THE MODE OF ACTION OF SPECIFIC SUBSTANCES WITH SPECIAL REFERENCE TO SECRETIN. BY W. E. DIXON AND P. HAMILL.

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Ehrlich and Langley have adopted the view that living protoplasm carries certain chemoreceptors, or contains chemoreceptive substances, and it is by combination with these that drugs induce specific effects. The validity of this hypothesis forms the subject of this paper.

The mode of action of secretin was investigated first, because this substance affects profoundly only one tissue in the body—the pancreas. It is true that it cannot be regarded as comparable with drugs proper since it belongs to the group of animal hormones which, as will be shown later, act differently from drugs of vegetable origin. As an example of the latter group strychnine was chosen since its action is specific and sharply confined to the medulla and cord.

Method. Dogs were employed in almost all experiments when the activity of the pancreas was being measured. They were anaesthetised

1 The Harben Lectures for 1907 contain a summary of Ehrlich's views.
2 This Journal, xxxvii. p. 285. 1908.
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with chloroform and urethane, and also received an injection of morphine. In a few cases the animals were killed by pithing after the induction of anaesthesia. Pancreatic emulsions and secretin were obtained from the tissues of the dog, but fresh pancreas and duodenum of the sheep were at times procured from the slaughter-house. The strychnine experiments were conducted on rabbits and frogs, and the spinal cord for making emulsions was also obtained from rabbits. All animals were anæsthetised unless otherwise stated. Frogs had their brains destroyed by pithing.

SECRETIN COMBINES CHEMICALLY WITH SOME SUBSTANCE IN THE PANCREAS.

(a) Some characters of secretin. Our first object was to obtain a reliable and standard secretin. This body can be obtained in considerable amounts from the duodenum of all vertebrates, and we fail to appreciate why Popielski still insists on the nervous hypothesis of pancreatic secretion; since nerve stimulation does not lead to secretion the necessity for such a hypothesis is wanting.

By pounding the mucous membrane of the duodenum in a mortar with sand, extracting with water, and filtering through a Berkefeld filter, a clear filtrate is obtained. If this filtrate is boiled with acid and neutralised, it never contains secretin. The secretin then should be in the precipitate on the Berkefeld; but if this precipitate is scraped off and similarly boiled with acid it also is found to be destitute of secretin. Bayliss and Starling first showed that prosecretin was not contained in the filtrate and we have confirmed their results and have also shown that it is not present in the precipitate. The hormone has completely disappeared. It seemed possible though à priori unlikely that secretin might be destroyed by passing through a Berkefeld filter. We have found that a freshly prepared, clear and active solution of secretin ceases to be active after passing through a Berkefeld filter, at a pressure of about 5 mm. of Hg. We propose to show on another occasion what becomes of this secretin.

If then the prosecretin be soluble in water it is possible to conceive that it may be destroyed in the filter in a similar manner. These

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1 Arch. f. d. ges. Physiol. cxxi. p. 239. 1908. Popielski asserts that pancreatic secretion produced by the action of secretin however carefully prepared is invariably accompanied by a fall of blood-pressure. We have confirmed the statement of Bayliss and Starling that secretin prepared from duodenal mucous membrane which has been extracted with hot alcohol produces active pancreatic secretion without producing any effect on blood-pressure.
experiments did not help in the preparation of a pure secretin, although
they have provided us with a ready means of separating secretin from
digestion mixtures, a method of which we have availed ourselves in later experiments.

If the duodenal mucous membrane of an animal be washed on a
filter paper for five or six days with running water the prosecretin does not pass out, and is not destroyed provided that a drop of chloroform is added daily to prevent putrefaction. From such washed mucous membrane we have removed the depressor substances by boiling alcohol and ether and have prepared a relatively pure secretin from the residue. Prosecretin is therefore insoluble in water, alcohol, and ether, but is readily soluble in dilute acids. In those experiments in which a pure secretin was necessary it was prepared in this manner.

If secretin is allowed to stand in unstoppered bottles in the laboratory during summer it is found to have practically disappeared within 24 hours. If, however, the freshly made secretin after boiling is preserved in a sterile tube, so as to prevent bacterial action, and is kept in the dark to inhibit oxidation, it will remain active almost indefinitely, and it is thus possible to use a standardised preparation. A dried acid extract of the mucous membrane also keeps well (samples tested after storage for a year were found extremely active), so that at any time a standard solution may be prepared by dissolving a weighed quantity in water and neutralising.

It has lately been stated by Modrakowski that choline causes a pancreatic secretion which is not antagonised by atropine; he says it is identical with secretin. It is true that choline is present in extracts of the mucous membrane of the duodenum as it is in most animal extracts, and we have shown that the injection of 0.02 grm. of choline intravenously into a dog induces a secretion of one or two drops of pancreatic juice. Anyone however who can seriously suggest an identity between these two substances, choline and secretin, can never have observed the effect of a properly prepared secretin. The choline action resembles that of muscarine and pilocarpine, though it is much feebler; the action of all these bodies on the pancreas is insignificant compared with secretin, and is eliminated by atropine while that of secretin is not. von Fürth and Schwarz also found that the

1 Latterly Parke, Davis and Co. provided us with an active secretin prepared by the method described.
2 Pflüger's Arch. cxxiv. p. 427. 1908.
3 Pflüger's Arch. cxxiv. p. 615. 1908.
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Pancreatic effect of choline is antagonised by atropine; they think however that some of the action of secretin may be due to choline. Their method of preparing secretin, though they state that it is after that of Bayliss and Starling, is unsatisfactory, since they appear to employ the whole duodenum rather than the mucous membrane.

(b) The combination of secretin with pancreas. If an emulsion of fresh tissue, such as salivary gland, liver, or muscle be added to a solution of secretin, the activity of the secretin is lost in a few hours. This was shown by Delezenne for intestinal mucous membrane, but it is also true for almost all tissues in the body. The presence or absence of secretin in this case is determined by boiling the mixture, filtering, and injecting the filtrate into the circulation of another animal, whilst recording the flow of pancreatic juice. If the secretin has remained in contact with the tissue for a period under half an hour but little destruction takes place.

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Fig. 1. Dog. Urethane, chloroform, morphine. Vagi cut. Shows the effect of injecting a mixture of 4 c.c. pancreatic emulsion with 4 c.c. very active secretin. At "A" the injection was made immediately after mixing the two, and at "B" half an hour later a like injection was made except that the solutions had been allowed to incubate mixed together for eight minutes. Pancreatic juice recorded as drops. Time = secs.
It will be seen from the experiment, illustrated in Fig. 1, that incubation of pancreatic emulsion with secretin destroys no more of the latter than is affected immediately the two are mixed, provided the incubation lasts no more than 10 or 15 minutes. Hence it is improbable that any considerable destruction of secretin, which occurs on mixing it with tissue cells for a strictly limited time, may be caused by oxidation.

In the present experiments the tissues employed to neutralise the secretin were cleaned, weighed, and ground up with normal saline solution to form an emulsion of known volume. Varying amounts of the standardised secretin were added to 10 c.c. of pancreas emulsion containing one gramme of fresh pancreas to 5—10 c.c. of water. The mixture was shaken for a period of one minute and was then boiled and filtered. The relative amount of secretin in the filtrate was determined by experiments on fresh animals suitably prepared to record the rate of secretion from the pancreatic duct. A long series of such experiments has shown that a definite amount of pancreas is capable of neutralising and destroying a fixed amount of secretin.

Fig. 2. (Exp. 5.) Dog. Chloroform, urethane, morphine. Record of blood-pressure and flow of pancreatic juice. Shows effect of injecting at "A" 5 c.c. secretin from the dog; at "B" 3 c.c. emulsion of sheeps' pancreas; and at "C" after a 20 minutes' rest a fresh mixture of 5 c.c. secretin and 2½ c.c. pancreas. Time = 30 secs.

Supposing that 10 c.c. of pancreatic emulsion just neutralised 5 c.c. of secretin, so that on boiling the mixture no secretin was present in the filtrate, then if 6 c.c. be added to the same amount of pancreas 1 c.c. of the secretin would be uncombined, and after boiling and filtering would induce a secretion of pancreatic juice comparable with that produced
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by 1 c.c. of the original secretin in the same animal. The two fluids neutralise one another quantitatively, compelling the view that the reaction is chemical in nature. This point is illustrated in Fig. 2, which shows, first at "A" the effect of injecting 5 c.c. secretin, and at "C" a mixture of the same quantity of secretin with pancreatic emulsion. The amount of pancreas employed has nearly neutralised the secretin since only four drops of pancreatic juice are produced in 5½ minutes, as the result of the injection. A slightly larger amount of the pancreas effected complete neutralisation.

If the emulsion of pancreas is boiled before secretin is added it has no longer the power of neutralising any of the secretin; although when pancreas and secretin are first mixed and then boiled the secretin ceases to exist as such. It may also be noted here that if the pancreas be frozen for one or more days, or if it be repeatedly frozen and thawed, its power of neutralising secretin remains unchanged.

The pancreas, however, from different individuals varies very considerably in this action. A normal dog in good condition had its pancreas thoroughly exhausted by intravenous injections of secretin. Twenty minutes after the last injection the animal was killed and the pancreas emulsified; it was found that this emulsion of pancreas had almost no power of neutralising secretin. Furthermore, it has chanced in two or three of our experiments that a dog's pancreas was used which had little power of attacking secretin, and at least in two of these experiments the dogs in question were ill-nourished or suffering from disease. It has been made quite clear to us from many experiments that not only does the pancreas vary greatly in different animals in respect of its power for neutralising secretin, but it varies, also, within wide limits in animals of the same species.

(c) The power of other tissues to destroy secretin. It now becomes necessary to consider whether this combination of pancreatic emulsion with secretin is specific, or whether other tissues exhibit a like action. For this purpose several different tissues were employed, but the most decided positive effects were obtained with the liver and salivary glands, and so we confine attention to these. Several experiments were performed in which definite quantities of standardised secretin were added to weighed amounts of liver, submaxillary gland and pancreas respectively. The emulsions after shaking for a definite period, 1 or 2 mins., were boiled and filtered and the amount of secretin in the filtrate was determined roughly, by injecting a known quantity into an anaesthetised animal. It was found that both the liver and salivary gland had
some power of destroying secretin, but that when a suitable pancreas
was used the destruction of secretin by this organ was out of all
proportion to that caused by the liver, salivary gland, or any other
tissue in the body. And sometimes so decided is this difference that it
is impossible to doubt that the effect of the pancreas is specific. In
Fig. 3 at "A" the effect of injection of liver emulsion and secretin is
shown upon a dog: it causes an active secretion of pancreatic
juice, less however than that induced by the same dose of secretin given alone.
When the same amount of secretin was mixed with a pancreas emulsion
equal in strength to that of the liver emulsion the intravenous injection
caused no flow of juice.

Several hypotheses might be suggested to account for the partial
neutralising action of liver and salivary gland emulsions upon secretin.
It may be pointed out in the first place that emulsions of organs injected
together with drugs materially influence the degree of activity of these
drugs. Thus an emulsion of liver extract containing pilocarpine injected
into a femoral vein induces less effect on the cardiac vagus than if the
pilocarpine had been injected alone. It appears to act rather by
delaying the absorption of the drug into the tissue upon which it acts
than by any chemical effect. Further and more complete evidence on
this point is given later, when the mode of action of strychnine on
the spinal cord is discussed. This criticism is not, however, significant
with emulsions of pancreas or liver and secretin, since these injections
were made, for the most part, after boiling and filtering. Perhaps the
most important fact in this connexion is that secretin has some action
on both the salivary glands and liver, and it is quite possible that some
of the destruction of this substance may be brought about during the
induction of physiological activity as, in our opinion, is the case with the
pancreas.

All these experiments then go to show that secretin combines in a
specific manner with some constituent of the pancreas. When the
secretin has once disappeared from the emulsion by no procedure that
we have adopted has it been possible to recover it. Some evidence has,
however, been obtained suggesting that secretin may sometimes combine
in a relatively loose way with some substance in the liver, this loose
combination tending either to diminish or annul the specific action of
the hormone.

This effect is illustrated in Fig. 3. In this experiment a liver
emulsion was added to standard secretin and the two were shaken
together for three minutes: the emulsion was then boiled and filtered
and the clear solution assayed for secretin. An equal volume of the clear filtrate was boiled with 0.4% HCl, neutralised and also assayed for secretin. It was found in the latter case that the amount of secretin was increased. This effect was noticed only in the case of the liver and it is not constant: since, however, we have observed the effect three times we record the fact. These results may be explained by supposing that a small amount of secretin enters into some loose combination with a constituent of the liver, the secretin being so masked as to lose its characteristic activity, and that this combination may be broken up by boiling with acid so as to liberate active secretin. Possibly the secretin enters into some condition of stable equilibrium such as prosecretin, but prosecretin cannot be the substance under discussion since prosecretin is insoluble in water whilst this substance is soluble.

![Fig. 3. Dog. Urethane, chloroform, morphine. Blood-pressure and flow of pancreatic juice. Shows the effect of injecting at "A" 7 c.c. of the filtrate obtained after emulsifying 5 grms. of liver, 10 c.c. of secretin and 25 c.c. of water, and boiling after three minutes. "B" shows the effect of injecting the same dose but the filtrate was in this case first boiled with acid and then neutralised. Time=seconds.](image-url)
(d) Is secretin recoverable? It now becomes necessary to determine what becomes of the secretin. It has been pointed out already that "in vitro" secretin combines with some constituent of the pancreas, and that to a much smaller extent it is destroyed also by the liver and salivary glands, and furthermore that a very small quantity of it may enter into so loose a combination with the liver that, for a time at any rate, it is recoverable by boiling with acid.

Dogs were anaesthetised and secretin was injected into the circulation during the course of two hours until it was found that the pancreas hardly responded to further injections. In this condition, 20 minutes after the last injection of secretin the animal was killed, and extracts were made of the pancreas, salivary glands, muscle and liver. These tissues when suitably emulsified were boiled with 0.4% hydrochloric acid, neutralised, filtered and assayed for secretin. Further, the considerable amount of pancreatic juice obtained by this procedure was boiled with acid, filtered and neutralised and also assayed. No secretin was detected in any of these preparations except in the case of the pancreatic juice; two or three drops were secreted from the pancreatic duct of one test dog when the juice, previously boiled with acid, was injected. In most cases, however, no trace of secretin could be detected and when it was present its amount was always quite insignificant, representing the merest trace; still it is not without interest that a trace of secretin, even under such abnormal conditions as those in which it was administered in these experiments, should find its way into the pancreatic juice, when the pancreatic cells contained no trace of the hormone.

(e) The injection of tissue extracts during pancreatic secretion. From these experiments it is clear that the secretin, which may be present at any one moment in the general circulation of an animal, should be capable of removal by the injection of a pancreatic emulsion. To investigate this effect dogs were anaesthetised and the pancreas was made to secrete by the administration of an intravenous injection of secretin. If now whilst the gland is actively secreting an injection of pancreatic emulsion is made into the circulation the secretion rapidly diminishes, and if the dose is sufficiently large ceases entirely for the space of some minutes, and this may occur when the blood-pressure is little affected. Usually after some minutes inhibition the pancreas commences to secrete again though it does not attain to anything like its former activity. In Fig. 2 "B" such an injection of pancreatic emulsion is shown whilst the pancreas is actively secreting and it will be seen

1 The animals were atropinised so that choline and allied bodies could have no effect.
that the secretion for the space of five minutes ceases entirely. The pancreas then recovers slightly and secretes sluggishly. After a rest active secretion is again readily obtained by the injection of further quantities of secretin.

Fig. 4. Exp. 2. Dog. Chloroform, urethane, morphine. Shows the relative effect of pancreatic and liver emulsion on an actively secreting pancreas. The emulsions were of equivalent strength. Time = seconds. Drop record of pancreatic juice.

Fig. 4 shows the comparative effect of injections of pancreatic and liver extracts on the activity of the pancreas. The injection of liver slows the rate of secretion somewhat but the pancreas completely inhibits secretion for some minutes after which secretion of juice gradually returns. The liver emulsion produces less effect in this experiment than usual.

This effect might be due to a direct inhibition of the living cell of the pancreas by the emulsion. This does not seem probable since an emulsion of salivary gland does not, to anything like the same extent, inhibit the flow of saliva in a cat or dog brought about by the previous injection of pilocarpine. That is, we have no reason to suppose that pancreatic or salivary extracts have any immediate specific inhibitory effects on their respective glands when injected into the general circulation of a living animal. Nor can the inhibition of secretion be explained by general circulatory changes since stoppage of pancreatic secretion is seen when changes in the circulation are negligible. The nucleo-proteid in the pancreas can hardly be the cause of the phenomena as other nucleo-proteids have not a like effect. The explanation is, \textit{prima facie},
that the pancreatic emulsion combines with the free and easily available secretin and renders it inactive, then when the "pancreas-secretin" molecule has been disposed of by the tissues such amounts of secretin as remain uninfluenced or possibly have been temporarily adsorbed in the liver or other tissues may be gradually liberated and free to act. The secretion now recommences, but owing to the destruction of a considerable amount of secretin is less active than before. When a drug is injected into the circulation of an animal some of it induces the specific effect on the particular organ upon which it acts immediately, and some of it is taken up by other tissues upon which it may have little or no action: the drug is then slowly secreted into the blood again and is eventually excreted by one or other of the natural channels. This is probably what happens to secretin. When a pancreatic emulsion is injected into the circulation of an animal it at once destroys all the free secretin in the blood, and the flow of pancreatic juice ceases. Now the combining constituent of the pancreas as will be shown later must be a highly complex substance possibly as complicated as protein, and hence is unlikely to be adsorbed into the tissues, but will be destroyed. So that as this substance disappears from the blood the secretin will gradually return to the blood and the flow of pancreatic juice may recommence.

Earlier in this paper it has been pointed out that the pancreas from different dogs varies greatly in its power of neutralising secretin and that the power may be very small, as for example in animals which are ill and emaciated or have had their pancreas previously exhausted by secretin. The pancreas of such animals when made up as an emulsion is unable to inhibit pancreatic secretion in the test animal, and so close is the parallelism between the neutralisation of secretin by different varieties of pancreatic emulsion on the one hand and the degree of inhibition of pancreatic secretion caused by injection of the emulsion on the other, that it affords valuable additional evidence that pancreatic emulsion checks pancreatic secretion by the neutralisation process. In other words, if an emulsion neutralises very little secretin it will have very little power of inhibiting pancreatic secretion and vice versa.

Further, if these deductions be trustworthy then emulsions of liver and salivary gland should also have this inhibitory action on the pancreas just in proportion as they neutralise secretin, and it has been found that this is roughly correct. Both emulsions of liver and salivary gland do inhibit pancreatic secretion, but as previously noted the effect weight for weight is less than that of the active pancreas.
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THE SUBSTANCES IN THE PANCREAS UPON WHICH SECRETIN ACTS.

The next step is to determine the nature of the substances in the pancreas with which the secretin combines. The most obvious constituents are the living protoplasm, and the granular matter which represents the proferments in the cells and which is extruded during the activity of the gland. Now if secretin should combine with these granules it should materially alter their nature and probably determine their secretion. It was our object therefore to inquire whether the substance in the pancreas representing the enzymes is the same as that in the pancreatic juice or whether it is altered by injections of secretin. We have studied the question by making emulsions of the ground-up gland and observing whether the addition of secretin causes any alteration in the enzymic activity of the extract. The following is the protocol of a typical experiment.

Exp. 1. The pancreas of a freshly killed cat was excised, ground with sand and chloroform-water, centrifuged and allowed to stand overnight in the ice-chest. Some secretin was prepared from the duodenum of the same animal and was carefully neutralised, a cold water extract of the duodenal mucous membrane of the same animal was also prepared and filtered through a Berkefeld filter, in order to destroy any secretin which it might have contained. By this procedure a clear filtrate was obtained.

Mixtures were set up in small sterile flasks as shown in the Table. Toluol and three Fermi’s tubes containing 12% gelatin coloured with methylene blue were added to each mixture, and digestion was allowed to proceed at room-temperature for 24 hours. At the end of this period the tubes were removed and the amount of digestion in each was measured. The figures given in the Table represent the total amount digested in each flask.

<table>
<thead>
<tr>
<th>Digestion in mm.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 1 c.c. P + 2 c.c. H₂O</td>
<td>0</td>
</tr>
<tr>
<td>(b) 1 c.c. P + 1 c.c. S + 1 c.c. H₂O</td>
<td>7</td>
</tr>
<tr>
<td>(c) 1 c.c. P + 1 c.c. D + 1 c.c. H₂O</td>
<td>16.5</td>
</tr>
<tr>
<td>(d) 1 c.c. P + 1 c.c. D + 1 c.c. S</td>
<td>36</td>
</tr>
<tr>
<td>(e) 1 c.c. P boiled + 1 c.c. D + 1 c.c. S</td>
<td>0</td>
</tr>
</tbody>
</table>


The results shown in the table indicate that whereas neither the emulsion of pancreas (a), nor the mixture of duodenal extract and secretin with boiled emulsion of pancreas (e) contains any active trypsin, a mixture of all three as in (d) represents a considerable amount of trypsin as measured by the digestion. It will be noticed that the addition of secretin or of duodenal extract alone to the emulsion causes the development of some proteolytic power, and that the duodenal extract is without doubt the more powerful activator. This is probably partly explained by the fact that in this experiment no especial care was
taken to avoid the inclusion in the pancreatic emulsion of those portions of the gland which from their proximity to the intestine are liable to be contaminated with intestinal juices, and to contain in their ducts small quantities of pancreatic juice. In later experiments in which particular attention was paid to these points, and in which the enterokinase was made from the lower portions of the intestine to avoid the more thoroughly any likelihood of contamination with secretin, it was found that the effect of adding either enterokinase or secretin alone to the emulsion of pancreas was insignificant in comparison with the effect of adding a mixture of the two.

Exp. 2. An emulsion of pancreas of dog was prepared in \(25\%\) \(\text{Na}_2\text{CO}_3\) solution. Enterokinase was taken from the ileum and secretin from the duodenum. Toluol and gelatine tubes were added as usual and the digestion measured after standing 40 hours at room-temperature. To a similar series of flasks Congo red fibrin\(^1\) was added. The colour of the mixture indicates the degree of digestion.

<table>
<thead>
<tr>
<th>Total digestion in mm.</th>
<th>Colour with Congo red fibrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 c.c. (P) + 2 c.c. (Ek) + 2 c.c. (H_2O)</td>
<td>3</td>
</tr>
<tr>
<td>4 c.c. (P) + 2 c.c. (S) + 2 c.c. (H_2O)</td>
<td>8.5</td>
</tr>
<tr>
<td>4 c.c. (P) + 2 c.c. (S) + 2 c.c. (Ek)</td>
<td>25.5</td>
</tr>
</tbody>
</table>

Other experiments have been performed in which the tryptic activity was tested on suspensions of casein: in these the residual nitrogen was estimated at the close of the period of digestion, after precipitation of the undigested casein, by means of tannic acid. These experiments show a still greater contrast between the amounts digested with and without the addition of secretin.

Exp. 3. Emulsion of pancreas and secretin were obtained from the dog. Solution of casein \(0.8\%\) in \(0.5\%\) \(\text{Na}_2\text{CO}_3\). Incubated at \(38^\circ\) C. for 24 hours in presence of toluol. 10 c.c. samples were treated with 5 c.c. \(H_2O\) and 10 c.c. Oathard's\(^2\) tannic acid solution and the nitrogen was estimated in 10 c.c. of the filtrate by Kjeldahl's method.

<table>
<thead>
<tr>
<th>Residual N at outset in c.c.</th>
<th>Residual N after 24 hrs. c.c.</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N\frac{10}{10}\text{H}_2\text{SO}_4)</td>
<td>(N\frac{10}{10}\text{H}_2\text{SO}_4)</td>
<td></td>
</tr>
<tr>
<td>(a) 100 c.c. (C) + 5 c.c. (S) + 5 c.c. (Ek) + 5 c.c. (P)</td>
<td>(0.9)</td>
<td>(3.63)</td>
</tr>
<tr>
<td>(b) 100 c.c. (C) + 5 c.c. (H_2O) + 5 c.c. (Ek) + 5 c.c. (P)</td>
<td>(1.5)</td>
<td>(1.5)</td>
</tr>
<tr>
<td>(c) 100 c.c. (C) + 5 c.c. (S) + 5 c.c. (Ek) + 5 c.c. boiled (P)</td>
<td>(0.6)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

\(C=\text{casein solution}\). Other symbols as in previous tables.

\(^1\) Roaf. *Biochem. Journ.* iii. p. 188. 1908.

\(^2\) This *Journal*, xxxiii. p. 462. 1905.

\(^3\) The digestion was almost complete.
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It is quite clear then that secretin exerts a profound effect upon the digestive mixture containing enterokinase and pancreatic emulsion. It can hardly be conceived as acting by affecting the enterokinase since a mixture of enterokinase and secretin does not activate pancreatic juice so efficiently as enterokinase alone. In order to make this point more definite we have performed some experiments in which pancreatic emulsion, enterokinase filtered through a Berkefeld, and secretin were mixed together in definite amounts and the rate of digestion determined. This was compared with a second digestion in which the enterokinase and secretin were first mixed together, then filtered through the Berkefeld and finally added to the pancreatic emulsion: in this case the filtrate was proved to contain no secretin.

Exp. 4. The following were prepared. 1. Pancreatic emulsion from a freshly killed cat. 2. Solution "A" by mixing 15 c.c. of enterokinase with 10 c.c. of standardised secretin and filtering through a Berkefeld filter. 3. Solution "B" by adding 15 c.c. of enterokinase to 10 c.c. of water and filtering through a Berkefeld filter.

The following digestion mixtures were made:—

P + 5 c.c. A + 2 c.c. water + toluol.

An equal weight of Congo red fibrin was added to each. After four hours' incubation at the laboratory temperature "A" showed no alteration in colour and "B" was pink. After 12 hours "A" was very slightly coloured and "B" was well coloured. Control experiments showed that the digestion in "A" was the same as if the secretin had not been added.

If the secretin acted on the enterokinase then the resulting filtrate (that is solution A) should digest at the same rate as solution B + secretin, which is not the case. Solution A contains apparently no secretin, it has been separated out by the Berkefeld as we have shown already: it appears to contain unaltered enterokinase.

It is of course well known that contact of secretin with an emulsion of cells from the small intestine brings about slow destruction of the hormone, but this change does not in any way affect the enterokinase since the activity of the latter remains unaltered. These experiments disprove any supposition of the secretin inducing its beneficial action on digestion by acting on enterokinase.

Secretin, then, acts upon certain dead granules in the cell, and we have obtained no evidence to show that it influences the general protoplasm.

The secretin might conceivably act either by chemical combination or after the fashion of a ferment, in which case a small amount of secretin should suffice to activate an indefinite amount of pancreatic
emulsion. Experiments were therefore undertaken to determine whether there is a stochiometric relationship between the quantity of secretin added to the emulsion and the resulting tryptic activity of the mixture.

Exp. 5. Pancreas and enterokinase prepared from the sheep, secretin from the dog. Mixtures were made up to 4 c.c., toluol and gelatine tubes being added as usual. The average digestion at each end of the tubes was measured after 15½ hours at room-temperature. The secretin was made comparatively dilute.

| 1 c.c. P | — | Water in each | 3 mm. |
| 1 c.c. P+1 c.c. S | — | case up to | 3 mm. |
| 1 c.c. P+1 c.c. Ek | — | 4 c.c. | 6 mm. |
| 1 c.c. P+1 c.c. Ek+0.1 c.c. S | 7.5 mm. |
| 1 c.c. P+1 c.c. Ek+0.4 c.c. S | 8 mm. |
| 1 c.c. P+1 c.c. Ek+0.8 c.c. S | 9.6 mm. |
| 1 c.c. P+1 c.c. Ek+1 c.c. S | 9.5 mm. |

Symbols as before.

In this experiment no precaution was taken when making the emulsion to exclude those portions of the pancreas which border on the intestine and this may account for the tryptic activity in the emulsion of pancreas alone.

The results show that the quantity of trypsin in the mixture, as measured by the amount of digestion, increases with the addition of secretin until a maximum is obtained. It has been found in other experiments that the addition of still further quantities of secretin produces either no effect or a slight inhibition of the activity. This experiment agrees with the results already shown that pancreatic emulsion destroys a certain definite amount of secretin.

It might be urged that the increase in tryptic activity in these experiments caused by the presence of secretin was due to the salts which are necessarily present in the secretin solution, for it is well recognised that electrolytes exert a great influence on ferment action. We have proved that this is not the explanation of the phenomenon. Two series of experiments were performed, the one in which the emulsions were made in physiological salt solution, the other in which distilled water was used, and to one of the latter flasks the residue obtained on evaporating and igniting some of the secretin solution was added. In neither class was the result different from those already quoted.

In view of these results it does not seem probable that the presence of calcium or other salts in the secretin solution exert any effect in

1 The pancreas and secretin were the same as those used in the Exp. illustrated in Fig. 2, by diluting with an equal volume of water.
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activating the pancreatic emulsion. Since Delezenne\textsuperscript{1} and more recently Zunz\textsuperscript{2}, however, have shown that calcium has the power of activating pancreatic juice, experiments were performed in which the digestion was accomplished in an excess of sodium oxalate thus ensuring the absence of ionic calcium.

Exp. 6. The details are the same as those in Exp. 2 above, except that the pancreatic emulsion contains 1% of sodium oxalate.

<table>
<thead>
<tr>
<th>Total digestion in mm.</th>
<th>Colouration with Congo red fibrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 c.c. P + 2 c.c. Ek + 2 c.c. H$_2$O</td>
<td>4.8</td>
</tr>
<tr>
<td>4 c.c. P + 2 c.c. S + 2 c.c. H$_2$O</td>
<td>22</td>
</tr>
<tr>
<td>4 c.c. P + 2 c.c. S + 2 c.c. Ek</td>
<td>30</td>
</tr>
</tbody>
</table>

(Symbols as in previous experiments.)

It is obvious then that the increased activity is not the result of calcium, since the effect is obtained equally well in the absence of the calcium ion.

Observations were next made on the other enzymes of the pancreas—the amylopsin and steapsin.

Exp. 7. In each of three flasks 5 c.c. of an emulsion of pancreas and 50 c.c. of well-boiled starch paste were placed. To two of these, 5 c.c. of neutralised secretin were added and to the third 5 c.c. water. One of the flasks containing secretin was heated to boiling point for a few minutes and used as a control. Toluol was added as an antiseptic and the vessels were corked and incubated at 37° C. for 15 hours. At the end of this time 50 c.c. alcohol were added to each to inhibit further change and the mixtures were left to settle for 24 hours. The supernatant fluid was then removed and evaporated on the water-bath. The residue and precipitate in the flask were repeatedly boiled with alcohol, filtered and evaporated. The alcohol-free filtrates were extracted several times with water and made up to 100 c.c. 50 c.c. were used for the determination of sugar by estimating the reducing power by Pfüger's method\textsuperscript{3}.

\[ Cu_{2}O \quad \text{in gr.} \quad \text{Equivalent dextrose} \]

\[ \begin{align*}
A. & \quad 100 \text{ c.c. St} + 5 \text{ c.c. P + 5 c.c. H}_{2}\text{O} & 0.0679 & 0.0253 \\
B. & \quad 100 \text{ c.c. St} + 5 \text{ c.c. P + 5 c.c. S (boiled as control)} & 0.0660 & 0.0243 \\
C. & \quad 100 \text{ c.c. St} + 5 \text{ c.c. P + 5 c.c. S} & 0.1012 & 0.0397 \\
\end{align*} \]

A - B = 1 mgr. \quad C - B = 15.4 mg.

The effect here is very considerable.

Another experiment was performed in which the alcoholic extracts were precipitated with phosphotungstic acid in order to remove reducing extractives, before being finally made up to known volume for estimation.


\textsuperscript{3} Arch. f. d. ges. Physiol. XVIII. p. 99. 1908.
Exp. 3. Same pancreas and secretin employed as were used for Exp. 3. 100 c.c. starch, 5 c.c. P, and 5 c.c. toluol used; they were incubated for 40 hours at 38° C. 50 c.c. were treated with 100 c.c. alcohol and the sugar extracted as before. The extracts were precipitated with phosphotungstic acid (which of itself does not reduce Fehling's solution), and were made up to 50 c.c. 25 c.c. were taken for each estimation of sugar.

\[
\begin{array}{ccc}
100 \text{ c.c. } \text{St} + 5 \text{ c.c. P} + 5 \text{ c.c. } \text{H}_2\text{O} & \text{Cu}_2\text{O} \text{ in gr.} & 0.1975 \\
100 \text{ c.c. } \text{St} + 5 \text{ c.c. P} + 5 \text{ c.c. S (boiled)} & \text{lost} & - \\
100 \text{ c.c. } \text{St} + 5 \text{ c.c. P} + 5 \text{ c.c. S} & (a) 0.2698 & 0.114 \\
& (b) 0.2715 & -
\end{array}
\]

In this experiment no special care was taken to obtain secretin-free pancreas, but in spite of this the action of secretin is well defined. The results show that secretin affects the precursor of amylase in the same way as it does the precursor of the proteolytic enzyme.

A qualitative experiment dealing with the action of secretin on the lipase of the pancreas may also be mentioned. An emulsion of pancreas was prepared and to it a few drops of \( \frac{N}{10} \) NaOH and some phenol-phthalein were added. Equal portions of this mixture were taken and mixed with 0.5 c.c. ethyl butyrate; to one tube carefully neutralised secretin was added and to the other an equal quantity of water, both were left at room-temperature. The red colour in the tube containing the secretin was discharged in one hour whereas the colour in the other was unaltered after 24 hours. Lowenhart\(^1\) and others have shown that bile salts influence the action of lipases on fats and many esters. Donath\(^2\) has recently shown that when an emulsion of pancreatin is deficient in or contains no lipase, the precursor of this ferment may be activated by the addition of cholic acid and that the activity of the solution within certain limits varies with the amount of acid added. Ordinary preparations of secretin contain varying quantities of bile salts and cholic acid so that for this experiment the secretin was specially prepared from dog's duodenal mucous membrane, which had been extracted in an extraction apparatus with hot alcohol and with ether. No reaction for bile salts could be obtained from this preparation.

The experiments given above, which may be regarded as typical of what occurs when proper precautions are taken, show conclusively that secretin acts upon the proferments of the pancreas, increasing their digestive power.

\(^1\) Journ. of Biol. Chem. ii. p. 391.

\(^2\) Hofmeister's Beitritte, x. 390. 1908.
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In considering the nature of this action it might be urged that the emulsions contain living intact cells and that the secretin stimulates them in some way, causing them to give up their contained zymogen. This is not the interpretation, since microscopical examination of the sediment obtained by centrifuging the emulsions shows that unbroken cells are absent or extremely rare. Further, the effect is produced with emulsions which before the addition of secretin have stood overnight with chloroform and toluol, a procedure which would effectually destroy still living cells. The quantitative relations obtaining between the amount of secretin added, and the digestive activity, force us to the conclusion that no hypotheses involving "vital" activity can account for the facts.

Before leaving this section it is necessary to point out that the addition of moderate quantities of secretin to pancreatic juice with or without enterokinase does not influence digestion; large amounts retard digestion.

DISCUSSION OF RESULTS.

From the facts recorded a clear idea is obtained of the mode of action of secretin. It was shown first that secretin combines quantitatively with some constituent of the pancreas, and as the result of this chemical change its characteristic features are lost. The secretin does not act upon the enterokinase. Secondly, it was shown that secretin exerts a profound influence on pancreatic enzyme action; that as secretin is added to an emulsion of pancreas and enterokinase the activity of the enzymes is increased until a maximum is reached after which further additions exert either no effect or slightly diminish the activity. Further, the amount of secretin necessary is proportional to the amount of zymogen present, i.e. double the amount of emulsion, and the quantity of secretin necessary to obtain the optimum activity must also be doubled. These facts must be interpreted as indicating that secretin acts upon and probably combines with the zymogens in the pancreas, and that the freshly formed substances are secreted from the cell. Accordingly the trypsinogen in the pancreatic juice is not the same as the zymogen in the pancreatic cell since the latter must be combined with secretin before it becomes trypsinogen; hence it is necessary to refer to the proteoclastic zymogen in the pancreas as protrypsinogen. Thus the series of changes resulting in the production of trypsin may be stated as follows:—
HCl × Prosecretin = Secretin,
Secretin × Protrypsinogen = Trypsinogen,
Trypsinogen × Enterokinase = Trypsin.

These experiments do not necessarily show the manner in which vegetable drugs produce a specific action, but rather the way in which physiological activity is normally induced. Adrenalin appears to exert its action in a somewhat similar manner to secretin—it ceases to exist as such when it induces physiological activity. Thus if adrenalin is perfused through the pulmonary vessels it is destroyed slowly and no effect is produced on the vessels, but if it is perfused through splanchnic vessels it is rapidly destroyed and the vessels become contracted.

Secretin acts mainly on the pancreas and hence it is of no great importance in what part of the body it is set free into the circulation, for eventually it will find its way to the pancreas and set free trypsinogen. But if judging by analogy we assume that all tissues are activated by the liberation of hormones such liberation into the general circulation would not be efficient. If for example muscle is caused to contract by the intervention of a hormone it is essential that this hormone should be set free at the spot at which it is to act, or it would affect other muscle, and if this suggestion is found to be supported by the discovery of other necessary hormones the development of the intricate system of nerve distribution assumes a new significance. In this connection attention might be drawn to the action of some of the purin derivatives which specially excite muscle contraction and which are produced in the muscle as the result of nerve stimulation.

Having shown that with two of the natural hormones the induction of physiological activity results in the destruction of the exciting substance, and in the case of secretin that this is accompanied by combination with certain chemical constituents of the cell, it becomes necessary to enquire whether this is the way in which vegetable drugs exert their specific action.

THE MODE OF ACTION OF STRYCHNINE.

Ehrlich held the view that alkaloids being foreign to the animal body were not capable of combining with protoplasm to form such stable compounds as those formed by toxins, but recently he lays stress

1 Elliot. This Journal, xxxii. p. 449. 1905.
2 Slade. Ibid. xxxv. p. 163. 1907.
upon the view that drugs which specifically affect certain tissues are bound to the protoplasmic molecule by certain atomic groupings, which he distinguishes from toxin-receptors by the term "chemo-receptors". The belief in the existence of receptors is based upon experiment: if to a solution of toxin, receptors suitable for anchoring it are added, the resulting mixture is innocuous. The validity of these experiments is not criticised here, although their significance has been challenged by Metchnikoff and others, but since they are accepted by Ehrlich and his school and are generally regarded as one of the foundations of the side-chain hypothesis, it is legitimate to extend this type of experiment to drugs. Those who uphold Ehrlich's view however admit that toxin may be fixed in other cells besides those on which its activity is most manifest. Thus the cells of the liver and spleen besides those of the brain and spinal cord may fix tetanus toxin.

Strychnine like tetanus toxin has a specific action on the cells of the spinal cord, and several attempts have been made to prove, by methods similar to those adopted by Wassermann for tetanus toxin, that strychnine acts by combining with the spinal cord or with one or other of its constituents. Widal and Nobécourt showed that when a solution of strychnine was added to an emulsion of brain, liver, or kidney, and injected into animals the toxicity of the strychnine was weakened. Von Brünnner also comes to the same conclusion and supports the side-chain hypothesis for the action of strychnine. Thoinot and Brouardel mixed strychnine with charcoal, talcum and starch and on injection have observed a diminution in the effect of the strychnine. The experiments of von Czyhlarz and Donath, who attempt to prove the fixation of strychnine by the tissues, are of little significance to us since their object was to show that the tissues of the limb fixed the strychnine, that is that the fixation was not necessarily specific. The interpretation which they give to their experiments, namely that the limb tissues can fix strychnine, has been shown by Meltzer and Langmann7 to be open to serious objections.

Still more recently Sano8 has returned to the attack; he says that

1 The Harben Lectures, p. 83. 1907.
3 La Semaine Méd. 1898, p. 83.
4 Fortsch. de Medizin, 1899, p. 7.
5 La Semaine Méd. 1898, p. 140.
7 Journ. of Medical Research, ix. p. 19. 1903.
8 Pflüger's Arch. cxx. p. 367. 1907. Ibid. cxxiv. pp. 369 and 381. 1908.
strychnine loses its effect when added to an emulsion of spinal cord and injected into frogs. He determined the amount of strychnine free to act in his emulsions by injecting into frogs and examining the reflexes. Sano concludes that strychnine is especially neutralised by the white substance of the cord, less strongly by the anterior portion of the gray matter, and still less by the posterior.

Strychnine mixed with an emulsion of cord shows no evidence of having entered into chemical combination since it can still be separated from the mixture by means of the ordinary solvents for alkaloids. In this respect then it behaves quite differently from tetanus toxin. Furthermore all these observations which we have mentioned can be more readily explained in another way than by supposing that the strychnine enters into some form of combination with the spinal cord.

If a lethal dose of strychnine is mixed with a solution of gum or with milk or even a suspension of chalk and injected into an animal, a diminution of the strychnine toxicity is observed, and this diminution is quite comparable with that produced when it is mixed with the emulsion of cord. We have performed a considerable number of experiments which have convinced us that emulsions of spinal cord added to solutions of strychnine do not interfere with the specific effect of the alkaloid except by delaying its absorption. If the minimal lethal dose of strychnine for subcutaneous injection in an animal is mixed with a solution of gum or an emulsion composed of the complete spinal cord of an adult rabbit, and is injected subcutaneously into that animal, it is seriously affected but does not die: if however a dose slightly in excess of the minimum lethal be given, death always ensues. Any tissue emulsion is found to have the same type of effect as nerve emulsion, and the more colloidal in nature the mixture the greater the delay in absorption of the strychnine, and therefore the less its toxicity. Spinal cord emulsion is in no way specific in neutralising the effect of strychnine.

Similar effects to these can be obtained with curare, if a dose of curare sufficient to produce motor paralysis be mixed with an emulsion of spinal cord and injected into a pithed frog the paralysis may never occur, not because the curare is destroyed or combined, since it retains its properties if the fluid is filtered, but on account of delayed absorption. Perhaps however the importance of delayed absorption is best appreciated by injecting a minimal lethal dose of strychnine subcutaneously into two animals as far as possible under the same conditions; in the one case let the strychnine be dissolved in the minimal amount of water and in the other in a large excess of water. Although the
amount of strychnine in the two cases is the same, the animal with the larger injection of water is very much less affected than its fellow. All these results tend to prove that nerve tissues have no power of combining with the strychnine, but that emulsions of spinal cord act by delaying absorption. Thus when the crucial experiment of the side-chain hypothesis is applied to vegetable drugs, and we have also performed a number of experiments with cocaine and morphine, it yields negative results so that no support is found for the conception of the existence of chemo-receptors for these bodies. It has been urged that the chemical combination is of such a delicate nature that the methods we have employed cannot be admitted: to which it might be replied, then why are they admitted in the case of toxins? But even allowing that the experiments are not valid, the surmise that drugs act by chemical combination with living protoplasm is likewise totally unsupported by direct evidence.

If drugs do not act by combining chemically with the protoplasmic molecule, how do they produce their effects? The specific effects of drugs are strictly comparable with normal physiological actions such as may be produced by electrical stimulation of nerves. For example, there is no inherent difference between the action of muscarine on the heart on the one hand, and electrical excitation of the vagus nerve on the other. So similar are the two effects that it is not unwarrantable, in the absence of any evidence to the contrary, to assume that they are brought about in the same way. If it is permissible to argue from analogy there is reason in the suggestion that excitation of a nerve induces the local liberation of a hormone which causes specific activity by combination with some constituent of the end organ, muscle or gland. If this be true of electrical stimulation it may be true also of drugs that they act by causing a liberation of the specific hormone, or in the case of paralytic agents preventing such liberation, and that they themselves, like enzymes and catalytic agents, take no part of a chemical nature in the ultimate changes. In any case the evidence at present available lends no support to the chemo-receptor hypothesis.

It is suggested therefore that the ordinary galenical drugs act quite differently from secretin and adrenalin. The latter are destroyed while inducing physiological activity and probably enter into chemical combination with some constituent of the tissues on which they act, the former do not form chemical compounds in the cell.

1 It is proposed to offer direct evidence on this point in a future communication.
It is well known that certain vegetable alkaloids which induce specific effects may be partly destroyed in the animal body, and further that by habituation the capacity of the tissues for destroying the drug may be increased, the condition of tolerance resulting. The destruction which takes place under these conditions is quite different from that of secretin and adrenalin; the hormones disappear with the production of physiological activity, i.e., they combine as soon as they reach the tissue activated, whereas the vegetable alkaloids are very slowly destroyed, and the destruction is not coincident with physiological activity. Evidence has recently been obtained that the destruction of such alkaloids as morphine and nicotine\footnote{Unpublished work from the Pharmacological Laboratory, Cambridge.} in tolerant animals is the result of enzymic activity.

It is true also that some drugs combine chemically with organic constituents of the body, and in so doing lose their specific action. Thus the important drug salicylic acid combines in the body to the extent of about 50\% with glycocoll, and the resulting salicyluric acid is innocuous, and possesses none of the specific therapeutic effects of salicylic acid. Similar examples are found in phenol and indol which combine with sulphuric acid, and camphor and its analogues which combine with glycuronic acid. In none of these cases does the combination bring about physiological activity, it is only a means by which the organism diminishes the toxicity and facilitates the excretion of the drug.

Conclusions.

(1) Enzymes exist in the pancreas as precursors—protrypsinogen, proamylopsis, prosteapsin. Secretin combines chemically with the precursor and liberates thezymogen in the case of trypsin, and the active enzymes in the other cases: these are secreted in the pancreatic juice.

(2) We have failed to obtain evidence that vegetable alkaloids exerting a specific effect in the body act by combining with chemo-receptor substances.

(3) It is suggested that physiological activity of muscle and gland is due in all cases to the liberation of specific hormones which combine with a receptor substance.

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