current was very brief, the ink soon emerging from the sub-branchial clefts, i.e. the spaces between the base of the thorax and the branchiostegite of each side.

Frequently no currents could be observed at all.

**Exp. II.**—When injected close to either sub-branchial cleft the ink was sucked in or inhaled throughout the whole opening, more especially round the coxa of the chela, and later it could be seen emerging from the
prostomium in the exhalent stream. As soon as the exhalent current ceased no more ink was inhaled.

At intervals the ink, instead of being inhaled, was expelled or exhaled from the vicinity of the cleft. This exhalent current was of short duration, however, the direction being again changed and the usual inhalent current once more established. On using a more concentrated suspension of ink the frequency of this momentary reversal of currents increased.

Exp. III.—A small opening, about a quarter of an inch in diameter, was made in the branchiostegite opposite the third thoracic leg, in order to determine the direction of the currents in the branchial chamber. An inhalent current was observed.

Experiments with Crabs immersed as before, but with the whole of the Respiratory Tract exposed.

The branchiostegite and the maxillipedes were removed on both sides in order to expose the scaphognathites and branchial chambers. The characteristic undulating movements of the former, described first by H. Milne Edwards (7) and later by Garstang (3), were observed in a postero-anterior direction. These movements were not rhythmical, being sometimes fast and at other times very slow.

Occasionally they were “reversed,” i.e. antero-posterior, when they became extremely rapid.

The action of the scaphognathites has already been noted to be irregular, while not infrequently it was entirely suspended. Often both sides did not work together; one side would stop or go more slowly than the other.

Exp. IV.—For this Experiment II. was repeated with the branchial chambers thus exposed. During the postero-anterior action of the scaphognathites the same result was obtained, i.e. an inhalent current was observed at the enlarged sub-branchial clefts. Following the current through the branchial chamber, the ink could be seen being drawn forward by the scaphognathite, between it and the roof of the pre-branchial chamber, and being finally exhaled through the prostomium.

When the action was “reversed,” injection at the prostomium showed the ink to be sucked inwards (inhaled) in the same manner as it was exhaled, i.e. between the scaphognathite and the pre-branchial roof. It then passed backwards through the branchial chamber and outwards through the enlarged sub-branchial cleft.

No current could be demonstrated when the scaphognathite was stationary.

VOL. XXXVIII.
EXPERIMENTS WITH CRABS HALF-BURIED IN SAND.

When the animals were placed in the sand basin already described, most "dug themselves in." The first procedure was to dig the thoracic legs into the sand and draw them up in a forward direction, at the same time elevating their bodies to an upright position, and then forcing themselves downwards and backwards. In this way sand was removed upwards, both in front and behind. It was only when the anterior accumulation of sand became too high, and therefore liable to fall back into the excavated space, that the chele were seen to sweep it aside. This process was continued until the posterior accumulation had risen over about half the carapace. The animals then remained in the horizontal position with their legs buried, in a position semi-flexed inwards at the merocarpal joints. The chele, however, were fully flexed, the amount of flexion being limited by the carpal spines (Garstang (4)); the coxo-basal flexion, however, may be such that there is a large exostegal channel between the inner side of the propodite and the adjacent carapace, or none at all.

The preceding experiments with ink were repeated.

EXP. V.—When ink was injected near the prostomial opening the usual exhalent current was observed. When a sufficient channel existed between the chele and the carapace a downward current was noticed, evidently going to the anterior ends of the sub-branchial eilefts; the ink could latterly be seen emerging in the prostomial stream.

EXP. VI.—Injection over the sand-covered posterior end of the carapace also resulted in the reappearance of the ink in the prostomial stream.

Occasionally reverse currents were set up, but of a longer duration than those previously seen. The ink was readily sucked in at the prostomium, and could be seen rising through the sand at the following points on both sides. Anteriorly, near the most lateral spine of the carapace, and posteriorly, in three "eddies" over the embedded postero-lateral border of the carapace—see fig. 1. When the current was again changed the ink was sucked in through these sand apertures.

Frequently the exhalent (prostomial) current was directed to one side, and when reversion occurred, exhalent "sand eddies" were observed on the opposite side only, thus indicating that only one scaphognathite was working.

EXPERIMENTS WITH CRABS ALMOST COMPLETELY BURIED IN SAND.

Small crabs and sometimes larger animals will continue to bury themselves until the whole carapace is covered with sand and there is only a triangular communication, by which the exhalent prostomial current
Respiratory Mechanism of the Shore-Crab.

escapes. The base of this triangle corresponds to the breadth of the inter-orbital projection. Experiments V. and VI. were repeated, with similar results.

Exp. VII.—Ink was injected into the sand half an inch behind and to the side of the animal about its own level beneath the surface, with the same result as Experiment VI.

Reversion currents were also observed, and were found to comply with the description in the last experiment.

Exp. VIII.—I covered some crabs completely with sand to a depth of an inch, and almost immediately the sand over the prostomium was seen to be thrown up by the exhalent current. The opening formed was gradually enlarged until the triangular communication mentioned above was attained. Experiments V., VI., and VII. were repeated, with the same results. One small crab, however, remained buried without forming the communication—the exhalent current being simply forced through the sand.

CHANNELS OF RESPIRATION.

The observation noted in Experiment VI. regarding the occurrence of "sand eddies" led me to determine the manner of their origin and hence to closely inspect the respiratory channels.

These are, briefly, the sub-branchial clefts, the branchial and pre-branchial chambers, and the prostomium: the inhalent apertures being the sub-branchial clefts, and the exhalent, the opening of the pre-branchial chambers into the prostomial region.

Each cleft is merely a slit, bounded medially by the base of the thorax,
and laterally by the indented free border of the branchiostegite. These indentations accommodate the coxae of the thoracic legs, while the intervening apices fit in between; but, whereas the former (with the exception of the concavity from the chela) are in close contact with the chitinous portion of the respective coxa, the latter are comparatively loosely applied to the soft arthrodial membranes. Thus the cleft may be divided into four separate “inter-coxal” passages, the first being the space for the coxa of the chela and the remaining three those between the apices and the lax joint membranes—see fig. 2.

On lifting the branchiostegite and exposing the posterior gills, one found them to be touching one another except at their origins, where they were distinctly separated by a small gap. It was also easy to note that

the last or 9th gill did not lie perfectly flat on the adjacent epimeron, but was slightly arched a short distance from its root. This arching was not peculiar to the 9th gill, but occurred in the 8th, 7th, and 6th as well, and is in fact mainly due to the flabellum of the third maxillipede.

Examination of the remaining gills showed that under the 1st gill was a large anterior inlet, kept patent by the commencement of the third flabellum. This inlet communicates with that under the 9th gill by means of a “tunnel” formed by the arching of the posterior gills. Leading from the anterior inlet but not in direct connection with the sub-branchial cleft, another gap was found between the 2nd and 4th gill origins. Besides causing this gap, the second maxillipede flabellum is also responsible for the arching of the 4th and 5th gills. The 3rd gill, arising as it does from the coxopodite of the third maxillipede, helps in the formation of the tunnel already described.

Altogether, therefore, there are five “inter-branchial inlets”—see figs. 3 and 4.
1917-18.] Respiratory Mechanism of the Shore-Crab. 53

1st being situated under the 1st gill and between it and the base of the 6th gill.

2nd being between the 2nd and 4th gill origins.

3rd " " 7th " 8th "

4th " " 8th " 9th "

5th " under " 9th gill.

Fig. 3.—Ventral surface of Carcinus. Branchiostegite and coxae of thoracic legs removed on both sides; third maxillipede entirely removed on the left side, on which side the first gill has also been removed.

Sc., scaphognathite; 1st-4th i.b., the first to fourth "inter-branchial inlets." Note commencement of flabellum of second maxillipede in 2nd i.b.

Fig. 4.—Oblique view of thorax and abdomen of Carcinus from the right side. Carapace and contained organs removed; also the coxae of the thoracic legs. Semi-diagrammatic.

1st f., flabellum of first maxillipede; 3rd f., commencement of flabellum of third maxillipede; 1st a., first gill; 1st, 3rd, 4th, and 5th i.b., the first, third, fourth, and fifth inter-branchial inlets.

X denotes the position, marked by the dotted lines, of the "tunnel" formed by the third maxillipede flabellum.

All excepting the second inlet communicate directly with the adjacent inter-coxal spaces. When a probe was introduced through each of the latter spaces into the corresponding inter-branchial inlets, it was found
to be directed postero-anteriorly in the case of the fifth space, and more medially in the anterior spaces till at the first space the direction was almost latero-medial—see figs. 1 and 6. (It is convenient for descriptive purposes to describe the inter-coxal space and the corresponding inter-branchial inlet together as the "sub-branchial inlet.")

It is now possible to explain the meaning of "sand eddies." When reversal occurred the exhalent stream was forced out through the sub-branchial inlets, the portion passing through the first appearing as the anterior eddy, while that passing through the remaining inlets as the three posterior eddies.

During the normal forward action of the scaphognathites the converse to the above holds true. Water may then be inhaled from behind and from the sides of the animal through the channels just described into the branchial chambers. The latter are somewhat triangular in transverse and crescentic in oblique antero-posterior sections.

The gills arise from an area which is roughly a segment of a circle—see figs. 5 and 6; so that, although they lie more or less latero-medially, their free medial ends almost meet. Their branchial septa occupy the plane above mentioned, while the lamellae are placed in the antero-posterior plane. Lastly, there is a greater space above than below the gills.

Of the pre-branchial chambers and their exits there is nothing of note.

**Conclusions.**

The normal direction of the respiratory current in decapods was shown to be postero-anterior by H. Milne Edwards (7). De Haan (6) at a later date remarked on the reversal of this direction in Portunus, though still
later Garstang (3) opposed this contention, while he himself described a similar reversal in Corystes, Atelecyclus, and Portunus (5). He, however, suggested that the phenomenon did not exist in such decapods as Cancer and Carcinus, and states positively (5), in the case of the latter, "I have found no indications of a reversal of the respiratory currents in the latter species" (Carcinus). His observation in this respect has been shown to be erroneous by Bohn (1), who seems to have proved that reversal is common to all decapods. My results confirm those of Bohn. They show that reversal occurs under all conditions, and that it is more frequent when the crab is buried or when a stronger ink suspension is used. On such meagre evidence I do not attempt to criticise this last authority, who argues that reversal is due to fatigue of the muscles which cause the forward direction of the scaphognathite.

I have also endeavoured to determine the direction of the respiratory currents in the branchial chambers. While investigating the cause of the "sand eddies" previously mentioned, I came to regard the position and relations of the gills as of first importance in the determination of this direction.

Claus (2) describes a backward flow due to the action of the maxillipede flabella. Pearson (8) agrees with Claus as regards direction, but fixes the cause to the presence of the "branchial ridge." Bohn, on the other hand, maintains that the direction is postero-anterior. The differences in opinion arise mainly in reference to the extent of the inhalent apertures; and since the two former workers believe that the whole sub-branchial cleft does not admit of a current, the aperture round the chela is considered to be the main inlet. This being situated far anteriorly, the backward flow becomes a necessity in order to thoroughly bathe the posterior gills. From my observations on the parts in question, it seems quite plain that the sub-branchial inlets will, to a large extent, predetermine the flow of the inhaled currents. The direction of these inlets veer from postero-anterior to almost latero-medial (from behind forwards), tending, therefore, towards the pre-branchial chamber or outlet—see figs. 1 and 6. Thus it is not difficult to allow that the inhaled currents must also follow this direction; additional evidence of this supposition being correct is supplied by Experiment III.

In connection with the above, there is another interesting feature in the radial arrangement of the gills and the direction of each of the sub-branchial inlets. These are so related that the gills lie more or less constantly at an acute angle to the direction of the inhaled currents, which, meeting with the opposition of the branchial septa and the gill lamelle, can only pass upwards between the individual lamelle to the roomy roof of the branchial chamber. Some will no doubt flow along the "tunnel"
described, and although I do not agree with Claus, I think that it is possible that the flabella disperse the currents laterally so that they may bathe the entire length of the gills more thoroughly. In this manner each sub-branchial inlet will supply, not only the adjacent gills, but those anterior to it. For example, the fourth inlet supplies mainly the 9th gill, but to a lesser extent the 8th, 7th, and so on; while the first inlet supplies the anterior five gills, the 2nd, 4th, and 5th in particular, by means of the second inter-branchial gap.

**SUMMARY.**

(a) The direction of the respiratory current is postero-anterior, whether the crab is above the sand or buried in it.

(b) Reversal of this direction also occurs and is more frequent when the animal is buried or when a strong ink suspension is used during an experiment.

(c) The sub-branchial cleft may be divided into four separate spaces which are in direct communication with gaps between certain gill origins, the whole constituting the sub-branchial inlets.

(d) The direction of these inlets varies from postero-anterior to almost latero-medial.

(e) They determine the direction of the inhaled currents within the branchial chambers.

(f) The relation between the position of the gills and these inlets allows for a convenient and maximal flow.

**LITERATURE.**


*(Issued separately April 16, 1918.)*
Period of Survival of the Shore-Crab (Carcinus mænas) in Distilled Water.

By Robert K. S. Lim.
IV.—Period of Survival of the Shore-Crab (Carcinus mænas) in Distilled Water. By Robert K. S. Lim. (From the Laboratory of Physiology, Edinburgh University.) Communicated by Professor Sir E. A. Schäfer, F.R.S.

(MS. received July 24, 1917. Read November 5, 1917.)

In some recent experiments on Ligia oceanica, Tait (13) found the period of survival of the animals in fresh water to be largely dependent on their moult-age, freshly moulted animals surviving longer than animals approaching new moult. It was of interest to know whether this result applies only to Ligia. At the suggestion of Dr Tait, to whom I am indebted for advice and encouragement, I have carried out somewhat similar experiments on Carcinus mænas (Pennant). That this crab does survive for an appreciable period in fresh water was first noted by Couch (3). At a later period, Plateau (10) showed that newly moulted Carcini (and other marine crustacea) succumbed earlier than the others; that survival varied directly with size; and that loss of salts, principally NaCl, caused the death of the animal. It was only after the present experiments had been completed that I learned of Plateau's paper, so that my results are mainly confirmatory.

DETAILS OF TECHNIQUE.

The experiments took place during the months of July, August, and September 1916, with the room temperature varying from 18° to 21° C.

The freshly caught crabs were kept without food under damp seaweed in a covered tank. Under such conditions they may survive for weeks; the animals used for experiment, however, were never kept longer than four days. The weight is more rapidly determined when they are in a semi-dry condition, for in spite of all manipulation a variable amount of water adheres to the gills and setæ of crabs taken straight from sea-water.

The immersion fluids consisted, in a few experiments, of tap-water, but in the others of distilled water. In tap-water the crabs survived, if anything, appreciably longer than in distilled water.

During each experiment the immersion fluid was kept aerated, the apparatus for the purpose being the same as that employed by Tait, with these two points of difference: viz. that it was found necessary to protect each capillary aeration tube with another tube of stout glass, and
that wide-mouthed bottles, provided with caps or lids to prevent escape, were used to hold the animals. A separate bottle was at first used for each crab; at a later stage selected groups of animals were immersed together in a large container provided with two or three aeration tubes.

Before immersion in its bottle, each crab was weighed; the breadth of the carapace between the two most widely separated points was also measured. Thereupon each animal was washed in a vessel, first under running tap-water, and then in three changes of distilled water. The washing process lasted about ten minutes; the time of immersion was reckoned from the first contact with tap-water. Tests carried out with silver nitrate solution showed that the preliminary washing suffices to remove all but the merest trace of externally adhering salts. About 0.01 per cent. of chlorides remained. The crab was finally immersed in 20 times its weight of distilled water, and there left till all respiratory movement had ceased, whereupon the animal was again weighed, after being shaken free of water and wiped with a cloth. Groups of crabs immersed in one dish were treated in essentially the same way. Over one hundred individual immersions, apart from group immersions, were made.

**Experimental Results.**

It being necessary to classify the crabs according to the phase of moult, a threefold grouping was adopted, viz. (1) those just moulted, (2) those intermediate between two mouls, and (3) those approaching new moult. These phases may, for shortness, be designated post-moult, inter-moult, and pre-moult respectively. Crabs in the post-moult phase are of course unmistakably distinguishable by the softness of the cuticle; they are brownish dorsally and yellowish ventrally. In the inter-moult phase the shell is conspicuously clean, the articular membranes are colourless, while the animal as a whole is green dorsally and white to green ventrally. Animals in the pre-moult phase have red articular membranes; they are dark green dorsally and orange to red ventrally, and sometimes—more especially the males—exhibit dirty-looking excavations and perforations of the carapace. According to Smith (11), those animals with red articular membranes are sexually mature. The most careful search failed to discover red pre-moult crabs below 5 cm. (males) and 3 cm. (females), indicating that maturity is only attained at these sizes. Five animals below 5 cm. were observed during the process of moultng. In two, the short pre-moult phase was characterised by the passing of the green colour of the inter-moult condition to one of greenish grey and finally to a dirty grey; in the remainder, no colour
change was noticeable. The exuviated cuticles of the former were translucent and almost devoid of green pigment, while those of the latter were identical with the cuticles of inter-moult animals. Above this size, there are no means of identifying immature pre-moult animals, hence it seems probable that maturity is reached before ecdysis. This, at any rate, is true of the females, which do not mate except in the post-moult condition. In the experiments here recorded, the pre-moult individuals employed were all sexually ripe examples.

The largest series of individual immersions was carried out with inter-moult crabs, the results being shown in the two appended tables. The following conclusions may be derived from these figures:

(a) *Size* has little influence on the result. This confirms Tait's findings (13) in the case of Ligia.

(b) *Size* has a marked influence, large crabs surviving longer than small. While this finding is also similar to Plateau's (10) and Tait's (13), the present results, obtained with a series of animals of greater range of size, enable one better to appreciate the enormous influence of this particular factor. As an illustration, one may perhaps compare the shortest survival period, one hour ten minutes in a crab less than 1 cm. in breadth, with the longest, twenty hours in a crab of 7-8 cms. breadth. The tables also show the extreme limits of variation for each particular size. Variation may be due in part to the animals being at the extremes of their size group, e.g. the group 1·1-2·0 cms. Of the sixteen animals used, eleven were below 1·4 cm., with an average survival period of 3 hrs. 30 mins.; one 1·5 cm., with a survival period of 7 hrs. 30 mins.; while the other four were above 1·7 cm., and had an average survival period of 8 hrs. 5 mins. Similarly, variation may depend on whether the animals are commencing or ending their moult phase. It has already been noted that mature pre-moult crabs below 5 cms. (males) and 3 cms. (females) were unprocurable; hence below these sizes it is possible that immature pre-moult forms were immersed along with inter-moult animals (from which they are not readily distinguishable on account of their similarity in colour), giving rise to low survival periods.

(c) *Moult-age* has also a decided influence, survival being shortest with the post-moult crab, longer with the pre-moult, and longest with the inter-moult. This result corroborates Plateau's (10) but would seem to negative Tait's observations (14), though, in comparing the moult ages of Ligia and Carcinus, one has to bear in mind that the former moult in two stages. Thus the post-moult phase of Carcinus does not correspond to that of Ligia, but rather to the phase, "between posterior and anterior moult," in which the posterior half of Ligia is in a post-moult condition, while its anterior
1917–18.] Period of Survival of Shore-Crab in Distilled Water. 17

half is imminently pre-moult. Post-moult animals harden rapidly and in a few days acquire inter-moult characteristics, including longer survival. A post-moult Ligia, therefore, being practically inter-moult posteriorly and

**RESULTS OF IMMERSION EXPERIMENTS—MALES.**

<table>
<thead>
<tr>
<th>Size in Centimetres</th>
<th>Number of Animals and Average Weight</th>
<th>Extreme Limits of Period of Survival*</th>
<th>Average Duration of Survival*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0·1 to 1·0</td>
<td>...</td>
<td>6</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0·68 grms.</td>
<td></td>
</tr>
<tr>
<td>1·1 to 2·0</td>
<td>...</td>
<td>16</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3·1 grms.</td>
<td></td>
</tr>
<tr>
<td>2·1 to 3·0</td>
<td>1</td>
<td>7</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>? grms.</td>
<td>4·4 grms.</td>
<td></td>
</tr>
<tr>
<td>3·1 to 4·0</td>
<td>...</td>
<td>11</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10·2 grms.</td>
<td></td>
</tr>
<tr>
<td>4·1 to 5·0</td>
<td>2</td>
<td>7</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>20·0 grms.</td>
<td>20·0 grms.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20·0 grms.</td>
<td></td>
</tr>
<tr>
<td>5·1 to 6·0</td>
<td>2</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>29·2 grms.</td>
<td>49·75 grms.</td>
<td>55·8 grms.</td>
</tr>
<tr>
<td>6·1 to 7·0</td>
<td>...</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>56·5 grms.</td>
<td>63·0 grms.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>63·0 grms.</td>
<td></td>
</tr>
<tr>
<td>7·1 to 8·0</td>
<td>...</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>99·0 grms.</td>
<td>88·1 grms.</td>
<td></td>
</tr>
</tbody>
</table>

* The time is in hours and minutes.

becoming rapidly so anteriorly, corresponds to an early inter-moult condition of Carcinus.

**BEHAVIOUR DURING IMMERSSION.**

The behaviour of the crabs during immersion was moderately uniform, although some were more active than others. The first noticeable effect was lessened activity, accompanied by weaker and slower movements. The reflex mechanism became gradually impaired, resulting in loss of response...

on stimulation. Attempt at unflexing the abdomen of a normal animal causes violent efforts to prevent the unbending, and, should this be accomplished, the animal is able to close the abdomen again. With an im-

**RESULTS OF IMMERSION EXPERIMENTS—FEMALES.**

<table>
<thead>
<tr>
<th>Size in Centimetres</th>
<th>Number of Animals and Average Weight</th>
<th>Extreme Limits of Period of Survival.</th>
<th>Average Duration of Survival.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 to 1.0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1.1 to 2.0</td>
<td>... 4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>3.5 grms.</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2.1 to 3.0</td>
<td>... 5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>3.6 grms.</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>3.1 to 4.0</td>
<td>... 3</td>
<td>1</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>13.2 grms.</td>
<td>7.0 grms.</td>
<td>...</td>
</tr>
<tr>
<td>4.1 to 5.0</td>
<td>1</td>
<td>6</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>2.0 grms.</td>
<td>21.0 grms.</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>20.0 grms.</td>
<td>20.6 grms.</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>14.35</td>
<td>7.30</td>
<td>...</td>
</tr>
<tr>
<td>5.1 to 6.0</td>
<td>2</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>34.0 grms.</td>
<td>29.2 grms.</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>33.0 grms.</td>
<td>29.6 grms.</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>11.30</td>
<td>9.30</td>
<td>...</td>
</tr>
<tr>
<td>6.1 to 7.0</td>
<td>... 1</td>
<td>3</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>49.0 grms.</td>
<td>50.0 grms.</td>
<td>...</td>
</tr>
<tr>
<td>7.1 to 8.0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

* The time is in hours and minutes.

mersed animal the response is feeble, and, once opened, the abdomen remains so; similarly, irritation of the oral margin and separation of the third maxillipedes produces no response. The righting reflex is also lost, i.e. the reflex which turns the crab on its ventral surface when it has been placed on its back. Respiration, as indicated by the movement of the scaphognathite, had already ceased, but on post-mortem examination the heart was found to be still acting.

**CEDEMA.**

Some time before death, flexion of the limbs commenced, causing the animal to be gradually raised from the ground. This continued, and, being
more marked in the two anterior limbs, forced the body of the animal into a sloping position with the anterior end downwards. The crab was thus enabled to turn over head first on to its back, in which position it remained till respiration ceased. Some of the animals immersed, however, were not found in this position.

When examined post-mortem, the arthrodial membranes were tense and the carapace was considerably raised from the thorax. The abdomen was often found unflexed, and the softer tissues swollen. This swelling was not invariable, as some animals appeared perfectly normal. The weight, however, was found to have increased. The cardiac area of the carapace was then removed, when an excess of fluid was found in the pericardium. As already mentioned, the heart was still beating, though irregularly, and continued to do so, although exposed, for about an hour afterwards. The obvious explanation for these changes is oedema. Increase of the body fluid was evident in the condition of the pericardium, and would naturally cause tension of the joints and lifting of the carapace. Semi-flexion of the limbs is similarly explained.

A number of crabs showing no evidence of respiratory or other movement were transferred to sea-water, and recovery resulted in a few. Similar observations were recorded by Tait.

Loss of Salts.

Examination of the immersion fluid showed definitely the presence of chlorides and traces of sulphates. The chlorides were estimated in a series of eight experiments by Mohr’s method. The following are the results:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Breadth</th>
<th>Original</th>
<th>Increase</th>
<th>Duration of Survival</th>
<th>Chlorides in Immersion Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.2 cms.</td>
<td>24.67 grms.</td>
<td>2.93 grms.</td>
<td>3 hrs. 30 mins.</td>
<td>0.026</td>
</tr>
<tr>
<td>F.</td>
<td>4.3</td>
<td>14.90</td>
<td>1.50</td>
<td>3, 30</td>
<td>0.038</td>
</tr>
<tr>
<td>M.</td>
<td>5.2</td>
<td>25.75</td>
<td>3.63</td>
<td>4, 30</td>
<td>0.040</td>
</tr>
</tbody>
</table>

|     | 6.5 cms.| 62.60 grms.| ...       | 16 hrs. 00 mins. | 0.038 | 0.472 grm. |
| M.  | 6.5     | 66.00      | 4.00 grms.| 17, 20             | 0.040 | 0.531      |
| M.  | 5.4     | 32.00      | ...       | 23, 40             | 0.040 | 0.256      |

|     | 6.6 cms.| 61.60 grms.| 3.40 grms.| 7 hrs. 40 mins. | 0.040 | 0.489 grm. |
| M.  | 5.8     | 40.70      | 4.70      | 11, 30             | 0.038 | 0.307      |
The total salts present in the fluid, half an hour after immersion, varied from 0.06-0.09 grm.; these were taken to represent the residue of external salts after washing, and have been deducted from the "total" results given.

The above shows that a definite percentage of chlorides is invariably lost, whatever the age of the animal, though the total chlorides necessarily depend on the gross weight. This plainly indicates the cause of death—diminished tonicity of the body fluid, due both to loss of salts and to oedema, or rather hydæmia. Hence, the period of survival will depend on the rate of diffusion of salts out of the crabs and on the onset of oedema, thus reducing the question to one of permeability of the membranes of the animal. Similar conclusions have also been reached by Plateau (10), by Loeb (6) working on Gammarus, and by Abbott (1) on the Fiddler crab. Loeb has not only shown that death is due to loss of salts, but that the onset of death depends on the disturbance of the antagonism between the sodium, potassium, and calcium ions of the animal's fluid. With the exception of Plateau, however, the question of moult has not been dealt with.

Smith (12) and Paul and Sharpe (9) have pointed out that the changes occurring during moult involve, not only the integuments, but also the general metabolism; and since it has been shown that the moult cycle profoundly modifies the duration of survival, the condition of the blood as well as that of the membranes is of obvious importance in considering the rate of loss of salts.

With regard to the blood, Cuénot (4) states that preparatory to moult a crab imbibes a considerable amount of fluid through its alimentary canal. Further information is supplied by Paul and Sharpe (9), who have shown that although the volume of the blood is increased, the calcium percentage remains unaltered; whether this holds true for sodium and potassium is not mentioned. This result, however, indicates that the total calcium has been increased; and if the total sodium and potassium were not similarly increased, according to Loeb the equilibrium would be disturbed, with resultant harm to the animal. Therefore it may be presumed, either that the three ions have been increased in due proportion, or that the excess of calcium in the blood is not in a dissociable condition (viz. calcium soaps—see Paul and Sharpe's paper). If the latter presumption be correct, the blood would be in less concentration than normal; whatever be the explanation, it is evident that under such conditions the animal suffers no ill effects.

The other factor affecting permeability is the condition of the membranes. The present experiments uphold Plateau's observations that the soft membranes of the newly moulted crab are extremely permeable. Histo-
logically, there is practically no difference in the structure of the membranes during any phase of the moult cycle (see Vitzou (15)), though it is possible that the post-moult membrane is looser in texture. It has long been known, however, that the calcium content of the membranes varies according to the moult phase. I conclude from Paul and Sharpe’s estimations that there is no calcium in the integument immediately after moulting, and that just before moulting the calcium content is much diminished.

From these considerations it would appear, since the concentration of the blood and the structure of the membranes seem to be little altered throughout the whole moult cycle, that the duration of survival depends on the amount of calcium in the membranes. Within limits, therefore, the greater the percentage of calcium, the less permeable are the membranes and the longer is the period of survival.

It is of interest to note that Bayliss (2), on the evidence of several researches (see those of Osterhout (8) and Lillie (5)), comes to a similar conclusion regarding cell membranes in general, viz. that the presence of calcium renders the cell membrane less permeable. Of more interest is the result which Meigs (7) obtained with an artificial membrane of calcium phosphate. He found that it was impermeable to the chlorides of sodium, potassium, and calcium. The observations just quoted further substantiate the conclusions already drawn.

I have to thank Dr J. H. Ashworth and Dr W. W. Taylor for the help which they have always willingly given.

Summary.

1. The period of survival in distilled water is shortest with post-moult crabs, longer with pre-moult, and longest with inter-moult.

2. Further, a relatively constant percentage of salts is lost, irrespective of size; hence one of the determining factors in survival is the rate of loss of these salts. The other factor is the rate of osmosis of water, causing oedema.

3. With regard to the moult cycle, the concentration of the blood and the structure of the membranes are seemingly little altered, but the amount of calcium in the latter varies with moult-age, being least in post-moult and most in inter-moult membranes.

4. It is concluded, that the higher the percentage of calcium, the less permeable are the membranes and the longer is the duration of survival.

5. Oedema and loss of salts naturally disturb the tonicity equilibrium of the body fluid, and thus cause death.
LITERATURE.

(3) Couch, Bell's *History of British Stalk-eyed Crustacea*, 1853, p. 78.
(14) Tait, *ibid.*, pp. 59-68.

*(Issued separately February 28, 1918.)*
THE EFFECTS OF ADRENALIN ON THE PULMONARY CIRCULATION. By Sir Edward Sharpey Schafer and R. K. S. Lim. (From the Department of Physiology, University of Edinburgh.) (With thirty-four illustrations.)
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(Received for publication 12th March 1919.)

CONTENTS.

INTRODUCTORY .......... PAGE 157
EFFECTS OF PERFUSING THROUGH VESSELS OF SURVIVING LUNG .......... 158
EFFECTS IN LIVING ANIMAL .......... Method .......... 172
Cat and Dog .......... 174
Rabbit .......... 175
SUMMARY .......... 194
LITERATURE .......... 196

INTRODUCTORY.

The intravascular administration of adrenalin may produce effects on the pulmonary circulation in four ways:—

1. An effect on the pulmonary arterioles, causing constriction, or in some doses dilatation, of these vessels; and thus a diminution, or increase, of the flow through the pulmonary system, and a rise or fall of the pulmonary arterial pressure.

2. An effect on the musculature of the heart (through the sympathetic) accompanying the passage of the autacoid through the coronary vessels. It is possible that the right and left sides of the heart may be differently affected.

3. An effect on the conducting system of fibres which lie immediately under the endocardium. No effect of this kind has hitherto been shown to occur, but it might conceivably be produced. In this way also it is possible that the two sides may be differently affected.

4. An effect may be produced on the pulmonary system as a consequence of the constriction of the systemic arterioles and the great rise which is thereby produced in aortic pressure. Such an effect may be termed indirect. It might be conceived to operate either backwards by obstructing the outflow from the left side of the heart, thus damming up the blood in the pulmonary system and causing rise of pressure in that
system, or forwards by diminishing the flow from the aortic system and thus diminishing the supply of blood to the right auricle: this would tend to cause a fall of pressure in the pulmonary system.

The effects produced upon the aortic system can be similarly caused in four ways:—

1. An effect produced by constricting (or in certain doses by dilating) the systemic arterioles.
2. An effect on the heart musculature through the coronary vessels.
3. A possible effect on the sub-endocardial conducting system of fibres.
4. An indirect effect through the pulmonary system of either a backward or forward character in the same manner as has been suggested above for the pulmonary system. Although this is not likely to be so marked as backward or forward effects produced through the aortic system upon the pulmonary, since the vasomotor system is far less developed in the pulmonary vessels than in the systemic, nevertheless its possibility must not be ignored, and it may account for some of the anomalous changes in aortic pressure which follow intravenous injection of drugs, which most authors have made no attempt to explain.

The Effects of Perfusing Adrenalin Through the Vessels of the Surviving Lung.

In attempting to analyse the changes in pulmonary pressure which are produced by intravenous injection of adrenalin in the living animal, it became of importance to determine in the first instance what variations might show themselves in different individuals of the species of animal used by us, by perfusion of the autacoid through the vessels of the surviving lung, and especially whether there are any constant differences due to age. This subject has already received attention from several authors, and has therefore a certain amount of literature relating to it.

The perfusion results obtained upon the surviving lung by previous observers are, however, considerably at variance. Thus Brodie and Dixon (1), who employed defibrinated blood as the perfusing medium and dissolved the adrenalin chloride in Locke solution, experimented on cats and dogs. They obtained no contraction; but got a slight dilatation from perfusion of 1 c.c. of 1:20,000 in the cat, or from 5 c.c. of 1:50,000 in the dog. Plumier (2), on the other hand, who experimented on twenty dogs, using defibrinated blood and dissolving the adrenalin in the same medium, obtained marked constriction even with doses of 0·5 c.c. of 1:10,000. He occasionally failed to obtain constriction, but never got dilatation.

Wiggers (3), perfusing with Locke and using cats and dogs, also obtained constriction: the minimum dose with which this was got was 0·02 mg. Wiggers ascribes the dilatation got by Brodie and Dixon
to the circumstance that they used as the medium of conveyance of the adrenalin a fluid of less viscosity than that which they were employing for perfusion. Argyll Campbell (4), working in this laboratory, made twelve experiments (upon the cat and rabbit), employing Ringer solution. He got in most cases only slight constriction on passing adrenalin through the pulmonary vessels. The preparation he used was Burrough & Wellcome’s “hemisine,” made up to a strength of from 1:60,000 to 1:120,000; from 15–30 c.c being injected at one time. Campbell also tested isolated portions (rings) of pulmonary artery (sheep and rabbit) as to the effect of “hemisine.” In confirmation of Meyer (5) and Langendorff (6), he records contraction. The same result was got from isolated portions of pulmonary vein (sheep), but to a less degree. The contraction of the pulmonary artery ring was greater than that of a ring from the subclavian.

In connexion with these “ring” experiments, the results obtained by Cow (7) demand attention. This observer found that when he took a series of rings from the pulmonary artery of a rabbit, one from the main artery, a second from a primary branch, a third from the root of the lung, and a fourth from a vessel within the lung, the contraction caused by the same solution of adrenalin was well marked in the first, less in the second, still less in the third, and not evident at all in the fourth. Cow draws the conclusion that the sympathetic nerve supply is confined to the larger vessels and is lacking in those within the lung. To us, however, it appears more probable that the true explanation is that the smaller rings had less power than the larger to raise the weight of the lever, and that for the same reason the smallest failed to cause any rise. Barbour (8) also was unable by this method to obtain evidence of contraction with any but the largest branches of the pulmonary.

Baehr and Pick (9) found no distinct effect to be produced in the guinea-pig’s lung by perfusion of Tyrode solution containing 001 mg. per litre. Fühner (22, p. 302) found, in confirmation of previous observers, that constriction was produced in the pulmonary vessels of the isolated dog’s lung by perfusion with adrenalin. Finally, Mrs Tribe (10), who experimented on a large variety of animals (cat, dog, rabbit, guinea-pig, rat, and ferret) and used defibrinated blood as the perfusing medium, obtained dilatation with very small doses of adrenalin (0.00002 mg.), and constriction followed by dilatation with larger doses (0.01 mg.). This author states that the solution of adrenalin chloride with chloroetone (Parke, Davis & Co.) which was used by Brodie and Dixon causes dilatation only, and she ascribes their result to the chloroetone. But Wiggers seems to have used this solution, and we ourselves have also employed it and have never got dilatation, but always constriction. It is possible that since Mrs Tribe employed defibrinated blood the reason suggested by Wiggers for the dilatation found by Brodie and Dixon may apply also to that found by her.

From the above it will be seen that whilst one or two investigators
report no effect, most have recorded a decided change in the direction of constriction, although this seems to have varied in amount, and to obtain it at all it has usually been necessary to employ stronger solutions than those which cause constriction of systemic vessels. With a very small dose some have found that dilatation may be caused, as is known to be the case with the systemic vessels, but it is not certain that this result has not been due to an alteration in viscosity caused by the solution used to dissolve the autacoid.

Since most of our experiments upon the action of adrenalin on the pulmonary circulation of the living animal have been made upon the cat, many of the perfusion experiments here to be recorded have also been performed upon this animal, but they have been extended to the rabbit. We have not used dogs for this purpose, but in any case the evidence that adrenalin produces constriction in the pulmonary vessels of this animal is fairly convincing.

The method of perfusion used by us is similar to that employed by some of our predecessors, but we have varied it in certain particulars. The details are as follows:—

The animal having been killed, cats usually by chloroform or carbon monoxide, rabbits by a blow over the medulla oblongata, the thorax was rapidly opened and an arterial cannula connected with the perfusion apparatus introduced into the main pulmonary artery (fig. 1, PA). Another larger cannula (O) was then passed into the left auricle through the auriculo-ventricular orifice, a cut having been made into the left ventricle to allow of its insertion: it was tied in by a tape passed round the base of the ventricles. From this (venous) cannula an indiarubber tube led to a seesaw tilter (fig. 2), which automatically recorded by means of an electromagnetic signal on the smoked paper of the kymograph the amount of fluid passing through the pulmonary system. The perfusion fluid used has been Locke solution, with or without the addition of gum arabic up to 6 per cent. (the amount recommended by Bayliss for transfusion in man). This addition prevents edema of the tissues perfused, but has the disadvantage that it sometimes has a spontaneous tendency for its flow to become gradually slower (with concomitant rise of pressure) owing to a fine sediment which it is liable to contain and which cannot be got rid of by repeated filtration through flannel.

The perfusion fluid was contained in a reservoir provided with a Mariotte tube, and was conducted through spiral glass tubing in a water-bath maintained at about 40° C.: the fluid reached the pulmonary artery at a temperature of about 38° C. Before opening the thorax a glass tube was tied into the trachea, and this tube was connected in an air-tight manner with a small thick-walled rubber bag, capable of being compressed at regular intervals of about thirty per minute by the aid of an electric motor. In this way regular inflations and deflations are kept up, thereby much facilitating the process of perfusion in the pulmonary capillaries; not
only rendering this more uniform in extent, but incidentally producing an intermittence in the pressure of the perfusing fluid, which may show itself in the form of small waves like heart-beats upon the tracing recording the pressure on the smoked paper of the kymograph. The apparatus for producing this record consisted of a water manometer, the excursions of which were registered by a piston recorder in precisely the same way as in the apparatus employed in the experiments recorded in the next part of this paper for determining the changes in the pulmonary pressure of the living animal. The apparatus is described by one of us in a previous article in this volume. The water manometer was connected laterally with the delivery tube near the arterial cannula: it showed the actual pressure at which the Locke solution was being passed into the pulmonary artery. If constriction of the pulmonary arterioles occurs, this pressure rises, if dilatation, it falls; the initial pressure at the reservoir being constant.

The arterial cannula was attached by a short length of rubber tubing to the five-way tube shown in fig. 1. Besides the branches of this tube connected respectively to the delivery tube, the manometer and the arterial

![Diagram](image-url)

**Fig. 1.—Perfusion cannula for pulmonary artery.**

PA, Cannula proper tied into root of pulmonary artery; I, limb of five-way tube bringing perfusing fluid from water-bath to artery; M, tube leading to water manometer, the pressure being recorded on the kymograph paper. Of the other limbs of the five-way one serves to introduce a thermometer, and the other as a trap to catch air. A, left auricle; V, left ventricle, with cannula passed through its wall into the atricle; O, tube leading to filter.
cannula, there are two others. One of these serves as a trap to catch and release air which may have entered the tube accidentally, as well as that which is liberated from the Locke solution as it becomes warmed in passing through the water-bath. The other branch is used to introduce a small thermometer which records the exact temperature at which the perfusion fluid is being delivered to the artery.

The adrenalin solution used for injection was made up with some of the same fluid as was employed in the perfusion. It was introduced into the perfusion fluid by means of a hypodermic syringe, the needle of which was made to pierce the wall of the indiarubber tube conveying that fluid just before reaching the water-bath. The amount of fluid injected was hardly ever more than 1 c.c.; in a few experiments it was only 0.5 c.c. The period of the actual injection was recorded by an electromagnetic signal on the smoked surface of the kymograph, and as the tilter is at the same time recording the amount of fluid which is perfused during that period, the extent of dilution of the adrenalin solution with the perfused fluid could be accurately determined. And since the injected solution has to traverse the spiral within the water-bath before it actually reaches the arterial cannula, it becomes thoroughly mixed with the perfusion fluid and warmed to the same temperature.

We have used Parke Davis's adrenalin chloride solution in all our ex-
periments. This contains a small amount of chloretone, but we believe that with the dilutions employed the effect of this may be disregarded.

A preliminary determination was made once and for all with the apparatus to determine the number of cubic centimetres of fluid which had to be perfused before the injected solution could reach the pulmonary artery. This determination was made by injecting a coloured solution at the place where the adrenalin was to be injected, and noting the number of movements of the tilter which occurred before the colour showed itself in the arterial cannula; in the apparatus used there were eight or nine such movements, representing a flow of about 25 or 26 c.c., the capacity of the tilter being 3 c.c.

The advantage of this method of introduction is that any substance so introduced is only acting for a limited period on the vessels. There is therefore obtained not only the immediate effect of the active substance whilst in contact with the tissues, but also the after-effect and the changes attending its substitution by the uncontaminated perfusion fluid.\(^1\)

To compare the results of perfusing adrenalin through the pulmonary system with those obtained upon the systemic vessels, a second arterial cannula has occasionally been introduced into the abdominal aorta and a second venous cannula into the inferior vena cava, with separate connexions to the perfusion delivery tube and to a second tilter respectively. With this arrangement the perfusion can at any time be directed from the pulmonary system to the aortic, and the effects of the substance upon the systemic vessels thus recorded independently; or the experiment can be conducted upon both systems at the same time. When aortic and pulmonary circulations are thus perfused simultaneously, the marked constriction of the systemic arterioles raises the pressure of the perfusing fluid, as recorded by the manometer. Nevertheless the outflow from the pulmonary in many cases is not accelerated. It may show either no appreciable change, or distinct retardation; both results indicate a constriction in this system also.

Fifty-eight perfusion experiments have been made in the manner above described; on twelve rabbits and twelve cats. The results are set out below in tabular form. In the first column are given the weights of the animals, so that a general idea may be formed of the relative age of the rabbits and cats employed. The second column records the amount of fluid actually injected as well as the strength of the solution. In the third column is given the dilution attained by this amount of fluid in becoming mixed with the perfusion liquid which was flowing along the tube during the period of injection. The fourth column is double, and shows the number of cubic centimetres of perfusion liquid per minute passing through the blood-

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\(^1\) The method here described is similar to that used by Argyll Campbell (4), but with the addition of the recording pressure gauge and the intermittent expansion of the lungs. This last device was used by Wiggers in some of his experiments.
vessels just before and just after the adrenalin reaches them. In the fifth column, under the head of "Remarks," attention is called to special features of the experiment; and in the sixth the general effect produced on the perfused vessels is recorded. Except when otherwise specifically stated, all the results given in the table were obtained from the pulmonary vessels.

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Amount injected</th>
<th>Dilution attained</th>
<th>No. of c.c. per minute</th>
<th>Remarks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before.</td>
<td>After.</td>
<td></td>
</tr>
<tr>
<td>1650 grm.</td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 24,000</td>
<td>9 c.c.</td>
<td>1·5 c.c.</td>
<td>Previously perfused with pituitrin.</td>
</tr>
<tr>
<td>1250 grm.</td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 21,000</td>
<td>6·9 c.c.</td>
<td>6·9 c.c.</td>
<td>A very slight rise of pressure began before the injection. It is a little accelerated by the adrenalin (fig. 3).</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 22,000</td>
<td>6·9 c.c.</td>
<td>6·9 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 23,000</td>
<td>7·2 c.c.</td>
<td>7·2 c.c.</td>
<td></td>
</tr>
<tr>
<td>870 grm.</td>
<td>1 c.c. 1 : 40,000</td>
<td>1 : 96,000</td>
<td>13·5 c.c.</td>
<td>13·5 c.c.</td>
<td>Shows effect of dosage.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 40,000</td>
<td>1 : 160,000</td>
<td>18·5 c.c.</td>
<td>18·5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 80,000</td>
<td>16·5 c.c.</td>
<td>16·5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 25,000</td>
<td>15 c.c.</td>
<td>10·8 c.c.</td>
<td></td>
</tr>
<tr>
<td>785 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 80,000</td>
<td>7·5 c.c.</td>
<td>4·8 c.c.</td>
<td>Previously perfused with weak BaCl₂.</td>
</tr>
<tr>
<td>770 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 60,000</td>
<td>P. 6 c.c.</td>
<td>A. 6·9 c.c.</td>
<td></td>
</tr>
<tr>
<td>750 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 25,000</td>
<td>...</td>
<td>1·5 c.c.</td>
<td>The effects produced were all prolonged in character. Marked variations in the pressure occurred after the last two injections (fig. 5).</td>
</tr>
<tr>
<td></td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 26,000</td>
<td>3·7 c.c.</td>
<td>1·5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 30,000</td>
<td>4·5 c.c.</td>
<td>1·5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 34,000</td>
<td>3 c.c.</td>
<td>2·7 c.c.</td>
<td></td>
</tr>
<tr>
<td>650 grm.</td>
<td>5 c.c. 1 : 10,000</td>
<td>1 : 40,000</td>
<td>6 c.c.</td>
<td>4·7 c.c.</td>
<td>Second dose. Pressure rose from 107·5 to 190 mm. (fig. 6), and remained high.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 64,000</td>
<td>6 c.c.</td>
<td>3·9 c.c.</td>
<td></td>
</tr>
<tr>
<td>628 grm.</td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 40,000</td>
<td>4·8 c.c.</td>
<td>3·9 c.c.</td>
<td>Previously perfused with pituitrin. Pressure rose from 185 to 210 mm.</td>
</tr>
<tr>
<td>514 grm.</td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 30,000</td>
<td>6 c.c.</td>
<td>4·5 c.c.</td>
<td>Previously perfused with pituitrin. Pressure rose from 80 to 115 mm. (3% gum).</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 40,000</td>
<td>7·8 c.c.</td>
<td>6·6 c.c.</td>
<td></td>
</tr>
</tbody>
</table>
The Effects of Adrenalin on the Pulmonary Circulation

RABBITS—continued.

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Amount injected</th>
<th>Dilution attained</th>
<th>No. of c.c. per minute</th>
<th>Remarks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before.</td>
<td>After.</td>
<td></td>
</tr>
<tr>
<td>470 grm.</td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 28,000</td>
<td>4.8 c.c.</td>
<td>2.7 c.c.</td>
<td>Pressure rose from 220 to 245 mm. (fig. 7).</td>
</tr>
<tr>
<td></td>
<td>5 c.c. 1 : 20,000</td>
<td>1 : 25,000</td>
<td>3 c.c.</td>
<td>1.5 c.c.</td>
<td>Pressure rose from 227 to 240 mm.</td>
</tr>
<tr>
<td>456 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 44,000</td>
<td>7.5 c.c.</td>
<td>4.8 c.c.</td>
<td>After second injection pressure rose from 100 to 160 mm. and did not fall again (6% gum).</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 40,000</td>
<td>6 c.c.</td>
<td>5.2 c.c.</td>
<td>Pressure rose from 135 to 145 mm. (6% gum).</td>
</tr>
<tr>
<td>450 grm.</td>
<td>5 c.c. 1 : 10,000</td>
<td>Tilted not recording regularly.</td>
<td></td>
<td></td>
<td>Pressure rose from 140 to 180 mm. (6% gum).</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

Cats.

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Amount injected</th>
<th>Dilution attained</th>
<th>No. of c.c. per minute</th>
<th>Remarks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before.</td>
<td>After.</td>
<td></td>
</tr>
<tr>
<td>3400 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 88,000</td>
<td>27 c.c.</td>
<td>27 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 44,000</td>
<td>31.5 c.c.</td>
<td>27 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 5,000</td>
<td>1 : 28,000</td>
<td>27.9 c.c.</td>
<td>31.9 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 2,000</td>
<td>1 : 12,000</td>
<td>25.5 c.c.</td>
<td>19.5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 5,000</td>
<td>1 : 25,000</td>
<td>24.6 c.c.</td>
<td>18 c.c.</td>
<td></td>
</tr>
<tr>
<td>3280 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 75,000</td>
<td>18 c.c.</td>
<td>18 c.c.</td>
<td>Very large doses with no result.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 31,000</td>
<td>18 c.c.</td>
<td>18 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 5,000</td>
<td>1 : 16,000</td>
<td>18 c.c.</td>
<td>18 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 2,000</td>
<td>1 : 6,000</td>
<td>16.5 c.c.</td>
<td>16.5 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 2,000</td>
<td>1 : 4,000</td>
<td>13.5 c.c.</td>
<td>13.5 c.c.</td>
<td></td>
</tr>
<tr>
<td>3250 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 60,000</td>
<td>13.8 c.c.</td>
<td>12.9 c.c.</td>
<td>Previously perfused with pituitrin.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 5,000</td>
<td>1 : 15,000</td>
<td>13.5 c.c.</td>
<td>12.6 c.c.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 2,000</td>
<td>1 : 5,400</td>
<td>13.5 c.c.</td>
<td>11.4 c.c.</td>
<td></td>
</tr>
<tr>
<td>3250 grm.</td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 20,000</td>
<td>7.8 c.c.</td>
<td>4.5 c.c.</td>
<td>Systemic vessels of same animal perfused.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 23,000</td>
<td>7.2 c.c.</td>
<td>3 c.c.</td>
<td></td>
</tr>
<tr>
<td>2850 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 60,000</td>
<td>10 c.c.</td>
<td>8.25 c.c.</td>
<td>Pressure rose from 85 to 90 mm. (fig. 8).</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 10,000</td>
<td>1 : 32,000</td>
<td>7.8 c.c.</td>
<td>6.9 c.c.</td>
<td></td>
</tr>
<tr>
<td>2800 grm.</td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 34,000</td>
<td>6.9 c.c.</td>
<td>2.25 c.c.</td>
<td>Systemic vessels alone perfused.</td>
</tr>
<tr>
<td></td>
<td>1 c.c. 1 : 20,000</td>
<td>1 : 16,000</td>
<td>5.25 c.c.</td>
<td>7.5 c.c.</td>
<td></td>
</tr>
</tbody>
</table>
### Table: Aorta and Pulmonary Constriction in Rabbits

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Amount injected</th>
<th>Dilution attained</th>
<th>No. of c.c. per minute</th>
<th>Remarks</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2500 grm.</td>
<td>2 c.c. 1: 20,000</td>
<td>1: 40,000</td>
<td>Before: 10:5 c.c.</td>
<td>Pressure of perfusing fluid about 85 mm. (6% gum), (fig. 9).</td>
<td>No appreciable effect.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>After: 10:5 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2300 grm.</td>
<td>1 c.c. 1: 20,000</td>
<td>1: 50,000</td>
<td>P. 10:5 c.c.</td>
<td>Aorta and pulmonary perfused simultaneously: the outflow from the systemic vessels was in each case less than 3 c.c. per minute, but the corresponding tilter failed to register. The pulmonary flow is unchanged.</td>
<td>Strong constriction of systemic vessels. No constriction of pulmonary apparent.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 9:75 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. 9 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 7:8 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P. 6:8 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 6:3 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2170 grm.</td>
<td>1 c.c. 1: 10,000</td>
<td>1: 40,000</td>
<td>13:8 c.c.</td>
<td>With 6% gum. Pressure tends to rise of its own accord.</td>
<td>No appreciable effect.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 10:5 c.c.</td>
<td>Pulmonary only.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6:75 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1870 grm.</td>
<td>1 c.c. 1: 20,000</td>
<td>1: 62,000</td>
<td>P. 10 c.c.</td>
<td>Systemic and pulmonary perfused simultaneously.</td>
<td>Strong constriction of systemic vessels. No constriction of pulmonary vessels apparent.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 12:5 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 10:5 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A. 10:8 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4:5 c.c.</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>A. 10:5 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10:5 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>A. 10:5 c.c.</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 c.c.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4:5 c.c.</td>
<td></td>
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<td>A. 7:8 c.c.</td>
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<td>4:2 c.c.</td>
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Explanatory.—Dilution attained = dilution after injection as calculated from the tracings. Since it takes, when one system only is perfused, from eight to nine tilts before a result is to be expected, when both aortic and pulmonary are perfused simultaneously only half that number are required. When not otherwise stated, the perfusion fluid used was Locke solution, without gum acacia.

It will be observed that in the twelve rabbits examined adrenalin produced constriction of the pulmonary vessels in all but one instance. In most the constriction was marked, and the greatest amount was obtained in the oldest animal of the series. On the other hand, the animal next in age yielded no appreciable result. The greatest dilution with which constriction was obtained was 1 in 80,000. No dilatation results are recorded. Usually there was complete recovery from the constriction.
although a certain interval always elapsed before it showed itself. In one or two cases there was little or no recovery.

In some of the rabbits fluctuations in the perfusion pressure were recorded, which took the form of waves on the pressure record (fig. 5). These were no doubt caused by a rhythmic contraction of the vessels. In connexion with this it is interesting to notice that Cow in his experiments on the action of adrenalin on arterial rings, above referred to (p. 159), noticed that this autacoid occasionally caused rhythmic contractions of such rings, which show themselves as waves upon the main curve of contraction.

Of the twelve cats examined, seven were employed for the pulmonary system alone. Five of these showed constriction of the pulmonary vessels, but in none was it considerable. Moreover, a relatively large dosage was usually required in the cat to obtain a positive result. In three animals there was no appreciable effect. In two, in which the aorta was perfused at the same time, there may have been some constriction of the pulmonary vessels, but it was masked by the simultaneous effect upon the systemic vessels. In the remaining two cats experimented on the systemic vessels alone were perfused, and gave the usual well-marked constriction. It will be seen that the pulmonary vessels of the cat are far less sensitive to the constricting action of adrenalin than those of the rabbit, in which a distinct effect was obtained in eleven individuals out of twelve. None of the experiments showed any dilatation.

Some of the actual results of these perfusion experiments are reproduced in figs. 3 to 9. In all of these \( p \) denotes the curve inscribed by the piston-recorder connected with the water manometer (as shown in Pl. I, opposite p. 158), \( t \) represents the movements of the tilter which records the rate of flow, the vertical strokes being marked by an electromagnet;
Fig. 4. Rabbit. Weight 770 gms. Injection of 1 c.c. of 1 in 20,000 adrenaline hydrochloride. Actual dilution 1 in 60,000. Perfusion through aortic and pulmonary systems simultaneously. Marked constriction in both.
Fig. 5.—Rabbit. Weight 750 grms. Injection of 0.5 c.c. of 1 in 20,000 adrenalin chloride. Actual dilution 1 in 30,000. Marked constriction in pulmonary vessels, with rhythmic variations shown on pressure curve.
Rabbit: Weight 650 gms. Injection of 1 c.c. of 1 in 20,000 adrenaline. Actual dilution 1 in 64,000. Marked constriction, with striking rise in pressure curve.
The Effects of Adrenalin on the Pulmonary Circulation

Fig. 7. Rabbit. Weight 470 g.m. Injection of 0.5 c.c. of 1 in 20,000 adrenalin chloride. Actual dilution 1 in 26,000. Marked constriction.
s is the signal line and has the period of injection marked upon it, the amount actually injected being printed immediately below; and \( m \) is the time record, the vertical strokes being separated by intervals representing minutes.

\[ \text{Fig. 8.—Cat. Weight 2850 grms. Injection of 1 c.c. of 1 in 10,000 adrenalin chloride. Actual dilution 1 in 32,000. Slight constriction.} \]

\[ \text{Fig. 9.—Cat. Weight 2500 grms. Injection of 1 c.c. of 1 in 2000 adrenalin chloride. Actual dilution 1 in 4000. No perceptible effect.} \]

**THE EFFECTS OF ADRENalin UPON THE PULMONARY CIRCULATION OF THE LIVING ANIMAL.**

The literature of this subject is limited. Cybulski (11) stated that intravenous injection of suprarenal extract produces a slight rise of pulmonary pressure, due to back action from the systemic vessels or accompanied by dilatation (passive) of the pulmonary vessels. Velich (12),
who likewise obtained a rise of pressure in the pulmonary system with adrenalin, also ascribes it to back action from the constriction of systemic arterioles damming back the blood in the left heart. Both these observers worked on dogs, which were curarised. The experiments of Gerhardt (13), who also worked with dogs, led him to take a similar view. Mellin (14) made a special study of the action of drugs (and incidentally of adrenalin) upon the pulmonary and aortic blood-pressures simultaneously. To record the pressure in the pulmonary artery he employed a silver cannula of peculiar construction, which he inserted through a slit in the wall of the main artery: during the insertion the vessel had to be clamped nearer the right ventricle to prevent loss of blood, but he states that it was generally only necessary to arrest the circulation for two or three seconds. Mellin used curarised rabbits, and found that adrenalin injected intravenously, although producing a great rise of systemic pressure, had practically no effect on the pulmonary circulation. But the pulmonary cannula in Mellin's experiments was connected with a mercury manometer, and since the pressure in the pulmonary system is not more than one-fourth or one-fifth of that in the aorta, variations in the height of the column of mercury must be relatively large to be appreciable. On this account a mercury manometer—although used by nearly all previous observers (Beutner (15), Lichtheim (16), Openchowski (17), Bradford and Dean (18), Bayet (19), Cybulski (11), Velich (12), Plumier (2), Wood (28), E. Weber (31))—to record pulmonary pressure, generally in connexion with a cannula inserted into the branch passing to one of the lobes of the lung—is not a suitable recording instrument for exhibiting fluctuations of pressure in this system. François-Franck (20) substituted a water manometer—or, to be precise, a manometer containing a water-solution of an anti-coagulant salt such as oxalate or bicarbonate of soda.—recording the fluctuations by connecting the open end of the manometer with a Marey tambour. Using this method, he was able to show with comparative ease that sympathetic fibres proceeding from the first thoracic ganglion have a distinct effect upon the pulmonary system, in which a marked rise of pressure was produced by their excitation, whilst the pressure in the left auricle and also that in the aorta fell: phenomena which he attributed—no doubt correctly—to the occurrence of constriction in the pulmonary arteries. But the sympathetico-excitant of suprarenal extract was unknown to François-Franck.

Plumier, employing the same method as most of his predecessors, viz. a mercury manometer connected with a branch of the left pulmonary of a dog, and measuring at the same time the pressure in a pulmonary vein, also with a mercury manometer, obtained, on injecting adrenalin intravenously, a slight rise in both manometers; he used a very large dose of adrenalin (2 c.c. of a 1 per 1000 solution), which caused powerful vagus action. When the vagi were cut there was a much
greater rise in the aortic pressure, but still only a small rise in the pulmonary. As the pressure in the left pulmonary vein fell slightly at the same time with the rise in the artery, he concluded that the rise caused by adrenalin was not due to back pressure from the aorta and left ventricle. Plumier used also the heart-lung preparation, connecting the aorta with the superior vena cava by way of the brachiocephalic and clamping off the part of the aorta distal to that branch, and got marked rise of both pressures on injecting adrenalin. That in the aorta must of course have been due entirely to increased cardiac action: that in the pulmonary was probably due to the same, but it may have been aided by vaso-constriction. E. Weber (31) also obtained rise of pressure in the pulmonary with adrenalin, and ascribes this to contraction of arteries, not to back pressure.

Petitjean (21), who also used the mercury manometer, obtained results similar to those of Plumier, but thinks they can be explained by "back action."

Fühner and Starling (22) employed the heart-lung preparation of the dog, after the method devised by Starling. They obtained evidence of constriction of the vessels of the lung on injecting 0.01 mg. of adrenalin into the perfusing fluid (defibrinated blood). They note that the effect upon the arteries is much more prolonged in this preparation than when the whole animal is employed.

Edmunds (29) and Desbouis and Langlois (23), using an electrical method, determined the rate of blood-flow through the lungs of the dog before, during, and after intravenous injection of adrenalin chloride. With small doses (0.025 and 0.05 mg.) the latter obtained slight acceleration, indicating dilatation of arterioles; with large doses (1 mg.) marked retardation, indicating constriction. The animals were under chloral hydrate, and had received an injection of Witto's peptone. The injection was made into the saphenous vein. The amount of fluid used to convey the adrenalin is not stated.

E. Anderes and M. Cloetta (30), who used a water-manometer, registering directly by a float and connected with a branch of the pulmonary artery (cat), state that adrenalin has no effect on the pulmonary system. The illustration they give (fig. 5 of their paper) seems, however, to show a rise of pressure in the pulmonary artery beginning before the rise in the aorta. They apparently ascribe all the effect obtained by them to "back action."

Method.

The instrument we have employed for recording the pulmonary pressure is that described by one of us in a previous paper in this volume (24). It is somewhat similar to that used by François-Franck, who, however, used a Marey tambour as the recorder. The piston-recorder has many advantages over the tambour, not the least being that
Fig. 10.—Cat. Weight 3200 grm. Vagi uncut. Anesthetic, chloroform. Effect of successive injections first into the aorta through one of the carotids, and second (4 minutes after) into the external jugular vein, of 1 c.c. of a 1 in 10,000 solution of adrenalin chloride. These were the sixth and seventh doses administered.

Pulmonary pressure: the scale in mm. Hg is marked at the left of the figure; ao, aortic pressure as shown by a mercury manometer connected with the other carotid; o, its abscissa; r, respiration line, showing no movements of natural respiration; s, signal line, with two marks showing the successive injections; t, time in minutes.

Simultaneous ordinates were described by the pulmonary and aortic writing points on the paper when stationary.
Reduced to 2/3rds of original.

SHARPEY SCHAFFER and LIM. "The Effects of Adrenalin on the Pulmonary Circulation."

To face p lib.

Figure 1. Cat. 1. Weight 500 gm. - Reduced by half. - Chloral hydrate. - Pulmonary and arterial pressures of four successive doses of 1 cc. of a 1 in 20,000 solution of adrenalin chloride. These doses were the first, second, third, and fourth respectively. The first dose was injected into the aorta through one of the carotids; the second into the jugular vein; the third also into the vein, and the fourth into the aorta through the carotid. Previously to this last dose the vago-sympathetic nerves were successively severed. Lettering as in fig. 10. Simultaneous ordinates are inscribed here and there for purposes of exact measurement of time intervals. Reduced to half.

Sharpey Schäfer and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
the pulmonary pressure and all its variations can be accurately measured on the record, when the relative sectional areas of the manometer and piston-recorder and the magnifying power of the lever of the recorder have once been determined. The method of introducing the pulmonary cannula through the wall of the right ventricle is new, and from its facility is a marked improvement on the procedures which have been adopted by previous workers.

It will be convenient to give the results of our experiments upon the cat and dog first and afterwards those upon the rabbit, since the effects in the latter animal are different in certain particulars from those produced in the cat and dog.

In the Cat and Dog.

The majority of our experiments have been upon the cat, the injections having been introduced either into the jugular vein or into the aorta through one of the carotids, the other carotid being connected with the mercury manometer for recording arterial pressure. In the dog the arterial pressure has been recorded by a cannula in the crural artery, and the material introduced into a comitant vein; but for intra-arterial injection one of the carotids was employed, as in the cat. Artificial respiration was always maintained by positive ventilation with bellows actuated by an electromotor, usually at a rate of about 30 per minute. The anaesthetic used was in all cases chloroform in the first instance, and this was sometimes used throughout, the anaesthesia being kept up by passing the (warmed) air through a Brodie chloroform apparatus. But in the dog we have usually administered a hypodermic injection of morphia sulphate to maintain anaesthesia; and in many experiments upon the cat and upon the rabbit the same result has been attained by a hypodermic injection of chloral hydrate: it being thus possible to drop the volatile anaesthetic and eliminate a considerable element of variation from the experiment.

Intravascular injection of adrenalin in the cat generally produces effects upon the pulmonary circulation which are in the main parallel to those produced on the systemic circulation (fig. 10). As in the well-known adrenalin curve of aortic blood-pressure, there occurs, in the pulmonary system also, a rapid rise of pressure—strictly comparable to the aortic rise in the rate of its development and often greater in its relative extent,—followed by a gradual fall. After a relatively small dose the original pressure is regained in two or three minutes. In some instances, however, there are differences noticeable in the two curves, the most common being that the fall of pulmonary pressure occurs at first more slowly than the aortic, so that there is sometimes a tendency to the appearance of a gradually sloping plateau immediately succeeding the highest part of the curve, although the original pressure is attained at about the same time in both systems (figs. 11, 15, 16). But it occasionally happens that the
Fig. 12.—Dog, young. Scotch terrier. Weight 7200 grm. Chloroform. Vagi uncut. Effect of injecting 0.5 c.c. of a 1 in 20,000 solution of adrenalin chloride into the aorta through the carotid (fourth dose).

p, pulmonary pressure rising from 200 mm. H₂O to above 400 mm. (the pen left the paper above this point); ao, aortic pressure, beginning to rise before the pulmonary but relatively much less (from 110 mm. Hg to 150); s, axis of aortic pressure; r, respiration line; s, signal; t, time in minutes.

This experiment contrasts with that illustrated in fig. 11 in showing a much greater relative rise in the pulmonary than in the aortic system.

Reduced to 4/5ths.
Fig. 13.—Cat. 8. Weight 2350 grm. Chloroform. Vagi uncut. Effect of injecting 1 c.c. of 1 in 10,000 solution of adrenalin chloride into aorta through carotid (first dose).

The rise in the aortic system begins 6 seconds before that in the pulmonary, but the relative increase is ultimately greater in the latter. The aortic tracing shows large fluctuations of pressure which are not reproduced on the pulmonary. The respirations are rendered at first more rapid, but are afterwards slowed, the inspiratory movements becoming gradually prolonged, and a permanent inspiratory tone being finally adopted.

Lettering as in previous figures. The pulmonary and aortic pens were in complete alignment.

Reduced to 4/5ths.
pulmonary pressure begins to fall whilst the aortic pressure is still maintained. This is the case in fig. 10, and is also shown in some of the other curves represented.

Occasionally there is a very considerable difference in the relative effects upon the blood-pressure in the two systems. In some cases, when such a difference occurs, it is in favour of the aortic pressure, which is raised relatively more than the pulmonary (fig. 11). But sometimes the reverse condition obtains, as in the experiment illustrated by fig. 12, where the injection of 1 c.c. of a 1 in 20,000 solution into the aorta through the carotid caused the aortic pressure to rise from 115 to 150 mm. Hg, whilst the pulmonary pressure rose from 200 to 400 mm. H₂O.

In other respects also the pulmonary curve is not always a mere replica of the aortic: either curve may show independent fluctuations as a result.
Fig. 15.—Cat. Weight 1950 grm. Chloral hydrate 0.6 grm. One vagus cut. Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into jugular vein. The antacoid has produced at first a slight fall in both pulmonary and aortic pressures, followed by a slight rise in the pulmonary and a pronounced rise in the aortic. In the pulmonary this rise is succeeded by a slight fall. Both curves show the marked fluctuations referred to in the text. It may be noticed that they are not synchronous with the natural respirations, which were in abeyance before the adrenalin was administered but start soon after the injections. Lettering and simultaneous ordinates as before. Reduced to 4/5ths.

Sharpay Schaper and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
Fig. 16.—Cat. 8. Weight 2350 grm. Chloroform. Vagi uncut. Effect of injecting 0·5 c.c. of a 1 in 80,000 solution of adrenalin chloride into aorta through carotid. There is a very slight rise followed by a prolonged fall of blood-pressure in both systems, but better marked in the pulmonary than in the aortic. Notice that the respirations which had ceased—in consequence probably of the positive ventilation—show a tendency to recommence. Lettering as in previous figures. Simultaneous markings at right of figure.

Reduced to 2/3rds.
The Effects of Adrenalin on the Pulmonary Circulation

Fig. 17.—Cat. 9. From the same animal as was used for the tracing shown in the last figure, and taken immediately before that. Effect of injecting 5 c.c. of a 1 in 80,000 solution of adrenalin chloride into aorta through carotid. The fall of pressure shown in fig. 10 is replaced by a well-marked rise in both systems, with fluctuations which are synchronous but by no means parallel. The effect of a much larger dose in this animal is shown in fig. 13.

Reduced to 2/3 size.
Fig. 18.—Cat. 3. Weight 3150 grm. Chloral hydrate. Vagi cut. Effect of injecting 1.3 c.c. of a 1 in 20,000 solution of adrenalin chloride into the jugular vein. First dose.

Lettering as in previous figures. Both pulmonary and aortic curves show, besides the respiratory waves, large irregular fluctuations during the action of the autacoid. In this experiment the pulmonary rise begins before the aortic. The respirations become slower, with prolonged inspiratory movement.

Reduced to 6/7ths.
The Effects of Adrenalin on the Pulmonary Circulation

Cause of the Blood-pressure Rise in Aortic and Pulmonary Systems.—When a sufficient dose of adrenalin is given to a mammal, the rise of systemic pressure which ensues is caused in a twofold manner, viz.

by (1) constriction of arterioles, and (2) augmentation and acceleration of heart-beats. When the pulmonary and aortic systems show identical curves, the latter is likely to be the main agent, since vaso-constriction in the two systems would probably be neither synchronous nor equal. Indeed, the rapidity which the rise of pressure generally exhibits is an indication that
it is due to cardiac action, for constriction of arterioles produced by adrenalin is a relatively slow process compared with the augmentation and acceleration of heart-beats caused by this autacoid. This probably explains why in most cases the commencements of the rise in the curves of aortic
Fig. 21.—Cat. 2. Weight 2320 grm. Chloroform. Vagi cut. Effect of injecting 1 c.c. of 1 in 20,000 adrenalin chloride into the jugular vein.

*p* pulmonary pressure, recorded by an Ellis piston-recorder with ordinary lever; the zero is marked by the lower end of the curved ordinates shown on the left of the curve. Each centimetre above this represents 5 centimetres water-pressure; *ao* aortic pressure measured from the carotid with a mercurial manometer; the abscissa is half a centimetre above the respiration line; *r* respiration (natural); these were slow and shallow before the injection of adrenalin and stopped as the immediate result, but afterwards became deeper and more rapid; *s* signal of intravenous injection; *t* time in minutes.

In this case the pulmonary curve began to rise some seconds before the aortic.
Reduced to 6/7ths.

"SHARPEY SCHAFFER and LIM. "The Effects of Adrenalin on the Pulmonary Circulation."
and pulmonary pressure are found to be simultaneous, and why the curves in both often run almost parallel. If the main cause were constriction of arterioles, we should expect with intravenous injection to find the rise commencing in the pulmonary system some seconds before that in the aortic; and with intra-aortic injection to find the rise in the aortic system beginning some seconds before that in the pulmonary. This does, in fact, sometimes occur, but in many instances the rise of the curve is to all appearance simultaneous in both. This can, as we have just explained, only mean that at any rate the beginning of the rise is cardiac in origin. But the exceptions prove that in some animals, at any rate, adrenalin exercises an independent action upon the two systems. Illustrations of this are shown in figs. 13 and 18.

One or two other illustrations may here be given:—In a young dog weighing about 6 kilog., and anaesthetised with morphia sulphate and chloroform, we were recording the effects of intravascular administration of adrenalin upon the pulmonary and aortic pressures. Having injected a dose of 1 c.c. of a 1:10,000 solution into a branch of the crural vein, it was manifest on watching the tracing that the pen recording the pulmonary pressure began to rise a very appreciable time before that recording the aortic pressure (fig. 19), and on timing the difference by a seconds watch it was found that there was as much as six seconds between the commencing rise in the two curves. The experiment was then repeated, except that the injection was made into the aorta through a long cannula passed down one of the carotids. A difference again showed itself in the time of commencing rise of the two curves, but the difference was now in favour of the aortic curve (fig. 20).

Another instance is shown in figs. 21 and 22 (from the cat). As in the last experiment, the aortic pressure was recorded in the usual way by a mercury manometer through a cannula in one of the carotids, whilst the pulmonary pressure was measured by a water manometer with the aid of an ordinary Ellis piston-recorder with simple lever. Injections were made (1) into the jugular vein (fig. 21), and (2) through the carotid into the aorta (fig. 22). In (1) the rise manifestly begins first in the pulmonary curve, and in (2) in the aortic curve, the difference in each case being about three seconds.

Fig. 23, also from the cat, in which the injection (1 c.c. of 1 in 20,000) was made into the artery, also affords a striking illustration of the difference in time of the rise in the two systems which sometimes occurs.

In cases such as these the commencement of the rise of blood-pressure is likely to have had a vascular origin. But in the majority of cases in the cat the beginning of the rise is simultaneous in the two systems, and in these, therefore, it seems clear that the immediate and main cause is cardiac, and brought about by the circulation of the hormone through the coronary vessels. Nevertheless, independence of the musculature (vascular or cardiac) of the two systems tends to show itself in
Fig. 22.—Cat. Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin-chloride into the aorta through one of the carotids.

This tracing is from the same animal as the last, and taken 5 minutes later. The respirations have become normal in rate and depth, but the effect of the second dose of adrenalin is at first to make them slower and shallower, although they afterwards become deeper and more rapid again.

In this case the aortic curve began to rise some seconds before the pulmonary.

Reduced to 6/7ths.
Fig. 23.—Cat, large. Chloral hydrate 0·6 grm. Vagi cut. Thorax open: artificial respiration by positive ventilation at about 30 per minute. Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into the aorta through one of the carotids. Lettering as in previous figures. The respiration record is of the natural contraction of the diaphragm. The movements are rendered somewhat irregular by the adrenalin. Simultaneous markings on the right and other small ones near the commencement of the curves.

Reduced to 6/7ths.

Sharpey Schafer and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
The Effects of Adrenalin on the Pulmonary Circulation

Fig. 24.—Cat. 9. Weight 2750 grm. Chloroform. Vagi cut. Thorax open and artificial respiration by bellows. Effects on pulmonary and aortic pressures of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into the jugular vein (fifth dose).

Lettering as in previous figures. Scale of water manometer on right. Although the injection was into a vein, the rise of pressure began first in the aortic system and fell when the pulmonary rise commenced.

Natural respirations are started by the adrenalin but again die off towards the end of its action.

Slightly reduced.
some cases by differences which manifest themselves in the further course of the curves; which, even if beginning simultaneously, may, as has been already pointed out, be by no means parallel as they proceed. This is illustrated in several of the curves reproduced in this paper.

We may here refer to an anomaly observed in certain of our experiments, which is more difficult of explanation. It was, of course, not surprising to find in some cases the rise of blood-pressure caused by adrenalin beginning in the pulmonary system when intravenous injection was employed, and in the aortic system with intra-arterial injection; we had, indeed, expected that this result would occur much more often than was actually the case. But it was somewhat surprising to see in two or three cases where the injection was intravenous a rise beginning in the aortic system an appreciable interval before it showed itself in the pulmonary system, this effect being got repeatedly in the same animal (figs. 24 and 25). In fig. 24 the deferred rise in the pulmonary system with an intravenous injection is very striking. And that the rise in pressure must here be mainly due to contraction of pulmonary vessels would appear evident from the effect which is produced in this case upon the aortic pressure curve, which falls as the pulmonary curve is rising, owing, it would seem, to the blood supply being cut down by the constriction which has been produced in the pulmonary system (see below, under the head “Forward Action”). Since this result was obtained five times in succession in the same animal, it could not have been the result of accident. Similarly fig. 25 shows the rise of pressure beginning in the aorta with intravenous injection as much as six seconds before that in the pulmonary, but when the pulmonary rise begins the constriction of the lung-vessels appears to check any further rise in the aortic system, although there is no actual fall of pressure in this case as in that illustrated by fig. 24. In the animal from which this curve was obtained the vagi were uncut, and there is evidence of cardio-inhibitory action in the tracings; in the animal from which fig. 24 is taken these nerves had been cut. In one instance we observed the rise of pressure in the pulmonary to precede that in the aortic system when the injection had been made into the aorta, so that the adrenalin must have passed the systemic capillaries before arriving at the heart and affecting the lesser circulation.

These anomalous—not to say paradoxical—effects are, as we have said, somewhat difficult of explanation. They indicate either that in the particular individuals and under the circumstances of the experiment the pulmonary and aortic arterioles differed greatly in their sensitivity to the action of the antacoid, or that the right and left ventricles were differently affected by it or took different times to react to its influence. We are so much accustomed to consider the two halves of the heart as contracting absolutely as one, that most physiologists would probably be inclined to repel the second suggestion. But it must not be forgotten that clinical observations on the heart have shown that there is not infrequently a want of coincidence in the relative force of contraction of the two ventricles in spite of their common musculature, and it is known that
Effects of Adrenalin on the Pulmonary Circulation

Fig. 25.—Cat. B. Weight 2550 grm. Chloroform. Vagi uncut. Effect of injecting 0·5 c.c. of a 1 in 10,000 solution of adrenalin chloride into the jugular vein. Lettering as in previous figures. The tracing of the pulmonary pressure was made by an Ellis piston-recorder with an ordinary lever, giving curved ordinates as shown on the left. Although the injection is into the vein, the aortic rise occurs first. Both curves show heart slowing, due to vagus action. Reduced to 4/5ths.
Experiments to determine the effect of compressing the abdominal aorta upon the pulmonary pressure.

Fig. 26.—Experiments to determine the effect of compressing the abdominal aorta upon the pulmonary pressure.

$p$, curve of pulmonary pressure; $ao$, curve of aortic pressure measured by cannula in carotid; $s$, signal line, indicating period of compression; $t$, time in minutes. The line above the signal line is abscissa of aortic pressure.

A, from a rabbit with vagi cut; B, from a cat with vagi intact; C, from a cat with vagi cut.

In all these cases the anaesthetic used was chloral hydrate.
the two halves of the heart are differently affected by certain drugs.\footnote{Cf. Knoll (25), Openechowski (26), Heger (27), and Gerhardt (13). The literature is given by Pletnew, Ergebn. d. inn. Med. u. Kinderh., 1909, iii. 429.} Indeed, the fact—which was familiar to Galen—that the right half of the heart, and especially the right auricle, will continue to contract after the left has ceased to beat indicates a certain independence of the two halves of the organ. Connected with this is the observation that the left heart may be found completely contracted and rigid post mortem whilst the right is still flaccid: this is common after death from asphyxia.

Back Action.—With reference to the possibility of mechanical “back action” upon the pulmonary system from the aortic as affording an explanation of the rise in pulmonary blood-pressure caused by adrenalin, this is sufficiently negatived (1) by the fact that the pulmonary rise may precede the aortic, and (2) that the curves of the two are in many instances by no means parallel; the pulmonary rise being indeed often proportionately far greater than the aortic. Striking instances of this disproportion are shown in many of the curves illustrating this paper—in particular that given in fig. 12 from a dog. In this the injection was made into the aorta through the carotid, and the rise began first in the aortic system, but the elevation in pressure in the pulmonary system is far out of proportion to that in the aortic system, and could not conceivably have been caused by any such “back action,” in which the increase of pressure would certainly not be proportionally greater than that which produces it.

In connexion with this subject we have made several experiments to determine the amount of increase due to such back pressure in the pulmonary artery when the pressure in the systemic vessels is raised by compressing the abdominal aorta (fig. 26). In most cases such increase in the pulmonary pressure is either altogether absent or is negligible and in all relatively small. Three illustrations are given in fig. 26, A, B, and C. Of these, A and B are from the rabbit, A with vagi cut, B with vagi intact. C is from the cat, with vagi cut. In the last there is a slight rise of pulmonary pressure followed by a fall; in A a very slight fall. It is impossible, therefore, to suppose that the rise of pressure in the pulmonary caused by adrenalin is due to “back action.” It must be remembered that the method used for recording the pulmonary pressure gives larger indications of relative variations in pressure than those exhibited by the mercury manometer used for the aortic system. Bradford and Dean, who used a mercury manometer for the pulmonary system as well as for the aortic, got no evidence of “back action,” even when the aortic pressure was much raised, as by compressing the thoracic aorta or by stimulating the splanchnic nerves.

Forward Action.—Regarding the probability of a “forward” effect of constriction of the vessels of the one circulation upon the blood-pressure in the other, it is clear that, when the arterial constriction is
strong, some such effect must occur, on account of the interference with the passage of blood thereby caused. It is seen in the rabbit, in which it may be quite well marked, but in the majority of cases in the cat and dog it is far less distinct. The explanation of this difference seems to be that in the latter animals the greater part of the effect on the blood-pressure is of cardiac origin and influences both circulations simultaneously. But exceptions occasionally occur, the autacoid producing a marked rise of pressure in the aortic system but none at all or even a fall in the pulmonary. Fig. 27, which is from the cat, may serve as an example. In this case, the pressure in the pulmonary system is very little influenced by the hormone, the chief effect of which has been to cause constriction of the systemic arterioles. The adrenalin (1 c.c. of 1 in 20,000) was injected into the aorta through the carotid. In the experiment recorded in fig. 28, as the result of an injection of 1 c.c. of 1 in 20,000 into the jugular vein, there was first produced a slow rise in the pulmonary pressure, then a slow fall, this fall being synchronous with a sharp rise in the aortic pressure; the latter was maintained for about a minute, and then gradually fell. The preliminary slow rise in pulmonary pressure is no doubt due to the direct action of the adrenalin on the pulmonary arterioles; the slow fall following it synchronises with the rise in the aortic curve; both these are probably caused by extreme constriction of systemic arteries damming back the blood and preventing its free access to the right side of the heart. There is a preliminary short fall in the aortic pressure, due perhaps to the constriction of the pulmonary arterioles similarly preventing the blood from freely reaching the left side of the heart. It seems clear that the cause of this preliminary short fall cannot be cardiac, as it does not show itself upon the pulmonary curve. The probability is that in this case—which is exceptional as regards the cat and dog but common in the rabbit—the effect of adrenalin in increasing the force of the heart has either not occurred or is small in proportion to its effect on the arterial musculature—exactly the reverse of the conditions which usually obtain in the cat and dog.

In another experiment on the cat already reproduced (fig. 24), in which 1 c.c. of 1 in 20,000 was injected into the vein, the aortic pressure began to rise well before the pulmonary, and the commencing rise of pulmonary pressure is synchronous with the commencement of a prolonged fall in aortic pressure. This was not due to any accidental circumstance (e.g. the lodging of an embolus in the pulmonary artery), for it was repeated five times with the same result. A somewhat similar effect is illustrated by the tracing shown in fig. 28. On the other hand, purely cardiac effects are well illustrated in fig. 29, from the cat. In this experiment, after injection of 0·5 c.c. of 1 in 20,000 adrenalin chloride into the aorta through the carotid artery, a simultaneous fall occurred in both aortic and pulmonary pressure, with slight recovery: all fluctuations in the one were produced in the other, except that the pressure in the pulmonary had risen after three minutes to
Fig. 27.—Cat. 7. Weight 3400 grm. Chloral hydrate 0-6 grm. Right vago-sympathetic cut. Lettering as in previous figures. Scale of pulmonary water manometer marked in mm. on left. Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into aorta through the carotid (second dose). A well-marked rise of pressure in aorta unaccompanied by a similar rise in pulmonary. Some tendency to vagal action is apparent in both curves. Rhythmic fluctuations in pressure also visible in both: probably a tone-rhythm of cardiac origin. The respiratory movements were arrested by the adrenalin.

Reduced slightly.

1 The first dose caused fibrillation of the ventricles. The circulation was maintained for 7 minutes by heart massage, but the fibrillation continued. On cutting the right vagus it instantly disappeared and the heart recovered completely.

SHARPEY SCHAFFER and LIM, "The Effects of Adrenalin on the Pulmonary Circulation."
FIG. 28.—Cat. 3. Weight 3450 grm. Chloroform. Vagi cut. Lettering as usual. Coincident markings on the left. Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into the jugular vein.

Slow rise of pulmonary pressure as immediate consequence of the injection, followed by gradual fall, the commencement of which is synchronous with the sharp rise of aortic pressure. This sharp rise is preceded by a slight and evanescent fall.

The respirations are almost unaffected in this instance. They are normal in rate and depth in spite of the double vagotomy.

Reduced to 6/7ths.

Sharpey Schaefer and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
Fig. 29.—Cat. $\delta$. Weight 3280 grm. Chloral hydrate. Vagi uncut. Effect of injecting 0.5 c.c. of a 1 in 20,000 solution of adrenalin chloride first into the aorta through a carotid, and 4 minutes later into an external jugular vein. A scale in mm. of water-pressure is appended to the record of pulmonary pressure. Lettering and corresponding ordinates as in previous figures. During this experiment, and in spite of the positive ventilation with uncut vagi, the natural respirations were maintained with great regularity, and are not appreciably affected by the dose of adrenalin, which is only half that given in most of our experiments.

Slightly reduced.

Sharpes, Schafier and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
Fig. 31.—Rabbit. Weight 2050 grm. Chloral hydrate 0·8 grm. subcutaneously. Lettering as before. Coincident markings at the left of each curve. At the signal marks 0·5 c.c. of a 1 in 20,000 solution of adrenalin chloride was injected (A) into the aorta through the carotid, (B) into the jugular vein. In A the beginning of the rise of aortic pressure slightly precedes that of pulmonary pressure, although there is some obscurity caused in the latter by a small elevation which was caused by the manipulation of introducing the syringe. In B the pressure in the pulmonary system begins distinctly before that in the aortic system.

Sharpey Schaffer and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
The Effects of Adrenalin on the Pulmonary Circulation

Fig. 30.—Cat. δ. Weight 3450 grm. Chloroform. Vagi cut. Effect of injecting 1 c.c. of 1 in 20,000 solution of adrenalin chloride into the aorta through the carotid. For description see text. Notice that the respirations are gradually slowed by the adrenalin, with a tendency to stop altogether in the inspiratory position.

Reduced to 6/7ths.
that before the injection, whilst the pressure in the systemic vessels showed no tendency to completely attain its original level. A second injection was now given into the jugular vein. This caused a second fall of the pulmonary pressure, whilst the aortic pressure, after a short depression followed by a short and sharp elevation, rose gradually to about its original level, and then again fell, at first slowly and afterwards more quickly, the fall being marked by large fluctuations, which are also reproduced on the pulmonary curve. As already insisted on, there is every reason to believe that in these cases of parallelism the fluctuations are of cardiac origin.

Another tracing—which seems to illustrate the "forward effect" which may be produced upon the pulmonary circulation by constriction of systemic arterioles is that shown in fig. 30. The injection in this case was into the aorta. The beginning of the rise in both systems is simultaneous, so that it is probable that a small amount of the autacoid found its way at once into the coronary arteries. But at a particular point, marked by a slight notch on the aortic curve, while the latter continues to rise, the pulmonary curve falls and continues to do so during the remainder of the time that the autacoid is causing contraction of the systemic arterioles. Here there must have been little or no action on the pulmonary arterioles, unless indeed they were actually dilating.

In the Rabbit.

We have not been able to perform as many experiments on the rabbit as on the cat and dog, owing to the difficulty experienced during the war in getting large specimens. But only rarely have we obtained the marked rise in the pulmonary pressure synchronising with that in the aorta, which is usually so characteristic a feature of the blood-pressure curves in the cat and dog. A tracing of this kind of effect in the rabbit is given in fig. 31. Here the injection was made, first, into the artery, and, second, into the vein. The effects on the two systems are independent of one another, and as the heart was already beating very fast, they are probably chiefly upon the vascular walls. But in most instances either there was only a trifling effect on the pulmonary circulation, in spite of an extreme effect on the aortic pressure (figs. 32, 33), or the result has been very similar to that described with the cat on p. 192, and illustrated in fig. 28. A very common effect in the rabbit is illustrated in fig. 32, in which there was a preliminary slight rise in the pulmonary pressure when the injection was made into the jugular vein, converted into a depression as the pressure rose in the systemic arteries (B). This effect is probably due to constriction of the walls of these arteries preventing the blood from reaching the right side of the heart. When the injection was made into the aorta through the carotid (A), the preliminary rise shown on the pulmonary pressure curve was absent. But the fall in the pulmonary pressure which immediately follows is shown, being succeeded by the same gradual recovery. In
FIG. 32.—Rabbit. W. Weight 2400 grm. Chloral hydrate 0·6 grm. One vagus only cut (the left). Lettering as usual. Effect of injecting 0'5 c.c. of a 1 in 20,000 solution of adrenalin chloride (A) into the aorta through the carotid, and (B) into the jugular vein. The respiratory movements are temporarily diminished in extent as the result of the injections.

Sharpey Schafer and Lim, "The Effects of Adrenalin on the Pulmonary Circulation."
The Effects of Adrenalin on the Pulmonary Circulation

Fig. 32.—Rabbit, J. Weight 2400 grm. Chloral hydrate 6 grm. One vagus cut (the left). Effect of injecting 1 c.c. of a 1 in 20,000 solution of adrenalin chloride into the jugular vein. Only a very slight elevation is visible in the pulmonary tracing, the pressure in which is 130 mm. H$_2$O, whilst an immense rise of pressure is produced in the aortic system (from 48 mm. Hg to 154 Hg). The respiratory movements are somewhat diminished.
fig. 33 there is a small, almost imperceptible, rise of pressure in the pulmonary system, synchronising with a large rise of aortic pressure.

Experiments like those shown in figs. 32 and 33 are sufficient to prove that the rise of pressure in the aortic system, even when extreme, is not felt backwards in the pulmonary system. They also suggest that the rise of pressure in the aortic system of the rabbit which is caused by adrenaline is in these cases not of cardiac but of vascular origin. If it were due to increased cardiac action, one would expect that it would be shown in both circulatory systems. There is, however, the possibility, already discussed on pp. 188, 191, that the one side of the heart may be more affected by the autacoid than the other.

The "forward action" of constriction of systemic arterioles upon the pulmonary pressure is further illustrated in fig. 34. The curve there reproduced shows the effect of two successive injections of 1:3 c.c. of a 1:20,000 solution into vein and artery respectively. That into the vein causes at first constriction of pulmonary arterioles and rise of pulmonary pressure, but as soon as the autacoid reaches the systemic arterioles and constricts them the pulmonary rise of pressure is promptly brought down and converted into a fall. With the injection into the artery only a fall is seen. The very slight rise preceding this was caused by the manipulation of introducing the syringe.

**Summary.**

1. In the rabbit adrenaline injected in moderate doses into the jugular vein at first either causes a rise of pulmonary blood-pressure, owing to its constricting effect on the pulmonary arterioles, or may show no effect upon the pulmonary system. But with the great constriction of the systemic arterioles which it produces when it reaches them, any rise in the pulmonary system is rapidly converted into a fall, gradual recovery following as the constriction of the systemic arterioles passes off. When injected into the aorta through the carotid the effects are similar, but the preliminary rise in pulmonary pressure due to the constriction of the pulmonary arterioles is absent. But sometimes the effects seen in the rabbit are similar to those ordinarily seen in the cat and dog (see below).

2. Although in the cat the effects which have just been mentioned as most common in the rabbit are sometimes seen, the usual result is the production—whether the autacoid be injected into the jugular vein or into the aorta through the carotid—of a sharp, well-marked rise in both pulmonary and aortic pressures, the rise being sometimes preceded in the case of the intravenous injection by a slower elevation of the pulmonary curve, and in the case of the arterial injection by a similar commencing rise in the aortic curve. These preliminary elevations, when seen, are no doubt due to commencing constriction of the respective arterioles; but the very marked and sharp rise which almost always occurs in both pulmonary and aortic curves, and which usually shows parallelism in both systems—
Fig. 34.—Rabbit. d. Weight 2040 grm. Chloral hydrate 0.6 grm. subcutaneously. Both vagi cut. Lettering as before. Scale of pulmonary pressure on left. Several coincident markings shown. Effects of injecting 1.3 c.c. of a 1 in 20,000 solution of adrenaline chloride first into the jugular vein and 1 1/2 minutes later into the aorta through the carotid. In the first case there is a preliminary rise of the pulmonary pressure, succeeded when the aortic rise begins by a fall. In the second only the fall is seen, synchronising with the aortic rise. In spite of the double vagotomy, the respiratory rhythm is rapid and the respirations are normal in character. They are temporarily diminished in extent by the adrenaline.

Reduced to 2/3rd.
not only in the main rise and fall but also in the fluctuations which are often seen—must be of cardiac origin.

With unusually small doses a fall of pressure may be obtained in both pulmonary and aortic systems.

3. In the dog there occurs, as the result of the intravenous injection of a fairly large dose of adrenalin, a great rise of pressure in both systems, sometimes running almost parallel, sometimes better marked in the pulmonary than in the aortic system. Occasionally, in the dog as in the cat and rabbit, when an injection is made into a vein, the commencement of the rise is very distinctly in advance in the pulmonary, and when into an artery, the commencement of the rise is seen first in the aortic system; but in many instances the rise is simultaneous in both. We have not observed in the dog either a fall in the pulmonary pressure with the rise in the aortic—which is the common effect in the rabbit and is occasionally seen in the cat—nor the simultaneous fall in both pulmonary and aortic pressures which we have occasionally seen in the cat. But the number of experiments we have made on dogs has been much more limited in number than those on cats.

4. The rise in the pulmonary system is not due to "back action" propagated from the aortic system. For there may be no rise at all in the pulmonary, with great rise in the aortic system. This is true not only for adrenalin, but also when the pressure in the aortic system is raised by mechanical compression of the aorta and in other ways.

5. In rabbits the chief effects of adrenalin upon both pulmonary and aortic pressures are produced upon the blood-vessels, but in most cats and in dogs the chief effects are produced by the action of the autacoid on the cardiac musculature. There are, however, distinct effects upon the vessels both of the pulmonary and aortic systems, but these are generally obscured by the cardiac effects, although in some individuals they become more prominent. Whether these differences in individuals have anything to do with the anaesthetic or not we are not at present in a position to determine.

6. Many of our tracings afford evidence that the musculature of the two sides of the heart may be differentially affected by adrenalin. It is possible that this difference of effect may be due to the action of the autacoid upon the Purkinje network of the ventricles.

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The Effects of Adrenalin on the Pulmonary Circulation

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Examples:—
### CONTENTS

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRAMER, W., and R. M’CALL</td>
<td>Carbohydrate Metabolism in Relation to the Thyroid Gland. IV. The Effect of Thyroid Feeding on the Gaseous Metabolism of Thyroid-ectomised Rats</td>
<td>97</td>
</tr>
<tr>
<td>HOUSSAY, B. A.</td>
<td>The Action of Blood Serum of the Dog in a Condition of Tetania Parathyreopriva on Voluntary Muscular Tissue compared with that of Normal Serum</td>
<td>111</td>
</tr>
<tr>
<td>HERRING, P. T.</td>
<td>The Adrenalin Content of the Suprarenals of the Female White Rat, and Changes in it brought about by Thyroid Feeding and other Conditions</td>
<td>115</td>
</tr>
<tr>
<td>BRINKMAN, R.</td>
<td>The Effect of Phloridzin on the Permeability to Glucose of the Frog’s Glomerular Membrane</td>
<td>125</td>
</tr>
<tr>
<td>SCHAFFER, Sir EDWARD SHARPEY</td>
<td>A New and Convenient Method of recording Pulmonary Blood-Pressure</td>
<td>133</td>
</tr>
<tr>
<td>GUGLIELMETTI, JOHN</td>
<td>The Effect of Adrenalin on Muscular Fatigue in Leptodactylus ocellatus (L. Gir.) and in Bufo marinus</td>
<td>139</td>
</tr>
<tr>
<td>CUSHNY, ARTHUR R.</td>
<td>Note on Strychnine Tetanus</td>
<td>153</td>
</tr>
<tr>
<td>SCHAFFER, Sir EDWARD SHARPEY, and R. K. S. LIM</td>
<td>The Effects of Adrenalin on the Pulmonary Circulation</td>
<td>157</td>
</tr>
</tbody>
</table>

*Papers for publication may be sent to any of the Editors.*
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.

IV. THE QUESTION OF A GASTRIC HORMONE.

V. THE SOURCE OF THE PROTEOLYTIC ENZYME IN EXTRACTS OF THE PYLORIC MUCOUS MEMBRANE.

VI. A NEW METHOD FOR OBTAINING A PURE PYLORIC SECRETION.

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

BY

Robert K. S. Lim.

(From the Department of Physiology, University of Edinburgh.)
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 insidiously, 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 (3 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 a/
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, are lymphatics and nerves. In addition to these, there 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 and absent in the human, pig, 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 continuous 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 (9), 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 described by Schafer and Williams (26) in the Kangaroo, and with 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 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 activity, the cell 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 (20) 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 fundamentally 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 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 cytoplasm contains granules in the fresh state (a fact already noted 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 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 Benesky, 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 size of the cell, contrary to Heidenhain's observation.

(2) Finely reticulated (granular) or mucoid cells. 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, XX1b). 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 continuous 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 crescentic. 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.

The observations which have just been described, show firstly, that the term "chief" or "central" cells is inadequate, since there are two types differing widely from each other. Secondly, that the cells of the cardia and pylorus are similar in structure and are continuous 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 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, and therefore, that is lined chiefly by mucoid cells (see fig. 5c). 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 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 contiguous 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 or 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 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.

Literature.


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(3) " , Amer. J. Anat., 1902, II, 105.

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

(6) Cobelli, Wiener Sitzungsb., 1886, LII, 250.


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(18) " , Parasitology, 1920, XII, 108.


(22) Pilliet, J. d. l'anat. n. et path., 1887, V.

(23) Prenant, Bouin et Maillard, Traité d'Histol., 1904, II, 793.


(27) Schaffer, Wiener Sitzungsb., 1897, CVI, 353.


(30) Zimmermann, Arch. f. mikr. Anat., 1898, LII, 546.
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.

a, 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).
a, 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.
a, 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 aterostomy 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 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. 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 parts mucoid to peptic elements in each tube varies in different 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 proper of the fundus in their regular shape and in exhibiting a red-staining reticulum with Mallory. The measurements of the cardia and pylorus are set forth below.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cardiac Cells</th>
<th>Cardiac &amp; Oxyntic C.</th>
<th>Pyloric Cells</th>
<th>Pyloric &amp; Oxyntic C.</th>
<th>Curvatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat.</td>
<td>0-4 mm.</td>
<td>3 mm.</td>
<td>15 mm.</td>
<td>20 mm.</td>
<td>Greater</td>
</tr>
<tr>
<td></td>
<td>0-3 mm.</td>
<td>&quot;</td>
<td>12-15 mm.</td>
<td>20-25 mm.</td>
<td>Lesser</td>
</tr>
<tr>
<td>Dog.</td>
<td>---</td>
<td>2 mm.</td>
<td>20 mm.</td>
<td>+ 40 mm.</td>
<td>Greater</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>3 mm.</td>
<td>25 mm.</td>
<td>+ 45 mm.</td>
<td>Lesser</td>
</tr>
<tr>
<td>Rabbit.</td>
<td>0-1 mm.</td>
<td>2 mm.</td>
<td>35 mm.</td>
<td>2 mm.</td>
<td>Greater</td>
</tr>
<tr>
<td></td>
<td>0-2 mm.</td>
<td>2-3 mm.</td>
<td>40 mm.</td>
<td>2-3 mm.</td>
<td>Lesser</td>
</tr>
</tbody>
</table>

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 reticulum being more constantly present and best seen towards the duodenum.

Rabbit:

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 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 muqueuses") 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 red-staining 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 cardiac—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. 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 bloodstream. 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 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 about\(^{(16, 23)}\) and even salivary secretion\(^{(19)}\) when these changes were brought 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 regarding 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 or 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 warm-
ing apparatus. The trachea is then opened and a two-way cannula tied in.
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 kept perfectly 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. 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 pyloro-duodenal 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 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, 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 containing 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.
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".

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (gm)</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes</th>
<th>No. of Drops Before:After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 31</td>
<td>3500</td>
<td>M</td>
<td>5cc. 5% (F)water. (followed 5 minutes after dose of secretion) 1cc. &quot;</td>
<td>2</td>
<td>2:12</td>
<td></td>
</tr>
<tr>
<td>Cat 32</td>
<td>2270</td>
<td>P</td>
<td>3cc. 5% (F)water. 6cc. &quot; (14 minutes afterwards) 2cc. 5% (F)acid. 5cc. &quot;</td>
<td>3</td>
<td>13:13</td>
<td></td>
</tr>
<tr>
<td>Cat 33</td>
<td>2200</td>
<td>M</td>
<td>3cc. 5% (F)water. 4cc. &quot;</td>
<td>3</td>
<td>20:13</td>
<td></td>
</tr>
<tr>
<td>Cat 34</td>
<td>2250</td>
<td>F</td>
<td>3cc. &quot;</td>
<td>3</td>
<td>20:0</td>
<td>P</td>
</tr>
<tr>
<td>Cat 35</td>
<td>3000</td>
<td>F</td>
<td>3cc. 5% (F)acid. 3cc. &quot; 3cc. &quot;</td>
<td>7</td>
<td>12:0</td>
<td>P</td>
</tr>
<tr>
<td>Cat 36</td>
<td>2500</td>
<td>F</td>
<td>3cc. &quot;</td>
<td>3</td>
<td>20:0</td>
<td>P</td>
</tr>
<tr>
<td>Cat 37</td>
<td>2500</td>
<td>M</td>
<td>3cc.10% (F)acid. D. 14(?) 6(?)</td>
<td>3</td>
<td>3:0</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3cc.10% (F)acid. S. (16 minutes afterwards)</td>
<td>3</td>
<td>40:3</td>
<td>P</td>
</tr>
<tr>
<td>Cat 40</td>
<td>3400</td>
<td>M</td>
<td>3cc. 5% (F)acid</td>
<td>3</td>
<td>14:2</td>
<td>P</td>
</tr>
</tbody>
</table>

* 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,
### Pyloric Extracts

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight grm.</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes</th>
<th>Duration of Secretion Before Drops</th>
<th>No. of Drops Before</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 31</td>
<td>3000</td>
<td>M</td>
<td>5 c.c. 5% (F) water. (Followed 6 minutes after dose of Adrenalin)</td>
<td>3</td>
<td>25</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. 5% (F) water.</td>
<td>2</td>
<td>15</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Cat 32</td>
<td>2270</td>
<td>F</td>
<td>3 c.c. 5% (F) water.</td>
<td>3½</td>
<td>10</td>
<td>1 (?)</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6 c.c. 5% (F) acid.</td>
<td>21</td>
<td>31</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 c.c. 5% (F) acid.</td>
<td>2</td>
<td>17</td>
<td>0</td>
<td>5 (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 c.c. 5% (21 minutes afterwards)</td>
<td>19</td>
<td></td>
<td>9</td>
<td>P</td>
</tr>
<tr>
<td>Cat 33</td>
<td>1850</td>
<td>M</td>
<td>4 c.c. 5% (F) water.</td>
<td>3</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 c.c. 5% (F) water.</td>
<td>3</td>
<td>20</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Cat 34</td>
<td>2200</td>
<td>M</td>
<td>3 c.c. 5% (F) acid.</td>
<td>6</td>
<td>13</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. 5% (F) acid.</td>
<td>3 (?)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat 35</td>
<td>3000</td>
<td>F</td>
<td>3 c.c. 5% (F) acid.</td>
<td>3½</td>
<td>11</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. 5% (F) acid.</td>
<td>7</td>
<td>15</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. 5% (F) acid.</td>
<td>5½</td>
<td>11</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Cat 36</td>
<td>2500</td>
<td>F</td>
<td>3 c.c. 5% (F) acid.</td>
<td>3</td>
<td>20</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Cat 37</td>
<td>2600</td>
<td>M</td>
<td>3 c.c. 10% (F) acid. D.</td>
<td>6 (?)</td>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14 (?)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. 10% (F) acid. S.</td>
<td>9</td>
<td>30</td>
<td>3</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(15 minutes afterwards)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat 40</td>
<td>3400</td>
<td>M</td>
<td>3 c.c. 5% (F) acid.</td>
<td>3</td>
<td>14½</td>
<td>2</td>
<td>19</td>
</tr>
</tbody>
</table>

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 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 9 drops per period of 10 minutes. Results which approach this standard are labelled either doubtful positive or negative.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight grm.</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes</th>
<th>Duration of Secretion</th>
<th>No. of Drops Before</th>
<th>No. of Drops After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 30</td>
<td>2600</td>
<td>?</td>
<td>2 c.c. 10% (D) water</td>
<td>3</td>
<td>14</td>
<td>0</td>
<td>11</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. &quot;</td>
<td>5? (?)</td>
<td>5 (?)</td>
<td>0</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4 c.c. &quot;</td>
<td>6 (?)</td>
<td>7 (?)</td>
<td>2</td>
<td>3</td>
<td>N</td>
</tr>
</tbody>
</table>
### Animal Weight

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (grm.)</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes.</th>
<th>Duration of Secretion</th>
<th>No. of Drops Before</th>
<th>No. of Drops After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 31</td>
<td>3000</td>
<td>M</td>
<td>3c.c. 5% (F) acid</td>
<td>2(?)</td>
<td>4(?)</td>
<td>2</td>
<td>5</td>
<td>P/N</td>
</tr>
<tr>
<td>Cat 32</td>
<td>2270</td>
<td>F</td>
<td>3c.c. 10% (D) water</td>
<td>2(?)</td>
<td>14(?)</td>
<td>5</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(17 minutes after dose of Adrenalin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (grm.)</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes.</th>
<th>Duration of Secretion</th>
<th>No. of Drops Before</th>
<th>No. of Drops After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 30</td>
<td>2600</td>
<td>?</td>
<td>4c.c. 10% (D) water</td>
<td>5½(?)</td>
<td>4½(?)</td>
<td>1</td>
<td>2</td>
<td>Vagi intact N</td>
</tr>
<tr>
<td>Cat 38</td>
<td>2500</td>
<td>F</td>
<td>3c.c. &quot;</td>
<td>2</td>
<td>15</td>
<td>11</td>
<td>11</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(19 minutes after dose of Cardiac ext.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat 42</td>
<td>2300</td>
<td>F</td>
<td>3c.c. 5% (F) acid</td>
<td>1½(?)</td>
<td>3½(?)</td>
<td>0</td>
<td>3</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3c.c. &quot;</td>
<td>2(?)</td>
<td>8(?)</td>
<td>3</td>
<td>3</td>
<td>P?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(6½ minutes afterwards)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

### Duodenal Extracts
Duodenal Extracts.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes</th>
<th>Duration of Secretion</th>
<th>No. of Drops Before</th>
<th>No. of Drops After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 34</td>
<td>2200</td>
<td>M</td>
<td>3c.c.10% (F) acid</td>
<td>10(?)</td>
<td>13(?)</td>
<td>0</td>
<td>4</td>
<td>N</td>
</tr>
<tr>
<td>Cat 35</td>
<td>3000</td>
<td>F</td>
<td>3c.c. &quot;</td>
<td>5(?)</td>
<td>6(?)</td>
<td>0</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td>Cat 40</td>
<td>3400</td>
<td>M</td>
<td>3c.c. 5% (F) acid</td>
<td>2</td>
<td>20</td>
<td>2</td>
<td>11</td>
<td>P?</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2350</td>
<td>F</td>
<td>1c.c.20% (F) acid (autonomous extract)</td>
<td>2½</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>17</td>
</tr>
</tbody>
</table>

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.

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.

<table>
<thead>
<tr>
<th>Cat 46 2850 M Recipient</th>
<th>Cat 47 1600 M Donor.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood transfused for 5 minutes - Carotid to Jugular.</td>
<td></td>
</tr>
<tr>
<td>Drops</td>
<td>Time</td>
</tr>
<tr>
<td>2</td>
<td>10 minutes before transfusion</td>
</tr>
<tr>
<td>2</td>
<td>5 minutes during transfusion</td>
</tr>
<tr>
<td>5</td>
<td>15 minutes after transfusion</td>
</tr>
</tbody>
</table>

Two subsequent transfusions did not show 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 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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight grm.</th>
<th>Sex</th>
<th>Dose and Source of Extracts</th>
<th>Latent Period in minutes</th>
<th>Duration of Secretion</th>
<th>No. of Drops Before</th>
<th>No. of Drops After</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 30</td>
<td>2600</td>
<td>?</td>
<td>1 c.c. 0.0001%</td>
<td>9</td>
<td>10</td>
<td>2</td>
<td>10</td>
<td>Vagi intact P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 c.c. &quot;</td>
<td>10</td>
<td>20</td>
<td>6</td>
<td>21</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. &quot;</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>5</td>
<td>P</td>
</tr>
<tr>
<td>Cat 31</td>
<td>3000</td>
<td>M</td>
<td>2 c.c. 0.0001%</td>
<td>5</td>
<td>12</td>
<td>6</td>
<td>10</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. &quot;</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>4</td>
<td>N</td>
</tr>
<tr>
<td>Cat 32</td>
<td>2270</td>
<td>F</td>
<td>2 c.c. 0.0001%</td>
<td>6&lt;sup&gt;4/5&lt;/sup&gt;</td>
<td>14&lt;sup&gt;3/5&lt;/sup&gt;</td>
<td>5</td>
<td>8</td>
<td>P?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(20 minutes after dose of Pyloric ext.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 c.c. &quot;</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. 0.00005%</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>P?</td>
</tr>
<tr>
<td>Cat 33</td>
<td>1850</td>
<td>M</td>
<td>1 c.c. 0.00005%</td>
<td>5(?)</td>
<td>12&lt;sup&gt;1/2&lt;/sup&gt;(?)</td>
<td>13</td>
<td>9</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(16 minutes after dose of Pyloric ext.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 c.c. &quot;</td>
<td>2(?)</td>
<td>9(?)</td>
<td>0</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td>Cat 43</td>
<td>3000</td>
<td>M</td>
<td>2 c.c. 0.00005%</td>
<td>2(?)</td>
<td>13(?)</td>
<td>3</td>
<td>7</td>
<td>P?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. Pyloric ext. acid + 2</td>
<td>16</td>
<td>7</td>
<td>15</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. Adren. 0.00005%</td>
<td>(15 minutes afterwards)</td>
<td>3&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>3&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 c.c. Cardiac ext. acid + 2</td>
<td>3&lt;sup&gt;1/2&lt;/sup&gt;</td>
<td>0</td>
<td>4</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 c.c. Adren. 0.00005%</td>
<td></td>
<td>0</td>
<td>1</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

The Adrenalin solution used was Parke, Davis & Co., Adrenalin Chloride, 1-1000.
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 partly for a few minutes. These results agree 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's 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 depressor evoked is about normal; note also that the pressor 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 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 CO₂ - 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 return gastric venous blood; but even if an exciting 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 for 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. Popielaki (24), Rothlin and Grundlach (26) and Keeton, Koch and Luckhardt (12) have shown that 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 cause 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.

Literature.

(2) Barger and Dale, J. Physiol., 1910, XLI, 499.
(4) Edkins, J. Physiol., 1906, XXXIV, 133.
(9) Hess u. Grundlach, Pflüger's Arch., 1920, CLXXXV, 121.
(10) Keeton and Koch, Amer. J. Physiol., 1915, XXXVII, 481.
(15) Maydell, Pflüger's Arch., 1913, CL, 390.
(16) Popielski, Pflüger's Arch., 1909, CXXVI, 483.
(18) Popielski, Pflüger's Arch., 1912, CXLIV, 135.
(19) Popielski, Pflüger's Arch., 1913, CL, 1.
(20) Popielski u. Panek, Pfluger's Arch., 1909, CXXVIII, 222.
(21) Popielski, Centralb. f. Physiol., 1910, XXIV, 635.
(22) Popielski, Centralb. f. Physiol., 1910, XXIV, 1102.
(24) Popielski, Pflüger's Arch., 1919, CLXXVIII, 214.
(26) Rothlin u. Grundlach, J. de physiol. e. d. path. génér., 1920,
(28) Starling, Recent advances in the Physiology of Digestion, 1906, London.
(30) Yakawa, quoted by Hess and Grundlach.

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 and 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.
Respiration

Cat. 36.9
2500 grm.
CHCl₃
9. XII. 30

Blood Pressure

Stomach Balloon

Drops from Stomach

Signal

Time (minutes)

3 c.c. 5% Pyloric ext. in 0.2% HCL, 2nd. dose.
Respiration

Drops from Stomach

Signal

Time 4 c.c. 5% Pyloric ext. in water.

Cat 33 ½ 1850 grm. CHCl₃ 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 g  CHCl₃
2500 grm.

Drops from Stomach

Signal

Time 3 c.c. 10% Cardiac ext. in water.

Respiration

Cat 43 g  3000 grm.  CHCl₃ 15, XII. 20

Blood Pressure

Stomach Balloon

Drops from Stomach

Signal 2 c.c. 0.00005% Adrenalin

Time 3 c.c. 5% Cardiac ext. (acid) + 1 c.c. 0.00005% Adrenalin.
Respiration

Blood Pressure

Cat 42 ♀
2300 grm.
CHCl₃
14. XII. 20

Stomach Balloon

Drops from Stomach

Signal

Time 3 c.c. 5% Fundus ext. in 0.2% HCl, 2nd. dose.
Respiration

Blood Pressure

Cat 40 ½
3400 grm.
CHCl₃
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 &
3590 gms
HCl 3
13.XII.20

Stomach Balloon

Drops from Stomach

3 o c, 5% jejunal ext. in 0.2% HCl.

Time

Signal
Respiration

Drops from Stomach

Signal

Time 2 c.c. 0.0001% Adrenalin Chloride, P.D. Salivation

I → (2nd. dose)

Cat 30½ 2500 grm. CHCl₃ Vagi intact.

Respiration

Drops from Stomach

Signal

Time II →
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 [Wasmann (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.

<table>
<thead>
<tr>
<th>Author</th>
<th>Animal</th>
<th>Activity of Pylorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grützner (6)</td>
<td>Dog</td>
<td>1/15-25</td>
</tr>
<tr>
<td>Ebstein (3a)</td>
<td></td>
<td>1/2-3</td>
</tr>
<tr>
<td>Glaessner (4)</td>
<td></td>
<td>1/21</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>1/17</td>
</tr>
<tr>
<td></td>
<td>Calf</td>
<td>1/20</td>
</tr>
<tr>
<td>Langley (12)</td>
<td>Mole</td>
<td>1/73</td>
</tr>
<tr>
<td>Greenwood (5)</td>
<td>Pig</td>
<td>1/40</td>
</tr>
</tbody>
</table>

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 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 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 Klemsiewicz (8) and Heidenhain (7). 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. Glaesner (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.

Methods.
Methods.

The stomach was opened as soon as the animal was killed, its interior thoroughly 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, 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.**

<table>
<thead>
<tr>
<th>Cats</th>
<th>Fundus</th>
<th>Pylorus</th>
<th>Hours since last meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>27 min +</td>
<td>1 hr.55 min +</td>
<td>5 hours</td>
</tr>
<tr>
<td>23</td>
<td>45 &quot; +</td>
<td>4 hrs. (negative)</td>
<td>1 &quot;</td>
</tr>
<tr>
<td>25</td>
<td>45 &quot; +</td>
<td>7 &quot; +</td>
<td>18½ &quot;</td>
</tr>
<tr>
<td>20</td>
<td>50 &quot; +</td>
<td>3 &quot; (negative)</td>
<td>14 &quot;</td>
</tr>
<tr>
<td>22</td>
<td>1 hour +</td>
<td>4 &quot; &quot;</td>
<td>19 &quot;</td>
</tr>
<tr>
<td>24</td>
<td>1½ hours +</td>
<td>5 &quot; &quot;</td>
<td>1 &quot;</td>
</tr>
</tbody>
</table>

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 every 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 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.
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 of both the peptic and pyloric cells vary considerably in animals apparently in the same stage of fasting or digestion. (I 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).

<table>
<thead>
<tr>
<th>Series III.</th>
<th>Frogs</th>
<th>Oesophagus</th>
<th>Stomach</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R. Esculenta</td>
<td>+ 45 minutes</td>
<td>+ 1 hr. 40 m.</td>
<td>Fed 5 hrs. ago</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>+ 55 &quot;</td>
<td>- 2 &quot; 30 &quot;</td>
<td>Unfed</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>+ 55 &quot;</td>
<td>+ 5 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>+ 1 hr. 15 min.</td>
<td>+ 5 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>R. Temporaria</td>
<td>+ 1 hour</td>
<td>+ 4 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>+ 55 minutes</td>
<td>+ 1 &quot; 35 &quot;</td>
<td>Dead parasite in stomach.</td>
</tr>
</tbody>
</table>

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).
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. Furthermore, 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 Glaesener 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 it 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.

Literature.

(2) Contejean, Centralb, f. Physiol., 1892, VI, 648.
(3) Ebstein u. Grützner, Pflüger's Arch., 1872, VI, 1; Ibid, VIII, 122, 621.
(3a) Ebstein, Arch. f. mikr. Anat., 1870, IV, 515.
(4) Glasersner, Hofmeister's Beit., 1901, I, 1.
(5) Grützner, Pflüger's Arch., 1879, XX, 385.
(7) Heidenhain, Pflüger's Arch., 1878, XVIII, 169.
(8) Klemsiewicz, Wiener Sitzungsb., 1875, 248.
(10) Klug, Pflüger's Arch., 1902, XCII, 281.
(14) Reach, Hofmeister's Beit., 1904 IV, 139.
(15) Swiecicki, Pflüger's Arch., 1876, XIII, 444.
(17) Wittich, Pflüger's Arch., 1873, VII, 18.
VI. A New Method for Obtaining a Pure Pyloric Secretion.

The necessity for the formation of a histologically 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 Klemieiewicz (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 (8) used both the Thiry and the Pavloff (5) procedures. The last method has the advantage of connections having both the nervous and vascular 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 investigated the pyloric secretion in the above manner. All these observers have found that the secretion is alkaline and proteolytic.

**Technique.**

The method which I have employed differs from both the procedures already described in several ways, and has not 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 broad, along a circular strip, about 10 mm. 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 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 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  Male  2800 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 1½ 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 1½ hours: recovered perfectly 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: Guff 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 an adequate 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.

(2) Heidenhain, Pflüger's Arch., 1878, XVIII, 162.
(3) Klemensiewicz, Jahresarb. ü. d. fort. d. Tierchem., 1875, V, 162.
(4) Kresteff, Rev. méd. d. L. Suisse Romande (quoted from Schemiakine)
Figure to show the method of 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 = Gastro-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.

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