THE NERVOUS CONTROL OF THE PANCREATIC GLAND IN THE RABBIT

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-by-

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C O N T E N T S

INTRODUCTION

The investigation of the secretory function of the pancreas in the rabbit has interest from different points of view. It is not only interesting from a purely comparative physiological aspect, but the peculiarities of the function of the alimentary tract of the rabbit provide an opportunity to approach the study of the mechanism of pancreatic secretion from a new point of view.

The great difference between the pancreatic secretion in herbivorous animals such as the rabbit and that of carnivorous animals such as the dog and cat, is that the secretion in the herbivors is continuous. This continuity of secretion was assumed by early investigators to be dependent on the unceasing digestive work of the stomach. In carnivors the stomach functions only for a few hours after a meal whereas in herbivorous animals the stomach is always more or less full.

The physiological mechanism of this spontaneous secretion has not as yet been adequately explained. The assumption of a direct and exclusive relationship between the continuous gastric activity and pancreatic secretion in herbivors has no experimental foundation. Other factors which may bear a part in the mechanism of the continuous secretion are neglected.

The respective parts played by the humoral and nervous mechanisms in the normal secretory function of the pancreatic

gland in the rabbit is, on reviewing the available literature, very confusing.

While the humoral mechanism is well established, the exact function of the nervous innervation of the pancreas in the rabbit in the process of normal secretion is not definitely established.

The pancreatic gland receives a nerve supply from both the sympathetic and para-sympathetic nervous systems. The sympathetic innervation comes from the splanchnic nerces to the coeliac plexus. From offsets of the hepatic, superior mesenteric and splenic plexuses networks of fibres reach the gland with the blood supply. The sympathetic is believed to supply the pancreatic gland with secretory and "trophic" fibres as well as vase constrictor fibres to the blood vessels.

The parasympathetic innervation is not well defined anatomically. It is believed by some that synapses of the vagus fibres take place in the coeliac plexus whence ganglionic fibres enter the gland along with the sympathetic plexuses. Other investigators claim that there are ganglion cells of parasympathetic origin in the glandular tissues which represent the terminal synapses of vagus fibres which reach the gland from the stomach and duodenum. Certain investigators (Hess & Polisk) 1926, claim on experimental evidence that the

parasympathetic ganglia of the pancreas are located in the vagus ganglia. Thus the investigation of the pancreatic secretion in the rabbit is one of interest as well as one of considerable physiological significance, and we have tried to elucidate the mechanism of the spontaneous secretion to determine what part the humoral mechanism is playing and what the nervous influences.

A brief review of the literature on pancreatic secretion will follow under the following divisions:

A. Humoral Mechanism,

B. Nervous Mechanism,

C. Pancreatic Secretion in the Rabbit.

LITERATURE

A. HUMORAL MECHANISM OF PANCREATIC SECRETION

The secretory function of the pancreas was established in the latter part of the 17th century by Regnier de Graaf. In 1671 he observed a flow of pancreatic juice from a quill inserted into the duct of Wirsung of a dog. Tiedmann & Gmelin 1826 also saw the secretion of a clear juice from the pancreatic ducts of the dog and sheep.

Leuret & Lassaigne, 1825, collected a large quantity of secretion from the pancreatic duct of a horse by means of a gum elastic bougie attached to the end of which was a bulb of the same material. A partial vacuum was produced in the bulb thereby exerting a gentle suction on the duct.

Claude Bernard, 1856, it was who first demonstrated the role of the pancreas in the process of digestion. He devised the first operation to secure a permanent pancreatic fistula, by tying a small silver canula into the duct of Wirsung. In this way he studied the pancreatic secretion of dogs and rabbits over a period of several days, and made the classic discoveries of the role of pancreatic secretion in the digestion of albumoses, carbohydrates and fats.

His technique was faulty, however, in that after a few days the ligatures holding the canula in the duct gave way and the canula was dislodged.

The next step in the investigation of the secretory function of the pancreas was made simultaneously by Heidenhain, 1880, and by Pavlov 1879, who developed an exact operative procedure in the preparation of permanent pancreatic fistulae. A small piece of the duodenal mucous membrane containing the papilla of the duct of Wirsung was excised and brought out to the surface of the abdominal wall. The duodenum was then

closed and attached to the abdominal wall with strong sutures to prevent undue traction on the newly transplanted papilla. In this way a permanent pancreatic fistula was prepared which would function for months and even years. The investigations of Pavlov and his school on dogs with this type of fistula have contributed much to the fundamental knowledge which we now possess of the physiology of the pancreatic gland.

The close interrelationship between digestive processes and pancreatic secretion was first noticed by Magendic 1817, and later emphasized by Claude Bernard.

The exhaustive researches of Pavlov and the Russian School of Physiologists have greatly clarified this co-agency. Walther 1899, observed that in a dog with a permanent pancreatic fistula, the flow of pancreatic juice was initiated $1\frac{1}{2}$ - $3\frac{1}{2}$ minutes after the ingestion of food. Studying the curve of secretion he found that it varied in amount and rapidity of secretion with the type of food ingested.

The fact that weak solutions of acid applied to the duodenal mucosa around the orifice of the duct of Wirsung provoked a flow of pancreatic juice was recorded by Leuret & Lassaigne in 1825. From this observation they postulated the hypothesis that the psssage of acid chyme from the stomach into the duod-enum must have a similar effect.

This very profound reflection was overlooked until a half

century later when Becker, 1893, and Gottlieb 1894, demonstrated that the introduction of a large number of different acids into the stomach produced a copious flow of pancreatic juice.

The direct injection of .5% HCl into the duodenum however resulted in a very abundant flow of juice. Popielski 1896, Wertheimer & Lepage 1901, Fleig 1903; Frouin & Marbe 1910,

The injection of acid into the blood was found to be without any secretory effect. Popielski 1901, This stimulating action of acid was not abolished by section of the vagi and sympathetic chains. Popielski 1896 or by section of the coeliac and superior mesenteric plexuses. Wertheimer 1901, Popielski 1901. On the basis of their experiments Wertheimer & Lepage 1901 concluded that the secretory property of acid injected into the intestine was due to a peripheral reflex mechanism probably sympathetic. This reflec diminished with descent down the intestine disappearing in the last few feet of the ileum. This reflex moreover was not abolished by atropine.

These observations attracted the attention of Bayliss & Starling who on the basis of their classic experiments proposed the secretin theory 1902. Their hypothesis was based on two experiments. First, they noted that when acid was injected into a completely denervated loop of upper ileum pancreatic secretion was initiated; secondly, they found in

acid extracts of the duodenal and jejunal mucosa a specific substance "secretin" which stimulated pancreatic secretion when injected intravenously. This momentous discovery was hailed with acclamation and many investigators repeated these basic experiments and some controversial evidence was presented.

Wertheimer 1902 - obtained confirmatory results but expressed doubt as to the completeness of the denervation. Fleig 1903 reported contradictory evidence with the injection of acid into the denervated loop. He suggested the possibility that secretin might be formed in the denervated loop but not absorbed into the blood.

Farrell & Ivy 1928 contended that the injection of acid into the denervated loop might indirectly change the blood flow through the pancreas by other means than through reflexes thereby augmenting the secretion; or that the strength of acid .4% might be considered abnormal and any result obtained unphysiological.

Now, then, arose the question as to how secretin acted and whether a local periphoral reflex was concerned or not. Popielski 1907 as a sequel to his experiments concluded that acid stimulation of the pancreas was due to a local nervous reflex. Babkin & Savitsch 1908 reported that atropine did not abolish the secretory property of acid injected into the duodenum which

was against the existence of a local nervous reflex. Wertheimer & Lepage 1901 & 1902, discovered that venous blood taken from a denervated loop containing acid would cause pancreatic secretion when injected intravenously into another animal. They reasoned, however, that both a peripheral reflex and a humoral mechanism were concerned. Popielski 1902 denied the humoral action of hydrochloric acid. According to him the transfusion of blood from a secreting dog to a non-secreting animal produced a very insignificant secretion. In some of the control experiments the blood of a fasting animal initiated a greater secretion than the blood of a secreting dog. Matsuo 1912, repeated the transfusion experiments. He found in experiments with carotid and jugular cross-circulation that acid injected into the duodenum of one animal produced a flow of pancreatic secretion in the second animal also. Carotid to jugular cross circulation being somewhat unphysiological, resulting changes in blood pressure to which the pancreas is extremely sensitive, must be considered in connection with Matsuo's experiments.

Luckhardt 1922 (unpublished) obtained negative results on cross circulating a secreting with a non-secreting dog, using a syringe canula method. Lack of uniformity of the results of these investigators may be accounted for by the technical difficulties of experimentation, and by the variation in the response of the pancreas of different dogs to stimulation under anaesthesia.

Transplantation of the tail of the pancreas has been used as a method of proof of a humoral mechanism for pancreatic secretion.

Hedon 1892 and Laguesse 1902 observed secretion in the auto-transplanted tail of the pancreas. These experimenters, however, did not observe any relationship between the secretion and the ingestion of food, and therefore did not attach any significance to their results.

Ivy & Farrell, 1926, have observed the secretion of the auto-transplanted tail of the pancreas on the ingestion of food, and regard this as conclusive proof of a humoral mechanism for pancreatic secretion. Further investigations from this aspect by Ivy Farrell & Lueth 1927, have offered more convincing evidence. These workers transplanted two six-inch loops of jejunum as well as the tail of the pancreas and found that acid injected into the loops produced a secretion from the pancreatic transplant.

Necheles & Lim 1928, have shown by the method of vivi-dialysis that a pancreatic secretory excitant is in the blood at all times, and is increased in amount during the digestion of a meal. Furthermore, this excitant is obtained more readily from the portal than from the carotid blood.

Thus the primitive experiments of Bayliss & Starling

proving the liberation of a pancreatic hormone from the intestinal mucosa on acid stimulation has been confirmed by more recent and exhaustive experimentation.

SPECIFICITY OF SECRETIN

The second postulate laid down by Bayliss & Starling was in regard to the specificity of the pancreatic hormone secretin. They believed that secretin was a specific hormone for pancreatic secretion and they prepared it from the intestines of different animals.

Subsequent investigators have questioned this specificity suggested by Bayliss & Starling. Popielsky 1901 & 1902 maintained that secretin was not a specific hormone for pancreatic secretion as other glands were stimulated by it - salivary glands, liver and gastric glands. Bottazzi 1904 and Popielski 1908 noted that the first injection of secretin gave a light secretion, and subsequent repeated injections rapidly became ineffective. Popielski suggested an "immunity" against secretin.

Launoy 1905, Motel & Terroine 1909 and Lalou 1912 have shown conclusively that pancreatic secretion can be provoked by repeated and continuous slow injection of secretin for as long as 12 hours.

Wertheimer & Lepage 1901 produced a flow of pancreatic juice with intravenous injections of extracts acid or saline

of the mucous membrane of the large intestines and also with extracts of the musculature of the large intestines.

Modrakowski 1910 found that extracts of the thyroid gland and Frouin 1912 extracts of the testicle initiated pancreatic secretion.

Popielski 1909 stated that secretin and vasodilatin - the substance which he regarded as being responsible for the abrupt fall in blood pressure produced by the intravenous injection of Bayliss & Starling secretin were one and the same, and that the flow of pancreatic juice depended on the fall of blood pressure. The careful work of Lalou 1912 showed that this view was incorrect. He compared the secretion obtained by extracts of different parts of the gastro intestinal tract and of other organs, and found that the amount of secretion produced by these other extracts was insignificant when compared with the secretory power of the duodenal jejunal extract. He suggested the existence in all tissue extracts of an unknown substance vasodilatin, which was responsible for the fall in blood pressure and the slight secretory effect produced by these different tissue extracts.

This hypothesis was confirmed by Barger & Dale 1911, who demonstrated that all tissue extracts contain a powerful vasodilator substance B - iminazolmethylamine (histamine). It has further been shown that histamine injected intravenously provokes a mild pancreatic secretion.

Subsequent investigators, Mellanby 1926, Volbroth 1925, and Weaver & Koch 1926, have made purified secretin preparations free from any vase dilating action. Thus it is an established fact that secretin is a distinct entity from histamine or from hypothetical "vaso dilatin".

Many workers have claimed to have isolated secretin from many animal: and even from plant tissues: from the stomach and colon Gley 1910, from muscle Popielski 1907. From brain, pancreas and blood Popielski 1909. From the thyroid Modra Kowski 1910, and from the liver, Rogers, Rohe Fawcett & Hackett 1916.

Dobreff, 1925 and Van Eweyk & Tannenbaum 1921, have reported secretin action of extracts of spinach, nettles and hydrolzsed egg white.

This apparently wide and non-specific distribution of secretin however probably has little physiological significance as these extracts undoubtedly contain vaso dilatin or histamine and until the exact chemical structure of true secretin is ascertained they cannot be regarded as containing specific pancreatic secretin. In fact Drewyer & Ivy 1929, have reported that extracts made from various body tissues and spinach by methods which regularly yield a vaso dilatin free secretin from duodenal mucosa, with the exception of the pyloric and colon they contain no specific pancreatic excitant.

Another experimental fact that seemed to endanger the stability of the secretin theory was that crude secretin preparations had much less effect when injected into the portal system as compared to their effect when injected into the systemic circulation. Halliburton and de Souza 1921, Lim & Ammon 1923, Matsuoka 1923. These results however, cannot be regarded as physiological as crude secretin preparations were used.

Mellanby 1926, confirmed the results for crude secretin but pointed out that purified secretin acted equally well on injection into the portal or systemic circulation.

On reviewing the mass of experimental evidence it would seem that the specificity of secretin for the pancreas has been conclusively proven. Starting with the crude preparation of Bayliss and Starling different investigators have by improved methods, and by elimation of the vaso dilatin factor, and by researches with this purified secretin, proven unquestionably the humoral mechanism for pancreatic secretion activated by introduction of acid into the duodenum.

Nervous Control of Pancreatic Secretion

That the parasympathetic and sympathetic nervous systems have a definite influence on the secretory function of the pancreatic gland is a well established fact. There are, however, many aspects of the action of these nerves which are not entirely clear.

The first real evidence that the parasympathetic (i.e. vagus nerve) nervous system had a secretory action on the pancreatic gland was presented by Pavlov 1893. The attempts of previous investigators to obtain pancreatic secretion by stimulation of the vagus nerve had been very unsuccessful. Pavlov ascribed their failure to the extreme sensitivity of the pancreas to any kind of sensory stimulation. To avoid these adverse stimuli, he devised a technique of sectioning the spinal cord below the medulla and introducing artificial respiration. Under these experimental conditions stimulation of the vagi in the chest below the heart resulted in a definite thought not very copious secretion of pancreatic juice. In addition to these acute experiments, Pavlov used dogs with permanent pancreatic fistulae. In these animals one vagus was cut asceptically in the neck 4 - 5 days before the experiment. This was done to secure the degeneration of the cardio and secretory inhibitory fibres. On the 4th or 5th day then, the wound was

opened and the vagus stimulated with an induction current without any manifestation of pain from the animal. A secretion of pancreatic juice resulted from this type of experiment also.

Both these experiments were criticized by Bayliss & Starling 1906, after their discovery of secretin on the grounds that stimulation of the vagus caused increased motility of the stomach with the passage of acid gastric juice into the ,duodenum and the liberation of secretin.

This supposition was not sustained, however, by subsequent investigators, Savitsch, 1909. Babkin & Savitsch 1908 who repeated Pavlov's classical experiments after ligature of the pylorus, thereby preventing the escape of gastric contents into the duodenum.

Recently Tonkich (1924) has proven the secretory action of the partly degenerated vagus nerve in dogs with permanent pancreatic fistulae. In all her experiments the stomach was separated from the duodenum by a circular suture in the mucous and sub-mucous membranes of the stomach near the pylorus.

The nitrogen content of the juice which is proportional to the enzyme content rose greatly during the vagus stimulation (e.g. from 0.318 to 0.865 after Kjeldahl). That is, the pancreatic juice acquired the characteristics of typical "nervous" juice. Therefore there is no doubt that the vagus nerve has

a definite relationship to the secretory elements of the pancreatic gland.

The secretory fibres of the parasympathetic (vagus nerve) are paralysed by atropine. Pavlov 1893, Savitsch 1909. Modiakowski 1906. Pavlov 1893.

Pilocarpine in the dog (Heidenhain (1883) and others - and in the rabbit. Gottlieb 1894; as well as physostigmine in the dog, Popielski 1896 and in the rabbit Gottlieb 1894, activate a secretion of pancreatic juice rich in organic matter and enzymes, Gottlieb 1894, Savitsch 1909, Wertheimer 1901 de Zelwa 1904 and others.

The pancreatic juice obtained from vagal stimulation is rich in organic substances and enzymes. This fact was established long ago in Pavlov's laboratory by Kudrewczki,1890. Savitsch l.c. and Babkin. Savitsch l.c. demonstrated that there is a notable difference between the juice obtained from vagal stimulation and that resulting from the introduction of a solution of H Cl. into the duodenum. The stimulation of the vagus gave a juice rich in organic material and a comparatively low content of ash. Its enzymatic power was high and two of the three enzymes, trypsin and lipase were partly in active form, i.e., they acted slightly on their substrates without being activated by enterokinase and bile respectively. Their full action was obtained however only after the addition of bile and enterokinase to the pancreatic juice.

The amylolytic power of the pancreatic secretion was powerful too, but, as always, amylase was secreted in an active form.

The pancreatic juice obtained from acid stimulation presented a markedly different composition. The organic constituents were poorer, the ash and alkalinity higher than those of the "nervous" juice. The enzyme content was low and the lipolytic and proteolytic ferments were secreted in an inactive form. Atropinehad no effect either on the amount or composition of the juice secreted under acid stimulation. Babkin & Savitsch L.c., whereas the stimulation of the vagi was ineffectual after this drug. Consequently, the secretion activated by H.C.l may occur without participation of the parasympathetic nervous system and may be looked upon as "humoral" or "hormonal" due to the formation of secretin.

The histological pictures of the pancreatic gland after vagal and acid stimulation were quite different too. Babkin, Rubaschkin and Savitsch 1909.

After the vagi were stimulated the number of zymogen granules greatly diminished, and vacuolae appeared in the cell body having different staining reactions from those of the granules. Thus the secretory process under control of the vagi would appear to be an active one. Following the secretory stimulation of H Cl, the granular content of the cells diminished only slightly. There was no vacuolar formation and no change in staining reaction, although the amount of secretion was greater than in the case

of vagus stimulation. Babkin, Rubaschkin and Savitsch therefore regarded the secretion activated by the introduction of H Cl into the duodenum as being due to a quite different mechanism from that initiated by stimulation of the secretory nerves. The humoral mechanism i.e., secretin formed by H Cl in the duodenum, acted by increasing the permeability of the secretory cells for water and some of the inorganic constituents of the blood. The organic substances and enzymes accumulated in the gland are "carried out" passively. Hence, the greater the flow of juice the pooer was the organic and enzyme content and vice versa. In the case of the action of the vagus the so-called trophic influence of Heidenhain is brought into play. That is, the content of organic substances and enzymes may vary independently of the rate of secretion of the fluid and inorganic salts of the juice. (✱)

Note:
(✱) Since the nitrogen content of the pancreatic juice varies directly with the concentration of organic substances and enzymes in it, Babkin & Tichonierow 1909, and since the three enzymes of the pancreatic juice are always secreted parallel, Babkin 1904, Savitsch l.c. the determination of one of these properties of the juice is sufficient to give a reliable indication of the composition of the juice. Different experimenters have investigated separate properties of pancreatic juice, and as evidenced by the literature, their results are comparable.

Pavlov's experiment proving the secretory action of the vagi in the dog were repeated by many workers.

Besides these, Sataki 1923 using Pavlov's methods obtained pancreatic secretion in the cat.

Savitsch 1918 could not demonstrate any increase in the spontaneous secretion of the rabbit after stimulation of the vagi, although Gottlieb 1894 in the same animal reported an increase in the volume of the juice, and a rise in the total solids following the injection of pilocarpine. A complete discussion of the data available concerning the pancreatic secretion in the rabbit will be included later on in this thesis.

SYMPATHETIC INNERVATION.

The secretory function of the splanchnic nerves was best demonstrated by methods which avoided the vaso constrictor action of these nerves to which the pancreas is very sensitive. Kudrewezki 1890 used Heidenhain's tetanometer or stimulated the splanchnic nerve 6-7 days after it had been cut asceptically to permit degeneration of the vaso constrictor fibres.

Later, Savitsch by the application of long continued stimulation of the peripheral end of the freshly sectioned nerve with an induction current, obtained a secretion.

The pancreatic secretion produced by splanchnic stimulation was very scanty and very rich in organic

substances and enzymes. According to Savitsch l.c. atropin inhibited the sympathetic secretion, but, however, Modrakowski 1906 denied the inhibitory action of this drug.

VAGUS INHIBITION

Besides its secretory action on the pancreatic gland, the vagus has an inhibitory effect as well. The secretion initiated by the stimulation of one vagus may be inhibited by stimulation of the other vagus. Mett 1889, Kudrewezki 1890. Furthermore, stimulation of the vagus may inhibit the secretion produced by H Cl. Popielski 1896 or by secretin, Pavlov 1908. Since it is well known that the vagus contains neither vasi dilator or vaso constrictor fibres and since the vagi were stimulated below the heart to avoid systemic circulatory changes, this inhibitory action of the vagus could not be ascribed to vascular changes occurring in the pancreatic glad. Pavlov's school (Mett, Popielski & Kudrewezki) held the belief that this inhibition of pancreatic secretion was due to the existence of special secretory inhibitory fibres in the vagus which had direct relationship to the secretory cells. Their action was directly opposite and antagnostic to the secretory fibres. Anrep 1914 as a result of further experimentation expressed a different view of the inhibitory action of the vagus on pancreatic secretion. He applied a plethysmograph to the pancreas in a dog and after stimulation of the vagus he observed an in-

crease in gland volume, but no secretion. Then, on the appearance of secretion this increase in gland volume disappeared.

Anrep explained this result by the theory that on stimulation of the vagus two phenomena occurred: First, the secretion took place, and secondly, a constriction of the pancreatic ducts occurred, and the juice was retained in the gland. Hence the gland volume increased, and on relaxation of the ducts and appearance of the secretion it abated.

Korowitzky 1923, confirmed Anrep's work, using a method of perfusion of the pancreatic gland. Under the influence of vagus stimulation or pilocarpine the main pancreatic duct contracted, and when atropine was injected it relaxed. According to Nakagawa, Vakita & Matsamoto 1925, the vagi possess fibres which control the tonus of the pancreatic ducts, it is probable that the contractile elements of the ducts are supplied with motor fibres by the vagus. This explanation of the inhibitory action of the vagus is at present more plausible than Pavlov's original theory of secretory and inhibitory fibres.

SPLANCHNIC INHIBITION

Stimulation of the Splanchnic nerves also causes an inhibition of pancreatic secretion. The mechanism of this inhibition is, however, purely vascular, due to the direct action of vaso constrictor fibres on the blood vessels of the

gland or through the action of adrenaline, Edmunds 1909 Mann & McLachlin 1917, The secretory cells of the pancreas are very sensitive to any diminution of the blood supply. Therefore, there is not sufficient evidence to agree with Pemberton and Sweet,,that Adrenaline is antagonistic to secretory stimulants of the pancreas and that its action is specific.

After consideration of the experimental evidence what conclusions can be made concerning the role of the secretory nerves in normal pancreatic secretion?

It must be admitted that our knowledge of this aspect of pancreatic secretion is still very fragmentary, and that existing data are somewhat conflicting. There is no doubt that there is a "reflex" phase of pancreatic secretion. The passage of food through the oral cavity activates the secretion of pancreatic juice in a short space of time - 15 minutes - Walther 1897 & Krewer 1899 proved this on dogs with permanent pancreatic fistulae. Miss Tonkick 1924, used for such experiments pancreatic fistulae dogs with the stomach separated from the duodenum by a circular suture through the mucous and sub mucous membranes near the pylorus. In her experiments the pancreatic juice secreted during the reflex phase was rich in organic substances and enzymes.

This reflex secretion does not occur if the parasympathetic nervous system is paralysed by a small dose of atropineor if the

vagi are sectioned in the neck.

Whether the reflex secretion is a true secretion or whether it is the juice in the ducts expelled by the muscular or other contractile element is a matter of controversy.

Walther & Krewer l.c. have noted that the secretion stops in about 15 minutes in spite of continued feeding of the animal. This observation is in favor of a motor action transmitted to the gland through the vagi. Babkin & Ishikawa 1912 explained the "periodic" secretion of pancreatic juice by the same mechanism. On the other hand, Miss Tonkick 1924 reported an increase in the nitrogen content of the "reflex" juice which is in favor of a true secretion through the vagi. Thus the phenomenon of reflex secretion requires further investigation before an accurate explanation can be offered.

The secretory action of such strong stimulants of pancreatic secretion such as fats and soaps are also influenced by the nervous system. The section of the splanchnics alone in a dog with permanent pancreatic fistula did not effect the concentration of enzymes in the pancreatic juice activated by the ingestion of 5% sodium oleate. When the section of the vagi was added the same solution of soap produced a secretion lower in enzymes in the first phase, but during the second phase the enzymatic power reached its former level. Buchstab 1904. More information about the role of the parasympathetic nervous system in the

normal process of secretion following the ingestion of fats and soaps, is given by Bylina 1911. Bylina worked on dogs with permanent pancreatic and gastric fistulae. In control experiments he introduced 100 cc of vegetable oil into the stomach and obtained a flow of juice abundant in organic constituents and enzymes. In his actual experiments during the secretion initiated by the introduction of oil into the stomach, he injected 5 mg. of atropine sulphate subcutaneously. There was no change in the volume of the secretion but the content of organic material diminished to one fourth of the original value. One interpretation of this result may be that soap produced a liberation of harmones which activated not only the secretion of fluid constituents of the pancreatic juice, but stimulated the endings of the parasympathetic nervous system as well. Atropine did not influence the first action of the harmones but inhibited the second.

A second theory is suggested by Mellanby 1925. He experimented on cats anaesthetized with urethane and stimulated by the continuous injection of secretin, and noticed a gradual fall in enzymatic powers of the juice. The addition of pilocarpine to the secretin brought about a rise in enzymes while atropine had the reverse effect.

Section of the vagi in the neck also caused a diminution in enzymes of the juice. Mellanby argues that the vagi are

sending a continual stream of "trophic" impulses to the pancreatic gland.

We may assume then that certain stimulants of procreatic secretion such as fats and soaps may act through the central nervous system. Whether this action is a type of nervous reflex or some special hormone stimulating the parasympathetic centres of the pancreatic secretory nerves is at present unknown. New researches on this fundamental problem are awaited.

C PANCREATIC SECRETION IN THE RABBIT.

Certain peculiarities of the pancreatic secretion in rabbits long ago attracted the attention of early investigators. They noted the spontaneous secretion but did not attempt to discover its mechanism.

Heidenhain 1877 used rabbits anaesthetized by curare. He observed that the pancreatic juice of the rabbit contained less solid constituents than that of the dog. He saw that the spontaneous secretion continued after 48 hours starvation but was not so copious, the average secretion per hour was .6 - .7 cc.

Gottlieb 1894 anaesthetized his animals with urethane. according to him the spontaneous secretion showed certain

irregularities which he thought were caused by rhythmic contractions of the pancreatic ducts. He found that the ferments presented great irregularities. The irregularities were probably due partly to the cruder methods of determination used at that time. Gottlieb also pointed out the important relationship between the blood supply and amount of secretion. He inhibited the spontaneous secretion by the injection of strychnine and then increased it again by the injection of chloral hydrate which relaxed the blood vessels constricted by strichnine. Since chloral hydrate not only paralyses the vaso constrictor centre but also stimulates pancreatic secretion, Wertheimer & Lepage 1901, Gottlieb's observations cannot be accepted unreservedly. After the injection of pilocarpine Gottlieb noted an increase in the amount of the secretion and also in the total solids. Injection of atropine had no effect on the rate of secretion or content of solid substances in the juice. He also saw that irritant substances such as mustard oil and sodium bicarbonate introduced into the stomach caused a great increase in secretion, and this effect was absent if the pylorus was tied. Pavlov 1878 reported also th t atropine had no effect on the rate of the spontaneous secretion in the rabbit.

Savitsch 1918 found that stimulation of the vagi in the neck in a rabbit with the spinal cord sectioned below the

medulla, had no effect on the rate of the secretion. Thus while the humoral mechanism of pancreatic secretion in the rabbit was well recognized, there was conflicting evidence as to the action of the parasympathetic nervous system. The influence of the sympathetic innorvation of the pancreatic gland in the rabbit has not been investigated.

EXPERIMENTAL METHODS

Adult rabbits were used in all experiments. Certain difficulties were encountered in the choice of anaesthetics, which it is well to point out, as they have an important bearing on the interpretation of experimental results. Those anaesthetics injected intraperitoneally such as dial and amytal (iso ethyl barbituric acid) produced a marked vaso-dilatation of the abdominal viscera and a sero-haemorrhagic exudate into the peritoneal cavity. As a result of this irritation an abnormally copious flow of pancreatic juice was produced.

Sodium luminal intravenously in doses sufficient for anaesthesia caused a serious depression of circulation and respiration and the animals frequently died from cardio-respiratory failure two or three hours after anaesthetization. Chlorarose gave good anaesthesia but it activates the secretion of adrenaline (Svaale Vincent & MacKay) which is greatly antagonistic to pancreatic secretion, due to vaso-constriction of pancreatic blood vessels.

Urethane intravenously 1 gram per kilo of a 20% solution in normal saline was found to give the most satisfactory anaesthesia. In spite of the fact that previous investigators have observed spontaneous secretion in rabbits, anaesthetized with various narcotics, experiments were done on decerebrate and decapitate preparations.

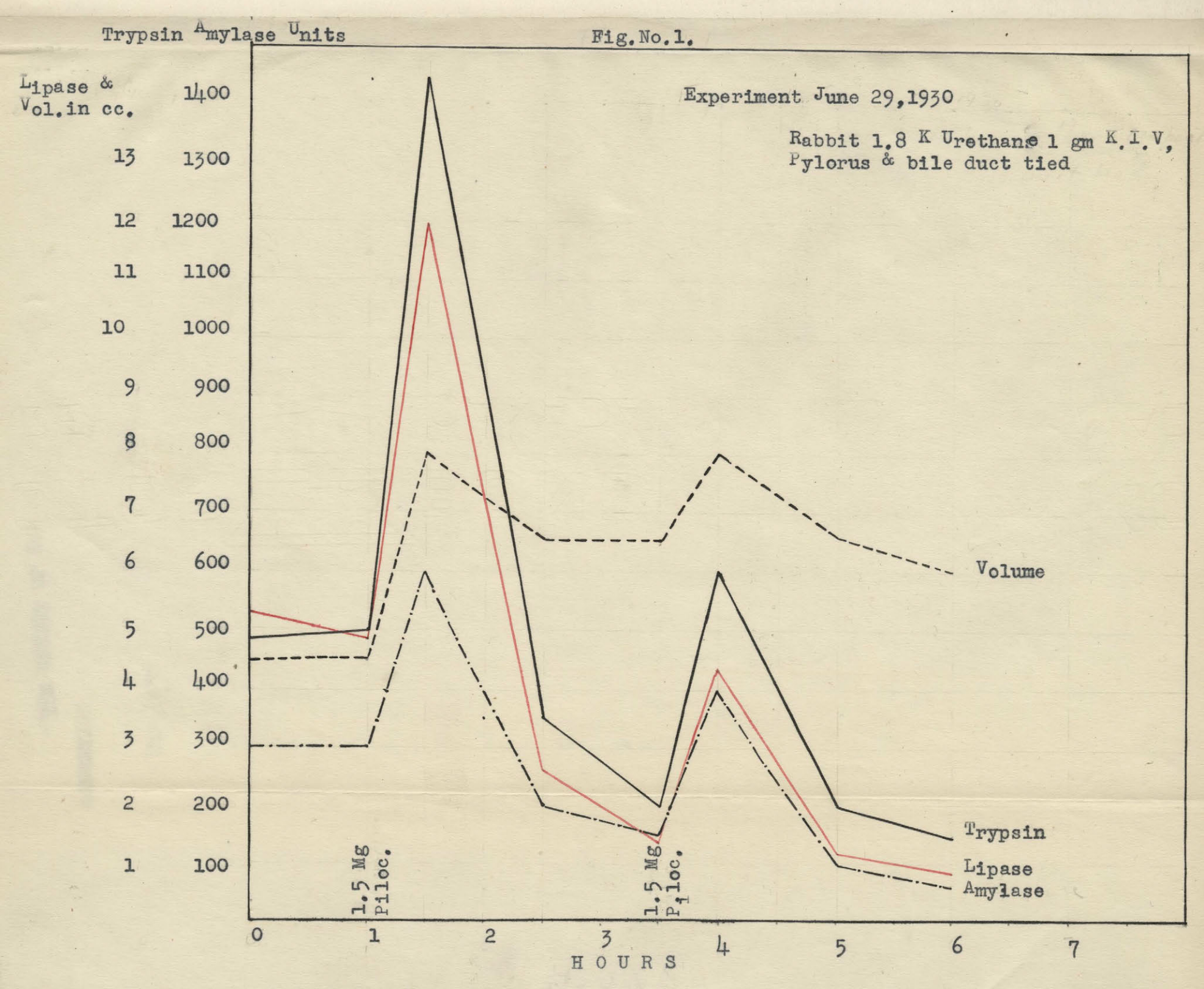
The pancreatic duct was canulated with a small metal canula and in experiments where a kymographic record of secretion was taken, the canula was connected with fine rubber tubing to a Gibb's drop-recorder. In other experiments the juice was collected with a fine glass pipette and kept in hourly samples. In this way an accurate correlation of the rate of secretion and enzyma content of the juice could be made.

In all experiments except when they were purposely left patent, the pylorus and bile duct were ligated. This was done to exclude the uncontrolled flow of gastric and biliary secretions into the duodenum, both of which are powerful excitants of pancreatic secretion. By employing this uniform technique the results obtained became more comparable.

DETERMINATION OF ENZYMES

Since as we have pointed out in the discussion of the literature all three enzymes are secreted in parallel concentration, we determined in the majority of experiments only the proteolytic ferment. A certain number of experiments were done to show the adherence of the pancreatic secretion in the rabbit to the general rule of parallel secretion of enzymes.

Fig. No. 1.


Trypsin Amylase Units
Lipase & Vol. in cc.
1400
13
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12
1200
11
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9
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300
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200
1
100
Experiment June 29, 1930
Rabbit 1.8 K Urethane 1 gm K.I.V,
Pylorus & bile duct tied
Volume
Trypsin
Lipase
Amylase
1.5 Mg Piloc.
1.5 Mg Piloc.
0
1
2
3
4
5
6
7
HOURS

Proteolytic Ferment.

To determine the Proteolytic Ferment the milk coagulation method developed by Mellanby 1912 was used. The rationale of this method depends on the fact that pancreatic trypsin is proportional to the rennetic activity of the juice, Pavlov & Parastuschuk 1909, Funk & Niemann 1910.

For substrate a 50% solution of milk and 1 N calcium chloride was used. The pancreatic juice with a suitable amount of enterokinase, 2 cc. of enterokinase solution for each cc of juice, was activated by incubation at 4C cC. for one hour on a constant temperature water bath.

The enterokinase solution was made by a uniform method of grinding 30 grams of intestinal mucosa with 100 cc. of distilled water, allowing to stand over night, filtering and adding a few cc. of toluine. This solution kept in a cool place retained its activity for several weeks. New solutions were always checked against the old for potency. To two cc. of milk solution .1 cc. of the activated juice was added and incubated at 40°C. The first appearance of discrete flocculent particles was taken as the end point which was quite sharp. This time in seconds was converted into an arbitrary value in units having an inverse

relationship to the time of coagulation. A series of duplicate determinations showed that the experimental error was less than 10%. This method which permitted of frequent determinations during the course of an experiment was used as the index of enzymatic power in most experiments.

Amylolytic Ferment.

To determine the amylolytic power of the juice the method of Waksman 1920 was found satisfactory. This method was based on the disappearance of the starch in the solution without considering the fact of just how much has been hydrolysed to dextrins and how much to sugars thus measuring as nearly as possible the first step in the hydrolysis of starch.

A 2% paste was made with raw potato starch which had been previously treated with 5% solution of neutral red which aids in determining the end point. To 5 cc. of this starch solution .1 cc. of pancreatic juice was added and incubated on a water bath at 40°C. The time of clearing of the starch solution was noted and converted by an appropriate formula into the amylolytic power of the juice.

Lipolytic Ferment.

To measure the lipolytic power of the juice the following method was adopted.

Commercial olive oil was used as substrate. It was previously made just alkaline to phenolphthalein with .1 N $NaOH$. The soap which was formed made an even suspension of the oil. To 2 cc. of this oil and soap suspension .2 cc. of pancreatic juice was added and incubated on the water bath at $40^{o}C$ for 1 hour. Then 2 cc. of absolute alcohol were added and the mixture titrated with .01N $NaOH$ until it was again just alkaline to phenolphthalein. Suitable controls were made with each determination and the necessary corrections made. The result expressed as cc. of .01N $NaOH$ gave a direct measure of the lipoclastic power of the juice.

The determinations of the lipolytic power of the pancreatic juice were always made as soon as the specimens had been collected, as auto-digestion of this ferment may take place although enterokinase had not been added, Mellanby & Woolley 1918.

EXPERIMENTAL WORK

1. Spontaneous Secretion.

The spontaneous secretion of pancreatic juice in the rabbit is as we have pointed out, one of the most interesting anomalies in the physiology of this gland. The first step in the investigation was to determine the influence of anaesthetics, if any, on the production of the spontaneous secretion. On reviewing the literature we find that different investigators have noted continuous secretion in rabbits anaesthetized with a wide range of narcotics: Gottlieb 1894 used urethane and chloral hydrate, Heidenhain 1877 with cruare, Pavlov 1878 on unanaesthetized animals and Savitsch 1918 on spinal animals.

In our own experiments we have used urethane, dial, amytal and sodium luminal, with all of which we have obtained a spontaneous secretion. These anaesthetics which produced low blood pressure chiefly due to vaso-dilatation such as dial intraperitoneally and sodium luminal intravenously tended to give a more copious secretion. This is due to the sensibility of the pancreas to low blood pressure.

Experiments with spontaneous secretion were also made on rabbits anaesthetized with ether and then rapidly

decerebrated or decapitated. The decapitate preparations proved more satisfactory as there was practically no blood loss, and with the introduction of artificial respiration the animals lived for 6 - 8 hours. In these preparations spontaneous secretion of pancreatic juice was traced for as long as 6 hours before the animal succumbed. Thus the fact of spontaneous pancreatic secretion in the rabbit is fully established both in animals anaesthetized with a wide range of narcotics and in decapitate and decentrate preparations.

The course of this spontaneous secretion proved very interesting. We have followed it in various experiments for as long as 12 hours. The rate of secretion as shown by the collection of hourly samples was often remarkably regular. The rate of secretion of pancreatic juice however is subject to so many external influences that frequently experimental conditions caused irregularities. The intravenous injection of urethane solution (10-15 cc.) frequently caused a more copious flow in the first 2-3 hours due partly to the increased blood volume and partly to the transient vaso-dilating action of this drug on the splanchnic blood vessels.

On the other hand, in rabbits with a high initial blood pressure, the secretion in the first 1-2 hours was less than in succeeding periods. Therefore, the first two or three

samples of each experiment, in which the juice was collected in hourly portions, were always used as a control period to establish the norm of secretion and enzyme power.

The enzyme content of the spontaneous secretion also gave important data which were of a novel character. It is a well known fact that the enzyme power of pancreatic juice collected by the repeated or slow continuous injection of secretin gradually and progressively diminishes. Mellanby 1925 has shown that there is a sharp fall in ferment power of the pancreatic juice of the cat provoked by the continuous intravenous injection of purified secretin solution.

In our experiments however in the rabbit the enzyme power of a normally secreted juice has been followed in hourly portions for 10-12 hours, and we have seen that with the exception of slight fluctuations the enzyme power remains remarkably regular.

In this connection must be mentioned the important relationship of the enzyme content to the rate of secretion of the juice. It has been well demonstrated that the enzyme content of the pancreatic juice of carnivorous animals provoked by one and the same stimulus is inversely proportional to the rate of secretion. This relationship is also present in the secretion of the rabbit but is not as sharp.

Another factor which influences the ferment power is the exhaustion of the gland. These important factors must be kept in mind when interpreting experimental results. Thus we have shown conclusively that there is in the rabbit a long continued spontaneous secretion which is secreted at a fairly uniform rate and contrary to accepted beliefs with an undiminished enzyme content.

These facts are illustrated in Table No.1.

Table No.1.

Experiment Feb.27, 1930

Rabbit 1.12 K. Urethane 1 gm. per kilo I.V.
Bile duct and pylorus tied.

Hour	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Vol.in cc.	.55	.3	.25	.35	.35	.35	.4	.3	.1	.1
Units Trypsin	240	200	200	150	200	175	185	200	170	200

This spontaneous secretion may be greatly enhanced by the injection of various substances into the duodenum. Gottlieb 1894 saw that irritant substances such as mustard oils and

sodium bicarbonate injected into the stomach caused a marked increase in the normal secretion of pancreatic juice in the rabbit.

The injection of .2% HCl alone gives an abundant secretion, which is illustrated in the following Table:

Table No.2.

Experiment Jan.16th,1930

Rabbit 1.6 K . Urethane 1 gm. per K. to I.V.
Pylorus and bile duct tied.

Hour	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	7-8	8-9
Vol. in cc.	.45	.8	.6	.55	.6	.6	.35	.2	.15	.2
Units Trypsin	480	800	600	600	300	100	133	150	170	240

5cc.
-2% HCl every ½ hour

In this Experiment the secretion was collected for the first hour and then 5 cc. of .2% HCl were injected into the duodenum every half hour. In spite of the fact that the volume of secretion rose almost double during the first hour of injection of acid the enzyme content rose considerably as well. Then during the next four hours of increased secretion there was a gradual and progressive fall to one-eighth of the former value. After this

the secretory power of acid was lost. During the next four hours with the lack of response to acid the enzyme content rose slowly. Thus the introduction of hydrochloric acid into the duodenum of the rabbit increases the volume of the pancreatic secretion, with rapid diminution of the concentration of enzymes. Coincident with the failure to respond to the stimulation of HCl the enzyme power rose. This was due not only to the diminished volume of secretion but to other factors as well, as the concentration of enzymes rose progressively although the rate of secretion was uniform.

A mixture of bile peptone amd .2% of HCl produced a copious secretion of pancreatic juice over a lengthy period of time. This fact is demonstrated in Experiment Jan.17. Table No.3.

Table No.3.

(See next page)

Table No.3.

Experiment Jan.17, 1930
Rabbit 1.4 K. Urethane 1 gm. per K. I.V.
Bile duct and pylorus tied. Canula in upper part of duodenum.

Hours	Time	Procedure	Vol.in cc.	Units Trypsin
	10.00	5 cc. 2% Peptone (Whitte's)		
1	11.00	in .36% HCl & 5 cc. .2 of HCl	.075	170
2	12.00	alternating in duodenum every 15 min.	.05	141
	12.05	3 cc. dog's bile in duodenum		
3				
	1.00	5 cc. .2 % HCl every half hour for succeeding 5 hours.	.1	109
	1.30	2 cc. bile in duodenum		
4	2.00		.5	133
5	2.45	2 cc. bile in duodenum		
	3.00		.9	150
6	3.45			
	4.00	1.5 cc. bile in duodenum	.9	70
7	4.45			
	5.00	1 cc. bile in duodenum	.8	77
8	6.00	5 cc. .2% HCl every half hour	.2	92
	6.05	2 cc. bile & 5 cc. peptone in .36%		
9		HCl.		
	7.00		.7	109
10	8.00	1 cc. bile & 5 cc. of 2% peptone	.6	150
11	9.00	in 36% HCl every half hour		
		in duodenum	.7	100
12	10.00		.65	92

During the first two hours the injection of a mixture of 2% peptone in .36% ~~mf~~ HCl and .2% HCl gave only a very scanty secretion. The addition of 3 cc. of bile in the third hour augmented the secretion and during the next four hours a mixture of bile and .2% of HCl provoked a copious secretion of juice with a marked fall in enzymatic power. (Compare hours 1-2 with 6-8).

In the eighth hour this mixture of bile and acid failed to stimulate the secretion, but the addition of 2% peptone again to the stimulant mixture returned the secretory power which continued undiminished for the next four hours.

In the tenth hour the enzyme value rose considerably over the preceding hours. This increased value coincided with the addition of peptone to the stimulant solution in the previous hour, and so could have been due to the addition of the new stimulus. This ability of peptone to return the secretion in the exhausted gland has also been demonstrated in the dog by Rasenkov, and also by Miss MacKay in our laboratory. The exact mechanism of this action of peptone is not clear, but it would seem that the presence of the products of protein digestion are required for the full sustained secretory effect of HCl.

Thus the enzyme content of the spontaneous secretion diminished only if the secretion was copious. The secretory effect of .2% HCl ceased after 4-5 hours, but a mixture of .2% of acid, .2% of peptone and bile stimulated the pancreatic

gland for ten hours giving a copious secretion in which the enzyme content diminished more than one half its original value.

The spontaneuus secretion is also augmented by drugs which act on the nervous mechanism of the pancreatic gland. Pilocarpine and acetyl choline both parasympathetic omimetic drugs, when injected intravenously produced an increase in secretion. Due to their action on the nerve endings of the vagus they also increased the enzyme content of the juice, having the reverse effect of humoral stimuli in this respect. These facts will be fully discussed in the Chapter "Nervous Control of the Pancreatic Secretion."

NERVOUS CONTROL OF THE PANCREATIC SECRETION.

A. Parasympathetic Nervous Mechanism.

Gottlieb 1894 found that pilocarpine injected intravenously caused an increase in the rate of secretion as well as in the amount of total solids of the pancreatic juice of the rabbit.

Savitsch 1918 stimulated the vagi in the neck of rabbits with the spinal cord sectioned below the medulla, and did not see any increase in the amount of secretion.

He concluded from his experiments that the vagus had no influence on the pancreatic secretion in the rabbit

and offered this fact as evidence in favor of the dualistic theory of pancreatic secretion. According to Savitsch in carnivorous animals such as the dog, the pancreatic secretion is regulated by nervous and humoral mechanisms; in the rabbit only the humoral mechanism is necessary for the secretory activity of this organ.

In the course of numerous experiments the vagi have been stimulated in the neck with rhythmic stimulation; in the chest below the heart and in the abdomen below the diaphragm, with an induction current.

Stimulation of the vagi in the neck is unsatisfactory as the stimulation has a detrimental effect on the heart, and causes abrupt changes in blood pressure. Electrical excitation of the vagi in the chest gave excellent results when the artificial respiration was properly adjusted.

Stimulation below the diaphragm was possible only if it could be accomplished without undue disturbance of the abdominal viscera. Stimulation of the vagi in either of these sites resulted in a definite augmentation of the pancreatic secretion preceded by a short inhibition.

In some of these experiments a kymographic record was taken; in another set of experiments, the juice was

collected directly from the canula in hourly or half-hourly portions. In the Kymographic tracing a short phase of inhibition was usually noted, and in accordance with Anrep's experments 1914, was probably due to a constriction of the pancreatic ducts. This inhibitory phase was followed by an acceleration of secretion. (Fig.No.2. & 2a)

In the experiments where the secretion was collected directly, the amount increased from twice to even three or four times that in control periods.

The enzyme content of the juice secreted after stimulation of the vagi rose tremendously to three or four times the control value. This result was observed consistently and is well illustrated in Table No.4.

Table No.4.

Experiment Jan.31, 1930

Rabbit 1.3 K Urethane 1 gm. per K. I.V.

(see next page)

Table No.4.

Experiment Jan. 31. 1930

Rabbit 1.3 K Urethane 1 Gm. per K. I.V.
Bile duct tied. Pylorus sewn with a circular suture in mucous and sub-mucous membrane.
Artificial respiration.

Time	Procedure	Vol.in cc.	Units Trypsin
10.15 11.15	Spontaneous Secretion	.2	600
12.15	Stimulation of alternate vagi in chest, 5 min. stim. & 5 min. rest Coil 10	.35	1800
1.30	Spontaneous Secretion	.1	1440
2.45	" "	.2	800
3.45	" "	.1	514
4.45	" "	.1	514
5.45	Stimulation of alternate vagi 5 min. stim. & 5 min. rest Coil 9	.3	1200
6.45	Spontaneous Secretion	.15	650

It is important to note in this experiment as well as in all others, with the stimulation of the vagi the digestive power of the juice secreted during the stimulation of the parasympathetic nerve increased in spite of the dilation of the juice with a greater volume of secretion. In other words, we may speak here about a "trophic" influence of the vagus using the word in that meaning which Heidenhain gave to it.

These experimental results indicate that in the rabbit the vagus has the same function as in the cat and dog, i.e. it contains secretory trophic and motor inhibitory fibres to the ducts.

Therefore, this investigation establishes definitely a parasympathetic nervous mechanism in an herbivorous animal in which previous investigators attributed the spontaneous secretion largely if not entirely to the humoral mechanism and denied any secretory activity to the vagus.

Thus while the basic physiology of the pancreatic gland in the rabbit must differ in some way from that of carnivorous animals, yet the essential effect of the parasympathetic innervation is analagous in both species.

Not only does the electrical stimulation of the vagus

augment the secretion and increase the enzyme content of the juice, but the parasympathetic omimetic drugs have a similar effect.

That pilocarpine and acetyl choline increase the amount of the spontaneous secretion has already been alluded to. However, these drugs also produce an increase in the enzyme power of the juice. That these drugs act on the nerve endings of the parasympathetic nervous system ~~system~~ is well known, and their action on the pancreas is merely one of their characteristic secretory effects. The secretory action of pilocarpine and acetyl choline is well shown in the Experiment of March 25,1930.

Table No.5.
Experiment of March 25, 1930
Rabbit 2.14 K. Urethane 1 Gm. per K.I.V.
Pilorus and bile duct tied

	1	2	3 A	3 B	4	5	6 A	6 B	7	8
Hour	10-11	11-12	12-12.30	12.30-1	1-2	2-3	3-3.30	3.30-4	4-5	5-6
Vol.in cc.	.4	.2	.25	.1	.4	.5	.35	.3	.4	.45
Units Tryp-sin	600	900	1800	1200	400	350	1050	450	300	350
			#				#			

#12.00
12.15 1/20 Mg Acetyl choline intravenously

3 P.M. 1 M.G Pilocarpine intravenously

. . . . .

After a control period of two hours, one-tenth of a milligram of acetyl choline was injected intravenously. The amount of secretion rose from .2 to .35 cc. and the proteolytic power of the juice was doubled. Then after another control period of two hours when the secretory rate and enzyme power became constant 1 milligram of pilocarpine was injected intravenously, and again there was a rise in the amount of the secretion from 5 cc. to 65 cc. in an hour, and the tryptic value of the juice increased from 350 to 1050 units.

The collection of the juice after the injection of the drugs in half hourly portions showed an interesting fact. It will be noticed that the action of acetyl choline is prolonged into the second half hour, while in the second period after the injection of pilocarpine the enzyme value had fallen practically to its normal level.

It would seem that acetyl choline is a stronger and more lasting stimulant for pancreatic secretion than pilocarpine.

(B) EFFECT OF SECTION OF PARASYMPATHETIC NERVOUS SYSTEM.

A series of experiments were carried out to determine the result of removal of the central impulses conveyed through the vagi, by section of these nerves either in the neck or below the disphragm. Section in the neck caused a disturbance of respiration and occasionally the rabbits died of respiratory failure.

Mellanby 1925 has reported a sharp decline in the enzyme power of the pancreatic juice of the cat, anaesthetized with urethane, secreted under the continuous injection of purified secretin solution. This also occurred after administration of atropine intravenously. Mellanby interpreted these results as due to the removal of the constant stream of central impulses reaching the pancreatic gland by way of the vagi. However, his conclusions are not especially convincing as in control experiments with vagi intact there was a distinct and progressive fall in the enzyme values in the juice secreted by the continuous injection of secretin. Furthermore, even though purified secretin was used in his experiments, the exact chemical composition of pure secretin being still unknown, we cannot be certain that it possessed a pure physiological stimulus and was free from other unphysiological constituents.

The physiological spontaneous secretion of the rabbit offered a good medium for this type of experiment.

In all the experiments involving the section of the vagi either in the neck or below the diaphragm, the bile duct and pylorus were not tied as we wished to ascertain the effect of removal of central vagal influences on the normal physiological secretion.

Section of the vagi either in the neck or below the diaphragm had no effect whatever on the spontaneous secretion in the rabbit. As far as could be seen, there was no change in the rate of secretion or in the content of the enzymes which remained remarkably steady. These results are shown in Table No.6.

Table No.6
Experiment June 5,1930
Rabbit 1.4 K Urethane 1 Gm. per K. I. V.
Pylorus and bile duct patent

	1	2	3	4	5	6	7	8
Hour	9-10	10-11	11-12	12-1	1-2	2-3	3-4	4-5
Vol.in cc.	.6	.8	.6	.8	1.1	1.1	1.1	1.0
Units Trypsin	100	100	100	105	105	102	100	110
cc. .01N N2OH Lipase	2.8	2.9	2.6 #	2.8	3.0	2.8	2.9	3.1

Vagi cut below diaphragm

The secretion showed no diminution of either the proteolytic or lipolytic ferment for 6 hours after section of the vagi below the diaphragm. The hourly volume of the secretion rose slightly during the last four hours of the experiment. Exactly the same results were obtained when the vagi were cut in the neck.

Thus the removal of the central impulses which travel by way of the vagi to the pancreatic gland in the rabbit by section above or below the diaphragm has no influence whatever on the rate of secretion or the enzyme content of the juice.

C. INFLUENCE OF THE SYMPATHETIC INNERVATION ON THE PANCREATIC GLAND IN THE RABBIT.

The sympathetic nerve supply to the pancreas comes with the blood vessels by plexiform offsets of the hepatic splenic and superior mesenteric plexuses which are relays of the coeliac plexus formed by the splanchnic nerves. Concerning the influence of the sympathetic innervation of the pancreatic gland in the rabbit we have no available data.

Electrical stimulation of the splanchnic nerves is complicated by the fact that the sympathetic system supplies the pancreas with vaso constrictor fibres and that the pancreatic gland is very sensitive to vaso constriction. However, the problem is not so acute in the rabbit as in the carnivorous experimental animals by reason of the spontaneous secretion in the former animal.

We have stimulated the left splanchnic nerve in the rabbit in the abdomen, the left being longer and easier to expose. After securing the nerve an incision was made into the left flank so that it could be stimulated with as little disturbance of the viscera as possible and without any movement of the canula which was connected to a Gibb's drop recorder and a signal marker on the kymograph paper. In this way a

kymographic record of the secretion was obtained as well as samples of juice for ferment determinations. Experiments were done in which the adrenal vein was tied as well as those in which it was left untied. The typical effect of stimulation of the splanchnic nerve is shown in Fig. No.3. Experiment June 30,1930.

The first three stimulations produced a typical inhibition of the secretion due to vaso constriction of the pancreatic vessels. During the next three periods of stimulation the inhibitory effect was not as marked as there were one or two drops during stimulation. On the 7th stimulation there was a definite increase in the secretion during the excitation of the nerve and no preliminary inhibition. The juice collected after stimulation showed over a two fold increase in enzyme power which is shown in Table No.7.

Table No.7.

Experiment June 30, 1930

Rabbit 1.8 K Urethane 1 Gm per K.I.V.

Pylorus and bile duct tied; adrenal vein not tied.

	1	2	3
Hour	11 - 11.35	11.35 - 12.35	12.35 - 1.15
Vol. in cc.	.7	.9	.3
Unit Trypsin	150	350	120
Lipase cc .OiN Na OH	1.4	3.6	1.3

#

Stim. Lt Spl

7 min. in 1 min periods

Fig.No.4. Experiment July 7, 1930 shows the action of the stimulation of the splanchnic nerve with the adrenal vein tied. The blood pressure rise was insignificant as compared with the previous experiment when the adrenal vein was not tied although the initial blood pressures were about equal.

In this experiment the secretory effect of the splanchnic nerve was beautifully shown, since on the third stimulation the acceleration occurred during the excitation without any inhibition and became more pronounced on each succeeding stimulation. The enzyme content of the juice also rose from 600 before to 900 after stimulation.

This increase in enzymes after stimulation of the splanchnic nerve cannot be explained on the basis of concentration as the rate of secretion undergoes little if any diminution due to vascular inhibition, and the increase in ferment power is two and even three times the control value. The initial inhibition followed by augmentation of secretion during stimulation is probably due to the well-known delicacy of the vaso constrictor fibres to electrical stimulation. They rapidly become fatigued and allow the more resistant secretory fibres to exert their true effect unconditioned by vascular inhibition.

Accordingly we can claim for the sympathetic innervation of the pancreatic gland in the rabbit an initial vascular inhibition which soon yields to a true increase in secretion with a definite and significant increase in ferment power of the juice.

An interesting experiment was also performed by estimating the effect of the repeated injection of small amounts of adrenaline

on the pancreatic secretion. The result is demonstrated in Fig. No. 5. Experiment July 2, 1930. The injection of ½ cc. of 1/10000 solution of adrenaline resulted in a tremendous rise in blood pressure, and an inhibition of the secretion, which was followed shortly by a well-marked acceleration of the secretory rate. The ferment power in the experiment after repeated injections of adrenalin rose from 60 to 150.

D. EFFECT OF SECTION OF THE SYMPATHETIC INNERVATION.

To complete the study of the action of the sympathetic on the pancreatic gland in the rabbit, a series of experiments were performed to show the effect of removal of the central impulses by section of both splanchnic nerves. On opening the abdomen the splanchnic nerves were identified then gently freed and isolated on strong threads. The canula was inserted and the incision closed. In this way after the usual control period the splanchnic nerves could be torn in two without opening the abdomen or disturbing the canula. As before, the pylorus and the bile duct were left open as we wished to demonstrate the effect of the central impulse on the purely physiological secretion.

In all our experiments the section of the splanchnics was

accompanied by a sharp immediate and lasting fall in enzyme power. This effect is well shown in Table No.8.

Experiment June 27, 1930.

Table No.8.

Experiment June 27, 1930

Rabbit 2.2 K. Urethane 1 Gm. per K. I.V.
Bile Duct & pylorus open.
Both splanchnics isolated on threads

	1	2	3	4	5	6	7	8
Hour	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6
Vol.in cc.	.6	.6	1.1	.9	.2	.6	.5	.7
Units Trypsin	300	300	78	66	54	48	70	70

Splanchnics torn in two

After the section of the splanchnic nerves the secretion rate rose two fold and the enzyme content diminished fourfold. This low enzyme value fell further and continued for 6 hours and even when the secretory rate had returned to its former level. Consequently the abrupt fall in ferment power could not be due to the transitory increase in secretory rate caused by the section of the splanchnics and resultant lowered blood pressure.

Accordingly, contrary to Mellanby's hypothesis of central impulses controlling the enzyme content of the pancreatic secretion in the cat, the impulses through the sympathetic system in the rabbit are of much greater importance in this respect. As a result of these findings the sympathetic innervation of the pancreatic gland assumes a new importance in the role of the normal physiological secretion.

11. ANALYSIS OF SPONTANEOUS SECRETION.

It has already been pointed out that in the rabbit there is a spontaneous secretion of pancreatic juice in regular rate and enzyme content. This fact was observed by the early investigators, but none of them tried to elucidate the mechanism of this spontaneous secretion. It was assumed by them to be due to a continuous humoral stimulus i.e. the constant presence of acid chyme in the duodenum and the liberation of secretin into the blood. Terroine 1913. The first step therefore in the solution of this interesting problem was to determine the effect of removing this constant humoral stimulus. This was most simply accomplished by ligature of the pylorus and common bile duct. Experiments carried out under this technique showed that the spontaneous secretion continued unabated and without any marked change in enzyme content for 10-12 hours.

Table No.1.

Experiment Feb. 27, 1930

Rabbit 1.12 K. Urethane 1 Gm. per Kilo I.V.
Bile duct and pylorus tied.

Hour	10-11	11-12	12-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Vol. in cc.	.55	.3	.25	.35	.35	.35	.4	.3	.1	.1
Units Trypsin	240	200	200	150	200	175	185	200	170	200

While it did not seem logical that the small amount of chyme left in the small intestine could activate the gland for 10-12 hours after ligature of the pylorus, it was decided to observe the effect of removal of the whole small intestine on the spontaneous secretion.

To accomplish this without unduly damaging the pancreatic gland which is very delicate and without interfering with the blood supply required special operative care.

The pancreatic gland in the rabbitreceives its blood supply from three main sources: one, the upper part of the body from the superior pancreatic-duodenal artery which is a branch of the gastro duodenal artery. Two, the lower

2/3rds of the body from the inferior pancreatico duodenal artery which is a branch of the superior mesenteric artery, and, thirdly, short branches from the splenic artery supply the tail of the pancreas.

Now, by tying the duodenal branches of the superior and inferior pancreatico duodenal arteries close to the duodenum, it was possible to separate the gland from the duodenal loop to which it was attached, without grossly interfering with the blood supply. A small section of duodenum containing the opening of the pancreatic duct was left intact as a support for the duct and canula.

In the Experiment of Jan. 27, 1930, (Table No.9.) the spontaneous secretion was recorded for 3½ hours following the extirpation of the whole small intestine. The amount of secretion in the experiment was scanty, and the enzyme content high. At the end of the third hour, the condition of the animal became poor and it died shortly after.

Table No.9.

Experiment Jan. 27, 1930

Rabbit 1.54 K. Urethane 1 Gm per K. I.V. small gut completely removed. Bile duct tied.

Hour	12-1	1-2.30	3.-3.30
Vol.in cc.	.15	.1	.1
Units Trypsin	800	1200	800

The importance of this fact is evident. It has been well demonstrated that the content of pro-secretin is present almost exclusively in the mucous membrane of the upper 2/3rds of the small intestine. There is no secretin in the cardiac pyloric mucous membranes and only very small amounts in the mucous membrane of the large gut. Furthermore, the injection of secretin preparations intravenously initiates a copious flow of juice which lasts only for a few minutes.

Thus according to our present knowledge of the humoral mechanism of pancreatic secretion, it would seem highly improbable that the small amount of secretin circulating in the blood at the time of removal of the small intestines, or the small amounts derived from the large gut could activate the gland for a period of 3½ hours.

Following up this clue, it was decided to attempt the removal of the stomach as well as the small intestine, and finally to completely eviscerate the animal in an endeavour to localize the stimulus for the spontaneous secretion.

The removal of the stomach required a careful study of the relationship of the gastric and pancreatic blood supplies. Finally, a technique for removing the stomach without impairment of the pancreatic blood supply was developed. The splenic branches to the stomach were tied close to that organ thus allowing

the short branches given off to the pancreas to remain undisturbed. Then the oesphagus was divided and the numerous branches of the left gastric and hepatic arteries were tied close to the stomach. Then following along the lesser curvature the gastric and gastro epiploic vessels were ligated, care being taken to avoid the superior pancreatico duodenal branch of the gastro duodenal artery. The bile duct was tied and cut. Now the pancreas was separated from the upper part of the duodenum and the first inch or two of the duodenum was tied and divided. By ligating any remaining vessels in the omentum the stomach could easily be removed without any serious loss of blood. Following the extirpation of the stomach the small gut was removed as before.

Table No.10, Experiment May 15, 1930, illustrates an experiment inwhich the secretion continued for 4 hours following the removal of stomach and small intestine.

Table No.10.

Table No.10

Experiment May 15, 1930

Rabbit 1.56 K. Urethane 1 Gm. per K.I.V.

Stomach and small intestine completely removed.

	1	2	3	4	5	6
Hour	10-11	11-12	12-1	1-2	2-3	3-4
Vol.in cc.	.25	.4	.45	.5	.7	.1
Units Trypsin	200	40	38	60	120	70
cc. .01N Na OH Lipase	4.8	1.8	2.6	2.4	2.6	-

Extirpation of stomach & small gut complete

The secretion in the first hour was collected while the stomach and intestines were being removed. Following the cessation of manipulation the secretion improved and continued at an even rate for 4 hours. The enzyme content of the juice showed a sharp decline following evisceration.

Table No.11, Experiment May 19, 1930, represents an experiment in which the complete removal of the gastro intestinal tract was accomplished. The pancreatic gland with intact blood supply alone remaining in the abdomen.

Table No.11

Experiment May 19,1930

Rabbit 1.8 K Urethane 1 Gm per K.I.V.

Complete extirpation of gastro-intestinal tract

	1	2	3	4	5
Hour	10-11	11.30-12.30	12.30-1.30	1.30-2.30	2.30-3.30
Vol.in cc.	.35	.2	.2	.15	.05
Units Trypsin	240	220	225	480	480

Extirpation of gut complete

From this Table it can be seen that the isolated pancreatic gland secreted at a steady rate for four hours after complete evisceration. As before, the secretion in the first hour was collected during the operation. Here there is no fall in

enzyme power but rather a rise towards the end of the experiment. The significance and probable explanation of these variations in enzyme content will be discussed later in this chapter.

In this experiment then the pancreatic gland secreted a juice with a relatively high enzyme content for 4 hours after the removal of every known source of secretin. As we have pointed out, the amount of secretin contained in the blood at the time of operation could not, according to current belief activate the secretion of the pancreatic gland for such a lenghty period.

Before drawing any conclusions from these unusual results it was considered necessary to further control the possible influence of anaesthetics in the production of this pancreatic secretion in eviscerated animals.

Consequently a number of experiments were performed on decerebrate and decapitate animals.

In Table No.12 is given an Experiment demonstrating spontaneous secretion in a decerebrate rabbit with pylorus and bile duct tied. The intestinal tract was not removed.

Table No.12.

Table No.12

Experiment May 28, 1930

Rabbit 1.4 K. ether decerebrated Bile duct and pylorus tied. Both splanchnics cut before decerebration, left vagus cut in neck after decerebration. Right vagus sectioned one hour later.

	1	2	3	4	5	6
Hour	10-11	11-12	12.-1	1-2	2-3	3-4
Vol.in cc.	.5	.2	.45	.4	.4	.2
Units Trypsin	170	70	63	60	68	100

Both splanchnics, Right vagus & left vagus cut.

In this experiment the spontaneous secretion was traced for 6 hours before the animal died. The rate of the secretion was quite even. The diminished amount in the second hour was probably due to the inhibition resulting from section of the right vagus . The enzyme value of the juice fell to less than one-half in the second hour and remained at a constant low level. The abrupt fall in the ferment power was due to the section of the splanchnics and vagi which was done to prevent any central inhibitory impulses from reaching the pancreatic

gland. Section of the vagi also improved the respiration in decerebrate preparations.

Accordingly in decerebrate preparations, and with the pyrorus and bile duct tied, the spontaneous secretion was traced for as long as 6 hours.

The next project was to observe the effect of evisceration in decerebrate and decapitate animals. Decapitation was found to be the more suitable procedure as there was practically no blood loss, and with the introduction of artificial respiration the animals lived 4 - 6 hours. Following decapitation the artificial respiration was adjusted and the animals warmed and left for 1/2 hour, to allow them to recover from the shock and to eliminate the ether from the lungs.

Table No.13, Experiment June 16,1930, illustrates the result of evisceration on spontaneous secretion in a decapitate rabbit.

Table No.13.

Table No.13.

Experiment June 16, 1930

Rabbit 2.4 K. Ether decapitated Gastro Intestinal Tract completely removed.

	1	2	3	4	5	6
Hour	11-12	12-1.00	1-2	2-3	3-4	4-5
Vol.in cc.	.4	.25	.3	.3	.2	.1
Units Trypsin	400	250	300	300	400	514

Evisceration complete

Here the secretion continued unabated for 5 hours after evisceration in a decapitate animal. The enzyme content was high at first, fell somewhat after evisceration but returned and remained at its original level. It was considered important to have a knowledge of the blood pressure in these experiments so that a correlation of blood pressure and secretory rate to enzyme might be possible.

Table No. 14, Experiment June 24,1930, reproduces such an experiment.

Table No.14

Experiment June 24, 1930

Rabbit 2.2K Ether decapitation

complete evisceration.

Time	Vol.in cc	Units Trypsin	B.P.	Remarks
3.00 P.M			45-50	After decapitation
4.00	.6	300		Evisceration complete
			35-40	After evisceration
4.30			30-32	
5.00	1.2	87	30-32	
5.30			24-26	
5.35			40-42	Aorta tied in lumbar region
6.00	1.1	60	30-32	
6.30			24-26	
7.00	.4	70	18-20	
7.15			14	
7.30	.1	87	10	Animal expired

In this experiment it will be noted the secretion continued for 3½ hours after evisceration. The relation between the blood pressure and secretory rate and anzyme content was very interesting.

After decapitation the blood pressure was 45-50 mm. Mercury. After evisceration was complete it had fallen to 35-40. Coincident with this fall in blood pressure and the cessation of operative procedures the amount of secretion doubled and there was a sharp fall in enzyme power.

As the blood pressure fell to 24 the abdominal aorta was ligated low down in the lumbar region. A few minutes later the vena cava was tied which allowed the blood to drain out of the lower extremities. The rate of secretion remained constant but the enzyme power fell slightly lower.

Finally as the blood pressure fell to 20 the secretion diminished, and with the slower rate of secretion the ferment power rose slightly towards the end of the experiment.

Comparison of these two experiments June 24th Table No.14 and June 16th Table No.13, will reveal the fact that there is a marked difference in the course of the enzymes. In the experiment of June 16th the value fell less than one-half in the first hour after evisceration, then gradually returned to the former level and remained stationery, whereas in

experiment June 24th the tryptic power fell precipitously to almost 1/4 in the hour after evisceration and even further during the remainder of experiment.

The same apparent discrepancy was noted also in experiments. May 15th and 19th. Tables 10 & 11. The most probable explanation for this abrupt fall in enzyme power in these experiments is that during the extirpation of the stomach and duodenum a certain amount of damage is done to the sympathetic nerve supply to the pancreatic gland.

The sympathetic supply comes as we have mentioned from offsets of hepatic, splenic, superior mesenteric plexuses, and follows the pancreatic vessels. In legating the vessels of the stomach and duodenum, it is quite possible that the connections to these plexuses may be destroyed having much the same effect as the section of the splanchic nerve which has already been discussed.

Referring to Table No. 9, Experiment January 27th, when only the small intestine was removed the enzyme content was very high.

Of course, in all evisceration experiments where the stomach is removed, the parasympathetic innervation to the pancreatic gland is served when the oesophagus is sectioned, but the removal of this innervation per se has no marked

effect on the enzyme content as previously noted.

To clarify this problem experiments must be done in which the splanchnics are cut before evisceration is commenced so that central impulses reaching the gland through the sympathetic system will be excluded from the beginning of the experiment.

Therefore, in summarizing these experiments we reach an important and interesting conclusion: the spontaneous secretion in the rabbit continues after the extirpation of the whole gastro intestinal tract in decapitate animals as well as in animals anaesthetized with urethane.

In view of the accepted beliefs concerning the action of secretin and the humoral mechanism of pancreatic secretion in the explanation of this fact is problematical. We must accordingly assume that the permeability of the secretory cells of the pancreatic gland in the rabbit for water and inorganic constituents of the blood is quite different from the permeability of the pancreatic cells in carnivorous animals.

Whether this is due to the innate properties of the cells, or whether secretin in the rabbit circulates in the blood for hours without being destroyed, only further investigations will reveal.

111. THE EFFECT OF INSULIN HYPOGLYCAEMIA ON THE PANCREATIC SECRETION OF THE RABBIT.

The inter-relationship between the internal and external secretion of the pancreatic gland has been known clinically for some time. It is generally believed that the external secretory activity of the pancreas is definitely diminished in cases of severe diabetes mellitus. Jones, Castle and Mulholland 1925, Labbe and Rechad 1926. The former investigators reported that effective insulin therapy reduced the external pancreatic secretory disturbance, a remarked improvement occurring on relief of the acidosis. It is difficult to say whether this effect is due to any specific action of insulin or whether it is due to the general action of insulin in improving or correcting the disturbed nutrition of the pancreas caused by the diabetic condition.

Deusch & Drost 1927 found that the injection of 10 units of insulin in 22 normal individuals produced an increased pancreatic secretion as judged by the increased amount of trypsin amylase and lipase in the duodenal secretion. They also stated that duodenal contents in the diabetics possessed a normal amount of ferments. On the other hand, Fonseca and Carvalho 1926 claimed that the injection of 20 units of

insulin in 10 cases produced no change in the pancreatic ferments estimated by Wholgemuth's methods, and they concluded that insulin had no effect on external pancreatic secretion.

The results of animal experimentation also gave conflicting results. Collazo & Dobreff 1924 reported that the injection of insulin intravenously in etherized dogs with the pylorus tied caused an increase in external pancreatic secretion during a few minutes after the injection.

Penau & Simonnet 1925, and Lambert & Hermann 1925, found that insulin injected intravenously had no secretory effect on the pancreatic gland in dogs although the pancreas responded to secretin.

LaBarre & Destree 1928 presented very interesting data on this problem. They experimented on dogs anaesthetized with chloralose, in which the pancreatic secretion was provoked by the continuous injection of Bayliss & Starling secretin 1 cc per minute. They obtained a continuous secretion of .35 - .5 cc per hour for 3-5 hours. On the injection of insulin they noticed a sharp coincident fall in the amount of secretion and ferments with the fall in blood sugar. Their work therefore explained the conflicting results of previous experimentors.

However, the mechanism of this inhibiting action of insulin is by no means clear and is further complicated by the effect of secretin on the blood sugar.

Freud & Saadi-Nazim 1926 found a definite fall in blood sugar in dogs anaesthetized with chloralose after the introduction of 60-100 cc. of .5 - 1% HCl into the duodenum. They also confirmed this result on unanaesthetized dogs after the introduction of a similar amount of HCl into the stomach.

Penau & Simonnet 1925 investigated this question thoroughly. They compared the effects of crude and purified secretin on the blood sugar of unanaesthetized rabbits. Their purified secretin gave a good secretion in small amounts and without any depressor effect on blood pressure.

The injection of the crude preparation .01 gm. per Kilo and of the purified .005 gm. per kilo in unanaesthetized rabbits gave a marked lowering of blood sugar, .115 to .05 and produced convulsions in some animals. They obtained the same results on unanaesthetized, normal and depancreatized dogs.

To all these data must be added an observation made long ago by Babkin & Savitsch 1921 that acid solutions of

sugar activated a section of the pancreatic juice in a dog with a permanent pancreatic fistula much richer in enzymes than the juice activated by a solution of HCl alone. The rate of secretion in both cases was the same.

Experiments in this field are influenced not only by the use of secretin but also by the diet of the animals used and the anaesthetics employed.

Page 1923 found that acid ash diets had a greater resistance to the hypoglycaemic action of insulin than basic diets, due to the fact that the former increased glycogenolysis in the liver. He concluded that diet in rabbits is an important factor in the blood sugar level.

Pinau & Simonnet 1925 noted that rabbits fed on hay were more sensitive to insulin than those fed on grain. Banting, Bast, Doffin and Gilchrist 1923 investigating the quantitative parallelism of insulin on man, dog and rabbit reported that there was considerable variation in the reaction of individual animals to insulin.

With regard to anaesthetics certain investigators have reported their results. It is well known that ether and chloroform produce a hyperglycaemia and glycosuria. Urethane

in a dosage of 1.2 Gm. per kilo caused a marked and lasting hyperglycaemia and glycosuria in the rabbit. Hirayama 1926. Tachi and Hirayama 1928. They also pointed out that there was a marked decrease in liver and muscle glycogen and that section of both splanchnics had no effect on liver glycogenolysis. Page 1923 reported that amytal, iso-amyl -ethyl-barbituric acid was an anaesthetic without influence in blood sugar regulation.

Accordingly there was a number of interesting observations regarding the inter-action of the internal and external pancreatic secretions and the effect of secretin on the blood sugar, but the underlying mechanisms of these phenomena were not known. Therefore it was decided not only to see the effect of insulin and hypoglycaemia on the volume and enzyme concentration of the pancreatic juice in the rabbit, but also the effect of the subsequent introduction into the circulation of glucose which form of the experiment had not yet been performed.

In the experiments we have done, an attempt has been made to clarify these points. Rabbits were used, fed on a regular diet of hay and carrots with water ad.lib. In the first few experiments, urethane 1 Gm. per kilo intravenously was used and and the high long sustained blood sugar was encountered. Large doses of insulin apparently had no effect whatever on the hypoglycaemia. In fact the blood sugar rose after the injection

of insulin. This antagonistic action of urethane and insulin is shown in Table No.15. Experiment April 10,1930.

Blood sugar determinations were made by the Schaffer Hartmann micro method. Insulin prepared by the Connaught Laboratories was used in all experiments.

Table No.15

Experiment April 10, 1930

Rabbit 2.0 K. Fasting 18 hours Urethane 1 Gm. per K.I.V.

Bile Duct and Pylorus tied.

Time	Vol.in cc.	Units Trypsin	Mg.% Bl. Sugar	Remarks
9.00			.097	
9.05				Urethane 1Gm per K.I.V 20% solution
9.15				Canula introduced
10.15	.25	800		
10.30			.177	
11.15	.1	720		20 units insulin I.V.
11.45			.266	
12.15	.1	720	.266	20 units insulin I.V.
12.45			.307	
1.15	.05	600	.288	40 units insulin I.V.
1.30			.317	
2.15	.1	720		

The fasting sugar of .097 % rose one and a half hours after the injection of urethane to .177 %. After 20 units of insulin at 11.15 - a half hour later, the blood sugar was .266 %, and in spite of repeated doses of insulin it finally rose to .317%.

The secretion was scanty and the enzyme value remained practically stationary. In direct contrast is the following experiment April 9, 1930, on an anaesthetized animal.

Table No.16

Experiment April 9, 1930

Rabbit 2.4 K. Unanaesthetized fasting 18 hours.

Time	10 A.M.	10.10	10.20	10.40	11.15	12.15	2.30
Blood Sugar	.117		.119	.078	.066	.052	.078
		# 10 units insulin intravenously				# 4 cc. 20% glucose I.V.	

Here it is seen that one half hour after the injection of 10 units intravenously the fasting blood sugar of .117% had fallen .078% and that two hours later it had reached .052% - almost the convulsive limit.

In all succeeding experiments Amytal 1/2 cc. per kilo injected intramuscularly was used as an anaesthetic. Occasionally, a few whiffs of ether were necessary to open the parietal peritoneum which is very sensitive in rabbits. The rise in blood sugar in some experiments after the anaesthetic was probably fue to this factpr.

Table No.17, Experiment Apr.16,1930, shows a typical experiment performed with this anaesthesia.

Table No. 17.

Table No.17

Experiment April 16, 1930

Rabbit 2.28 K. Fasting 18 hours. Amytal 1.5 cc. 1M ether to open abdomen. Bile duct and pylorus open. Stomach and duodenum partly full.

Time	Vol.in cc.	Units Trypsin	Blood Sugar	Remarks
9.00				
9.30			.120	
9.45				1.5 cc. amytal I.M.
10.00				Canula introduced
11.00	.4	400		
12.00	.6	300	.281	15 parts insulin I.V.
12.30			.300	
1.00	.6	266	.288	
1.30				40 units of insulin I.V
2.00	.55	240		
2.30			.285	
3.00	.7	266		
3.30				
4.00	.4	30	.177	
4.30			.085	Secretion stopped
5.00			.066	
5.30				5 cc.20% glucose I.V saline
6.00				
7.00	.05	20	.124	
7.30				5 cc.20% glucose saline I.V
8.00	.1	24		
9.00	.25	100		

This experiment presents dome interesting facts. It will be noted first that the fasting blood sugar of .120 % rose to .281% after anaesthetization. After the intravenous injection of 15 units of insulin the blood sugar rose slightly to.300%. The secretion rate had become steady and the enzyme value centered around 250. 40 more units were injected at 1.30 p.m.

By 3.30 P.M. the blood sugar had fallen to .177%. The secretion during this hour was .4 cc and the tryptic value of the juice had fallen over 8 times to 30. This is the more remarkable in that the same volume of secretion was secreted between 10 and 11 a.m. and trypsin units were 400.

During the next two hours there was no secretion at all and the blood sugar fell to .066% at 5.00 P.M. At 5.30 5 cc of 20% glucose saline were injected and from 6 to 7 the secretion returned. At 7 o'clock the blood sugar was .124%; at 7.30 5 cc of 20% glucose saline were again given by vein and the secretion rose during the next two hours to .25 cc. The enzyme value also increased to 5 times the extremely low value of 20.

Here then is corroboration of La Barre and Destree's work on dogs for thepancreatic secretion in the rabbit.

hypoglycaemia induced by the intravenous injection of insulin inhibits the spontaneous secretion in the rabbit and causes a tremendous drop in enzymatic power of the juice.

Following this inhibition of secretion which lasted for two hours the injection of 5 cc of 20% glucose saline and the return of the blood sugar to its normal level there was an immediate return of the secretion and a partial restoration of the ferment power of the juice in the succeeding 2 hours. Whether this action was due to the insulin itself or to the accompanying hypoglycamia must be decided by further experimentation.

However, the prompt return of the secretion and enzyme content following the administration of glucose saline is a very suggestive fact.

DISCUSSION

The special features of this study on pancreatic secretion are 1st, The proof of the action of the parasympathetic nervous system on the pancreatic gland in the rabbit; 2nd. The investigation of the role of the sympathetic innervation of the pancreatic gland in the rabbit. 3rd. The new data concerning the spontaneous secretion. 4th. The effect of insulin hypoglycaemia and of hyperglycaemia on the pancreatic secretion. These sections will be taken up in this order in the discussion of experimental results.

A. Parasympathetic Innervation.

The proof of the action of the parasympathetic nervous system on the pancreatic gland in the rabbit has cleared up discordant data in the literature. Gottlieb 1894 it will be remembered reported an increase in amount and total solids of the pancreatic secretion in the rabbit after the intravenous injection of pilocarpine. Savitsch 1918, on the other hand affirmed that in his experiments the vagus had no action on the spontaneous secretion. We have established without doubt that the vagus acts as a secretory nerve for the pancreatic gland in the rabbit since electrical stimulation of the nerve produces not only an increase in the amount of the secretion but also a tremendous rise in the enzyme power of the juice.

Section of the vagi on the other hand, and the consequent removal of any central impulses transmitted by these nerves to the gland, contrary to the experiments of Mellanby 1925 on the pancreatic secretion in the cat, has no effect whatever on the rate of secretion or enzyme content of the juice. Thus the exact function of the parasympathetic nervous innervation in the production of the spontaneous secretion of the pancreatic gland in the rabbit is difficult to define. The new significance attached to the sympathetic innervation of the pancreatic gland in this animal would seem to indicate that the vagus is a secondary or subsidiary nerve supply, and consequently does not play an important poart in the production of normal secretion.

B. SYMPATHETIC INNERVATION.

The study of the sympathetic innervation of the pancreatic gland revealed some new facts. Very little was known of its action in the cat or dog as experimentation in these animals is full of technical difficulties owing to the fact that there is no spontaneous secretion. Furthermore, electrical stimulation of this nerve in the freshly divided state results primarily in a marked vaso constriction of the abdomonal blood vessels which is, of course, very antagonistic to pancreatic secretion. To overcome these difficulties dif ferent methods have been devised. Kudrewezki 1890 stimulated the

splanchnic nerve in dogs, 6-7 days after it had been cut asceptically to permit degeneration of the vaso constrictor fibres and thus obtained a scanty secretion of juice very rich in enzymes. As an alternative procedure he employed Haidenhain's tetanometer, for mechanical stimulation does not excite the vaso constrictor nerves.

Savitsch after long continued electrical stimulation of the freshly divided splanchnic nerve in dogs obtained a small secretion of a juice potent in enzymes.

These experimental difficulties are greatly facilitated in the rabbit by the fact of the spontaneous secretion. Furthermore, the separation of the vaso constrictor secretory components of the splanchnic nerve in this animal is apparently more easily accomplished by electrical stimulation.

Accordingly the influence of the sympathetic innervation of the pancreatic gland in the rabbit revealed new and interesting data. Electrical stimulation of the freshly divided nerve first of all produced inhibition of secretion followed by an augmentation. The inhibition soon disapeared and a definite acceleration of the secretion during the period of excitation, and for a few minutes following occured. Besides this acceleration of secretion there was a definite and sharp rise in the ferment index of the juice following repeated stimulation of the splanchnic nerve.

Section of the splanchnic nerves and removal of the central impulses conveyed by them to the pancreatic gland had immediate and definite results. there was a transient increase in the secretion rate due to the dilatation of the visceral blood vessels which followed this procedure. But more important was the abrupt and profound fall of the enzyme index which occured immediately after section of the splanchnic nerves and very often independently of the rate of the secretion. Consequently the splanchnic nerve has been established as a secretory nerve of importance in the rabbit, and apparently plays a more important role in the production of the normal spontaneous secretion than the parasympathetic nervous system.

C. Spontaneous Secretion

The fact of the spontaneous secretion was well known to previous experimentors but concerning its causation little or nothing was established.

It was assumed, a priori, that the spontaneous secretion was due to the continuous humoral stimulus. In direct contradiction to this supposition we have shown that the spontaneous secretion continues for 5-6 hours in decapitate animals in which the whole gastro intestinal tract has been removed.

The significance of these experiments and their bearing on the secretin mechanism as we now interpret it is astounding.

Either this mechanism in the rabbit differs fundamentally from other animals and the small amount of secretin circulating in the blood at the time of evisceration is capable of activating the secretion for long periods, which is extremely unlikely, or the basic cellular physiology of the pancreatic gland in the rabbit differs radically from that of carnivorous animals. In other words, the secretory cells of the rabbit's pancreatic gland are permeable at all times for water and salts which pass through the blood carrying the organic material and enzymes with them.

D. The effect of insulin hypoglycaemea on the pancreatic secretion of the rabbit.

In a field of conflicting evidence one piece of experimental work by La Barre & Destree 1928 showed that there was an important inter-relation between the external and internal pancreatic secretion in the dog. The demonstrated that the intravenous injection of insulin in a dog secreting under the constant influx of secretin caused a marked diminution of the pancreatic secretion with a pronounced fall in enzyme power of the juice which was coincident with the hypoglycaemia. Whether this effect was due to a specific action of insulin or to the hypoglycaemia itself they did not determine.

In further experimentation with insulin hypoglycaemia, we have confirmed the inhibitory action of the hypoglycaemia

on the rate of secretion and the enzyme index of the juice in the rabbit.

It is important to note that the pancreatic secretion was influenced after injection of insulin not by the absolute concentration of sugar in the blood, but by its relative value as compared with the blood sugar concentration before the injection of insulin. Therefore it seems that the pancreatic secretion was inhibited more by insulin itself than by a low concentration of sugar in the blood.

However, it has been demonstrated that the injection of glucose solution intravenously not only restores the secretion but tends to bring about a gradual return of the enzyme power of the juice.

Accordingly on present evidence it would appear that both factors i.e. hypoglycaemia and insulin itself are responsible for the depressing effect on the pancreas although the exact mechanism of this result is not yet known but will be investigated in future researches.

Consequently in this study on pancreatic secretion in the rabbit, we have brought out interesting facts in the nervous mechanism as well as in the humoral and hormonal regulation of the secretion.

CONCLUSIONS

1. There is in the rabbit a regular spontaneous secretion with undiminished enzyme content which is continuous after the ligature of the pylorus and bile duct. It is observed in animals anaesthetized with a wide range of narcotics as well as in decapitate and decerebrate preparations.

2. The spontaneous secretion is stimulated by HCl but particularly by an acid bile and peptone mixture.

3. The vagus nerve, contrary to previous belief, is a true secretion nerve to the pancreatic gland in the rabbit and possesses secretory "trophic" and motor fibres to the ducts.

4. The pancreatic secretion in the rabbit is also stimulated by parasympathetico mimetic drugs, pilocarpine and acetyl choline.

5. Section of the parasympathetic nerve supply to the pancreatic gland has no apparent result on the rate of secretion or content of enzymes.

6. The sympathetic nerve supply to the pancreatic gland in the rabbit exerts a hitherto unproven trophic influence in addition to a vascular inhibition.

7. The repeated injection of small amounts of adrenaline produces the same results as the stimulation of the splanchnic nerve on the pancreatic secretion in the rabbit.

8. Section of the sympathetic nerve supply produces a profound immediate and lasting fall in enzyme content of the pancreatic secretion in the rabbit.

9. The spontaneous secretion continues after the removal of the whole gastro intestinal tract in decapitate preparations as well as in animals anaesthetized with urethane.

10. The hypoglycaemia resulting from the intravenous injection of insulin produces a sharp inhibition of pancreatic secretion in the rabbit with an abrupt coincident fall in enzyme power.

11. The injection of glucose saline restores the secretion and brings about a gradual return of the enzyme content of the juice.

12. Urethane 1 gm. per kilo injected intravenously in a 20% solution causes a high sustained blood sugar which is not affected by large doses of insulin.

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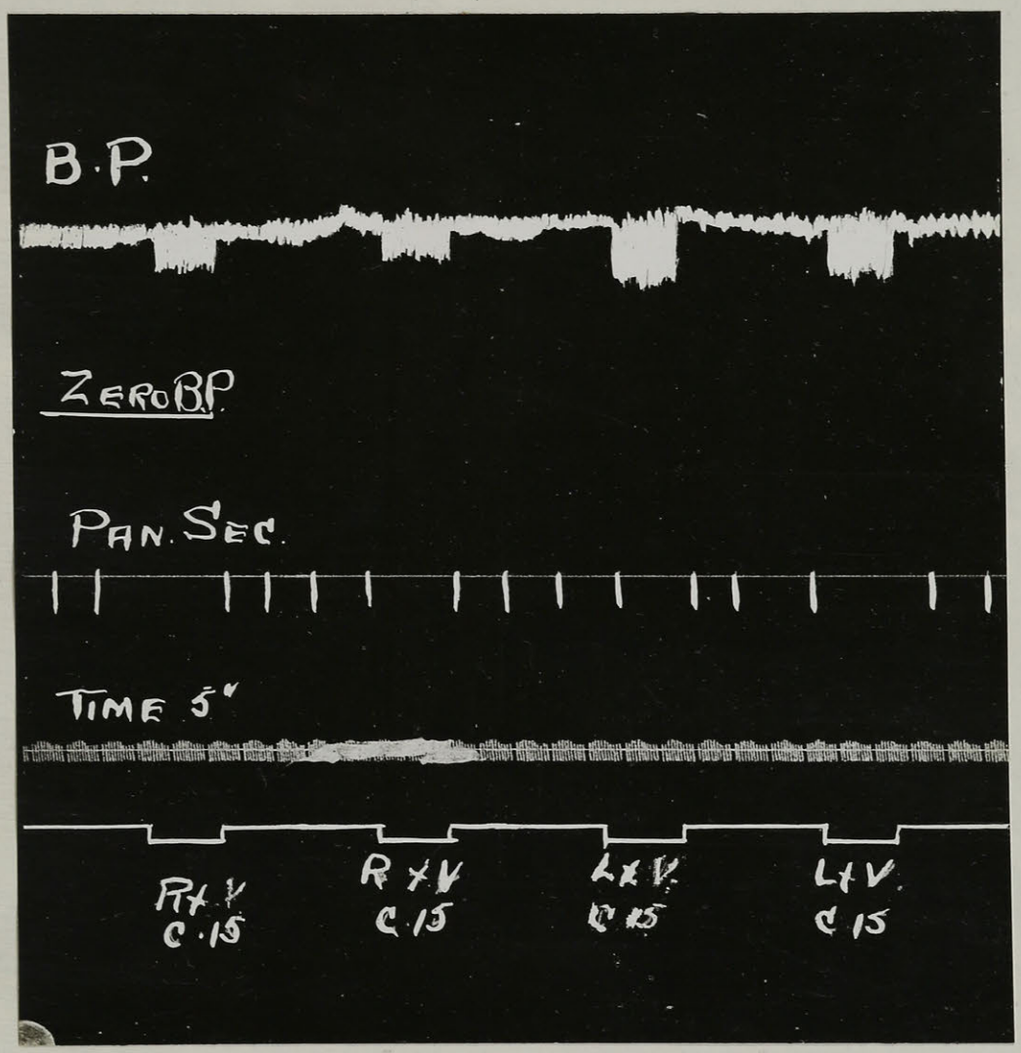


Fig. No.2.

Rhythmic stimulations of the vagi in the neck. Coil at 15 cm. Shows inhibition of secretion following stimulation.

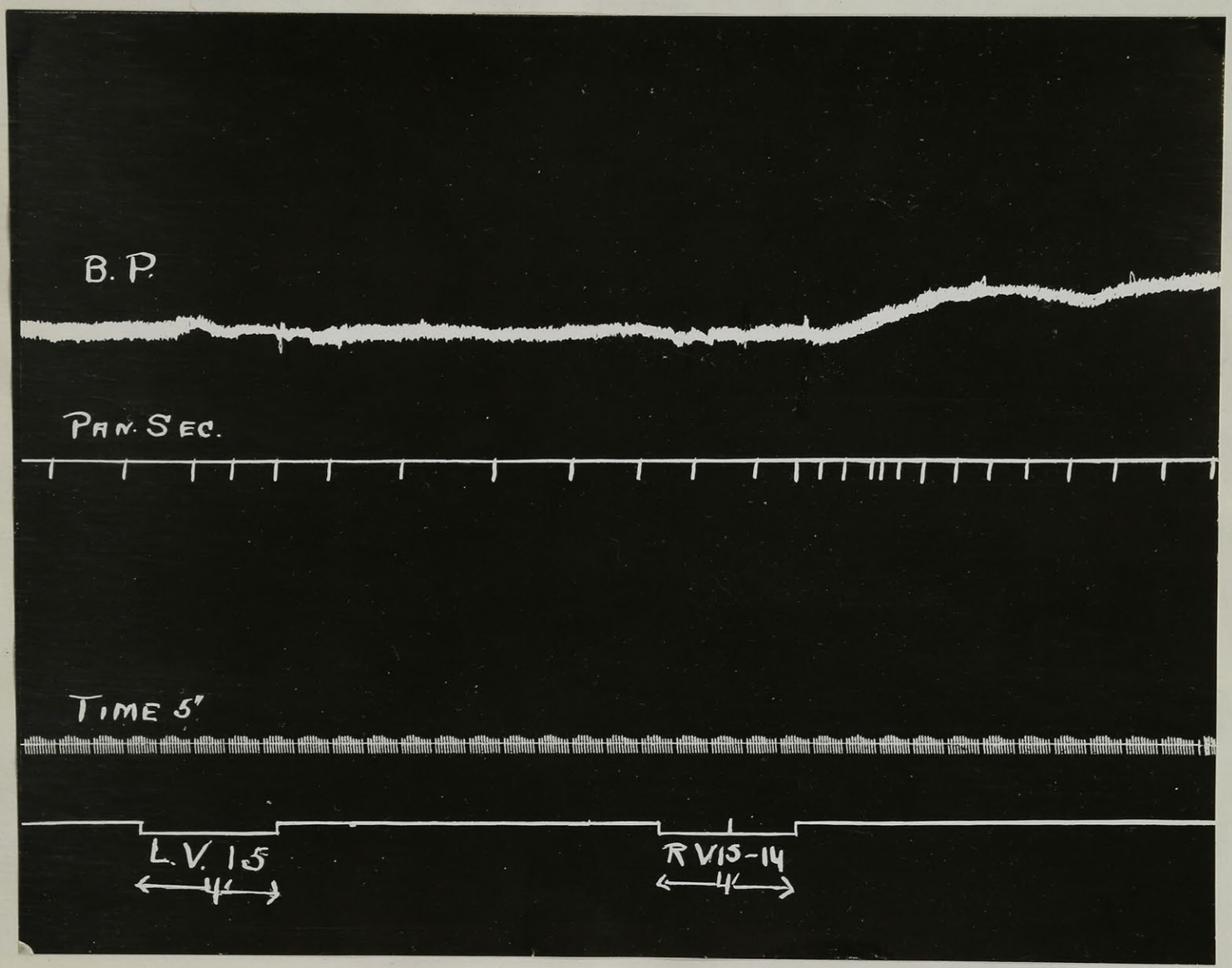


Fig.No.2 a.

Stimulation of vagi in the chest below the heart with an induction current. Illustrates acceleration of secretion following a four minute period of stimulation.

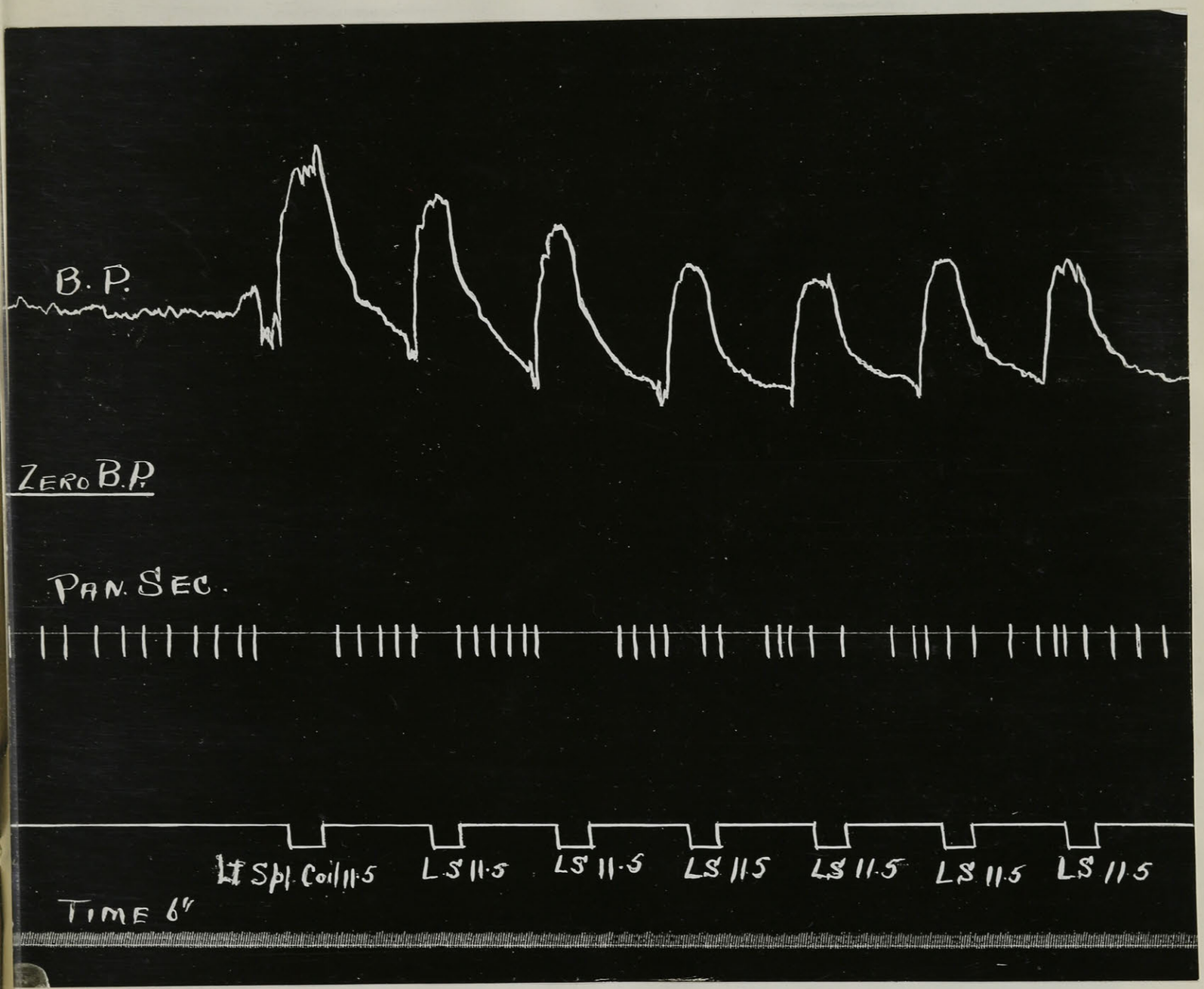


Fig.No.3.

Excitation of left splanchnic, adrenal vein not tied. Pylorus and Bile Duct ligated. Demonstrates initial vascular inhibition which is followed by a pure secretory effect.

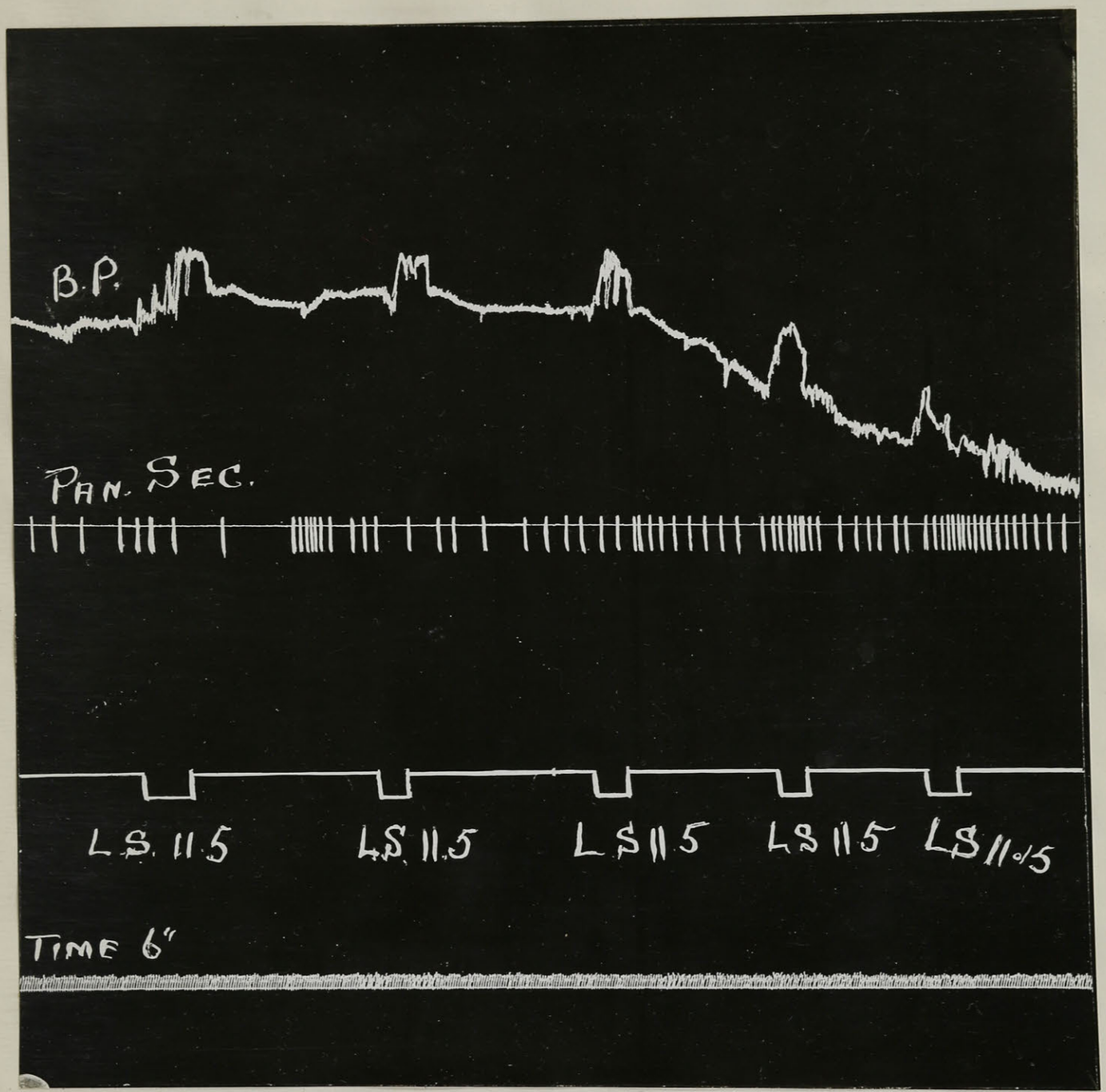
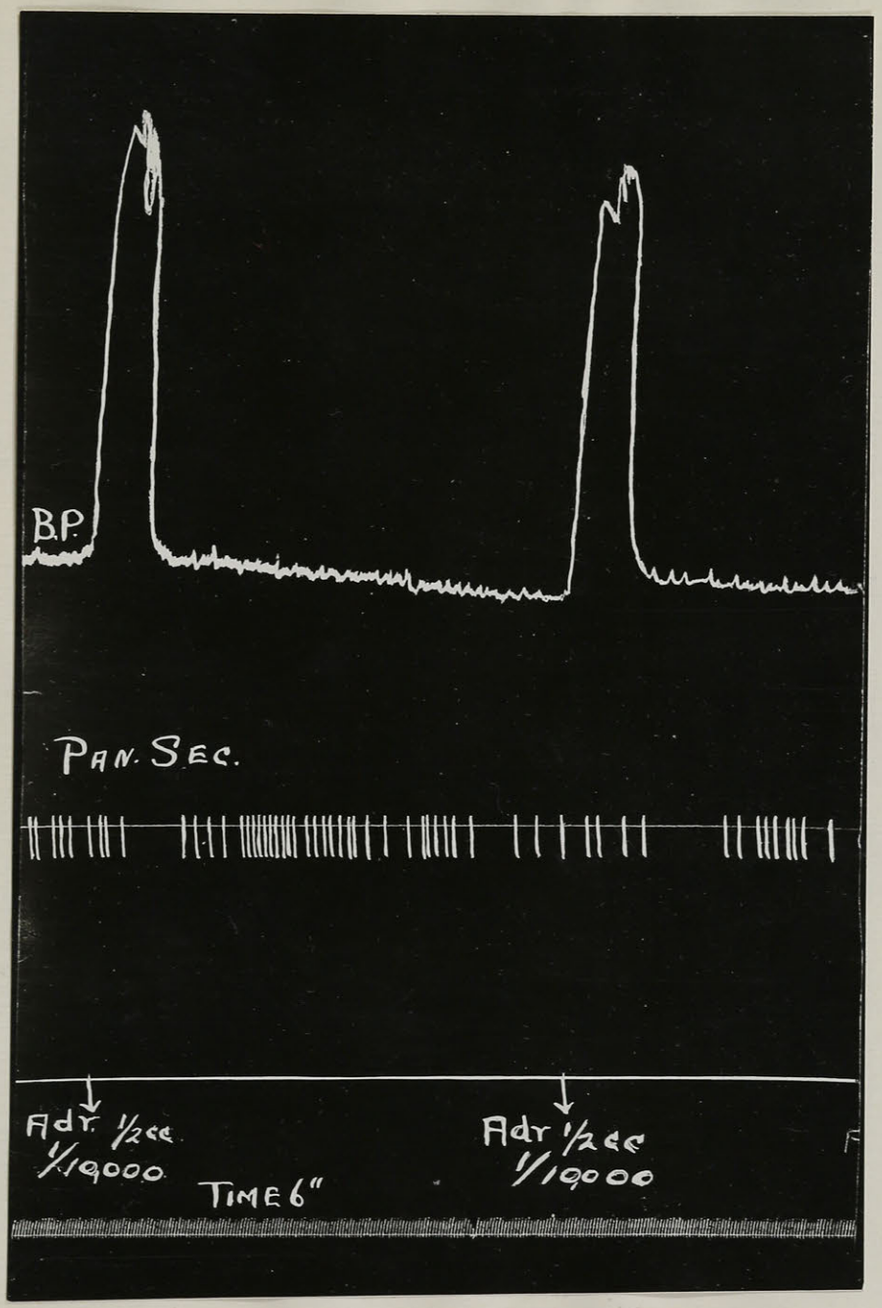


Fig.No.4.

Stimulation of left splanchnic nerve, adrenal vein tied, Pylorus and Bile Duct ligated. Blood pressure rise insignificant, marked secretory action of sympathetic.



, Fig. No.5.

Repeated injection of adrenaline. Pylorus and Bile Duct tied, tremendous rise in Blood Pressure with vascular inhibition followed by acceleration of secretion.