
Physiology. — "*Investigations into the internal secretion of the pancreas*". By N. WATERMAN M. D. (Communicated by Prof. C. A. PEKELHARING).

(Communicated in the meeting of February 22, 1913).

In the following paper we shall attempt to summarize some series of experiments in connection with the internal secretion of the pancreas, which, though highly probable, is still a matter of conjecture.

1st *Series* (*A*).

Upon evidence with which we need not here concern ourselves, it has been concluded that the pancreas subserves the carbohydrate metabolism through some product of internal secretion. This being admitted it is quite natural that we should be induced to ascertain whether stimulants that increase the activity of this organ, also exert an action on the carbohydrates of the organism.

Among the agents that considerably promote the function of the pancreas we selected the most potent, viz. secretin, discovered by BAYLISS and STARLING. Our experiments showed results that gave rise to the question whether we could trace any influence of secretin on the amount of sugar in the blood. The fluctuations in this amount were taken as an index of the changes in the carbohydrate metabolism as the latter manifest themselves in the former.

Experiments.

1. *Preparation of the secretin.*

Dogs, kindly procured by the "Society for the prevention of cruelty

to animals" were, immediately after being killed, deprived of the duodemum and the proximal part of the jejunum; the mucous membrane of these organs was rubbed up in the ordinary way and mixed with 0.45% HCl, macerated for half an hour, then boiled, neutralised with sodium and finally acidulated with some drops of acetic acid.

2. *The laboratory animal.*

My first experiments were made on rabbits. I used this animal because its amount of blood-sugar both in normal and abnormal condition was pretty well known to me from earlier researches, in which also the technique of the operation (ligature of the carotid), presented hardly any difficulty. According to the size of the animal from 8—15 c.c. of secretin were given intravenously (per auricular vein); at different intervals of time after the injection the amount of sugar in the blood was estimated.

3. *Estimation of blood-sugar.*

The estimation was performed by cautious titration after FEHLING. Beforehand the Fehling-liquid had been accurately tested on invert-sugar. Of the copper test solution 10 c.c. were diluted to 100 c.c. and the reducing power of the blood-sugar solution was repeatedly tested on 5 cc., until the liquid being passed through a wet double filter, gave no longer a red discoloration with an acetic acid solution of potassium ferrocyanid.

4. *Removal of proteins.*

Proteins were separated from the blood after the old method of BERNARD (Na_2SO_4 etc.).

The following table shows the results obtained in this manner:

TABLE I.

Number	Date	Secretin	Blood-sugar	Remarks
1	Dec. 1911	12 cc	after 45 min. 0.14 %	
2		12 „	„ 60 „ 0.082 „	
3		14 „	„ 90 „ 0.077 „	
4		15 „	„ 120 „ 0.032 „	
5		12 „	„ 120 „ 0.087 „	
6		8 „	„ 115 „ 0.071 „	With absolute alcohol substances lowering the bloodpressure were extracted from the mucous membrane.
7		10 „	„ 195 „ 0.078 „	
8		15 „	„ 120 „ 0.076 „	

1*

The following values obtained with normal animals without injection of secretin are given for purposes of comparison:

TABLE II.

Number	Date	Secretin	Bloodsugar	Remarks
9	27 Nov. 1911	—	0.14 %	—
10	1 Dec. „	—	0.11 „	—
11	3 Dec. „	—	0.091 „	—

In some other experiments the effect of secretin on the amount of sugar in the blood was determined while the animal was subjected to the action of *levo-rotatory* suprarenin (HÖCHST).

It is well known, that suprarenin has the property of producing an excessive formation of sugar in the blood by splitting the glycogen in the liver and of exciting glucosuria. By extensive experimentation it has already been pointed out that the rise of the sugar-content in the blood of a rabbit, injected subcutaneously with $^1/_2$ mgr. of suprarenin, amounts to about 100 %, i.e. the percentage content of the blood-sugar rises from ± 0.12 % to 0.25—0.30 % in $1^1/_2$ hour.

The following table illustrates the results obtained, after I had given the animal secretin intravenously five minutes before a subcutaneous injection of $^1/_2$ mgr. of levorotatory suprarenin.

TABLE III.

Number	Date	Secretin	Suprarenin	Bloodsugar	Urine in 24 hours
12	Jan. '12	11 cc	0.5 mg.	90 min. after secr. 0.205 %	58 cc ad 0.3%
13	„ „	16 „	„ „	„ „ „ „ 0.151 „	120 „ „ 0 „
14	„ „	13 „	„ „	„ „ „ „ 0.181 „	60 „ „ 0.6 „

In the next series I extracted the distal portion of the ileum instead of the duodenum and the proximal portion of the jejunum and tried to detect whether this extract, which should be devoid of secretin, none the less keeps down the percentage of glucose in the blood, as well by itself as when acting concomitantly with suprarenin. The results of four experiments, carried out in this way, implied that this was altogether out of the question.

From the foregoing experiments (18) we arrive at the following conclusions:

Injection of secretin in most cases lowers the amount of sugar in the blood, the average fall being 30 %. This faculty of secretin asserts itself even in counteraction to suprarenin, which of itself produces an increment of 100 % at the end of $1^1/_2$ hours, but only 50 % under the given circumstances.

However, glucosuria appeared in all cases but one. That the results communicated here are due to the sole action of secretin has been distinctly shown by the experiments, which yielded negative results with injection of other intestinal extracts.

These experimental investigations led me to endeavour to find out whether an internal secretum of the pancreas could actually be elicited and whether injection of secretin excited its activity.

Second series (B).

It has often struck me, that only few workers have strenuously exerted themselves to obtain in vivo the internal secretum of the pancreas, putting aside of course BIEDL's [1]) and DE MEYER's [2]) experimentation. The former examined the anti-glucosurian action of the lymph from the thoracic duct. The latter the effect of pancreatic extracts or of fluids which had been made to pass through the pancreas artificially, on the glycogenesis of the liver. These researches constitute the best work that has been done in this field, as the other investigations were restricted to biological experiments with blood from the pancreatic vein, especially as regards its fermentative properties.

Our purpose was rather to furnish large quantities of blood from the pancreatic-duodenal vein for further experimentation on carbohydrate metabolism. Let it be stated beforehand, that in large dogs such quantities are not difficult to procure from this vein.

Method. In large dogs of 10—15 K.G. an incision is made through the abdominal wall parallel to the arcus costalis to get access to the pancreas, which is drawn out by the hook-shaped finger and immediately wrapped in cloths soaked with a warm physiological common salt solution.

It is then easy to find the pancreatic-duodenal vein, which is ligatured as near the trunk of the portal vein as possible. Subsequently a cannula is inserted, which cannot always be managed. In case of failure the vein is simply cut through. At the same time

[1]) BIEDL. Wiener Kl. Wochensch. 1907.

[2]) DE MEYER. Archiv. Intern. de Phys. 1910.

20—40 cc. of secretin is injected intravenously or subcutaneously. Most times the blood is discharged rapidly, sometimes it issues in a jet, the blood flow can still be accelerated by kneading the pancreas with the full hand and by massage. In this way 80—250 cc. of blood can be collected in about $^3/_4$ hour. In some of our experiments the blood was allowed to coagulate and we used the serum separated in the clotting. Most often, however the blood was defibrinated at once.

With this blood or with this serum the following experiments were performed:

Specimens of this blood varying from 5— 20 cc. were used upon rabbits[1]) subcutaneously, in which also $^1/_2$ mgr. of suprarenin[2]) was injected. After the lapse of $1^1/_2$ hours the blood-sugar percentage was determined. The experiments however varied in some details according to the various treatment of the pancreas blood that had been administered. Usually three specimens of dog's blood were given separately to three rabbits of equal weight. The specimens, however, were all different inasmuch as either the blood had flowed from the vein prior to the injection of secretin, or this had occurred concomitantly with the injection, or the blood had been heated for half an hour at 56° C., or finally the blood had been liberated from its proteins by addition of three times its volume of alcohol, the alcoholic filtrate having been evaporated down to its original volume.

We subjoin a table illustrating the results of some of our most successful experiments.

By the side of these experiments others were made upon rabbits with homologous serum. It being, however, very difficult to draw an adequate quantity of blood from the rabbit's pancreatic vein, we have to content ourselves with reporting only one satisfactory test. From the pancreatic vein of a rabbit, weighing 3 K.G., 8 cc. of blood was obtained. Four cc. of serum were separated. Subsequently 12 cc. of secretin were injected per vena auricularis; and 15 cc. of blood, furnishing 7 cc. of serum, were drawn from the pancreatic vein.

Next day 2 rabbits, weighing 1300 grms were injected intravenously. The first got the 4 cc. of serum of the first sample and immediately after 1 mgr. of levorotatory suprarenin. The percentage of sugar in the blood was 0.297 after $1^1/_2$ hours. The urine passed in 24 hrs. was 15 cc. with 0,6 % of sugar.

[1]) In order to avoid complications the foreign serum was injected only once into each rabbit.

[2]) In this experiment as in all others only fresh suprarenin was used from vials that had just been opened.

TABEL IV

Weight	Pancreas blood injected	Suprarenin	Blood-sugar	Urine in 24 hours	Details
K.G.	5 cc serum	0.5 m.g.	0.195 %	40 cc à 6½% glucose	Serum from pancreas acted upon by secretin.
„	5 „ (blood)	0.5 „	0.183 „	not collected	Blood from pancreas before injection of secretin.
„	4.5 „ „	0.5 „	0.156 „	contains much glucose	Blood from pancreas acted upon by secretin.
5 „	10 „ „	0.5 „	0.134 „	40 cc à 0.12% glucose	Blood under the influence of secretin.
„	10 „ „	0.5 „	0.189 „	presence of glucose	Blood from pancreas before injection of secretin.
„	10 „ „	0.5 „	0.177 „	40 cc +	Blood from pancreas acted upon by secretin.
„	10 „ serum	0.5 „	0.153 „	> 30 cc à 0.3%	Blood under the influence of secretin.
„	20 „ blood	0.5 „	0.185 „	50 cc à 1.8%	Blood under the influence of secretin.
5 „	20 „ heated for half an hour at 56° C.	0.5 „	0.145 „	30 cc a 1.68%	Blood under the influence of secretin. Next day hemoglobinuria appears.
„	23 cc. liberated from protein with 3 × its volume of alcohol 96% and evaporated down to its original volume	0.5 „	0.151 „	70 cc à 1.5%	Blood under the influence of secretin. No derangements.

The 2nd rabbit got 2 cc. of the serum obtained under the influence of secretin, also intravenously. A few bubbles of air, injected along with the substance were responsible for a slight degree of dyspnoea in the animal, which, however, disappears within a quarter of an hour; 5 minutes after this intravenous injection ½ mgr. of levoratatory suprarenin was administered subcutaneously. At the end of 1½ hrs. the amount of sugar in the blood is 0,178 %. After 24 hrs the quantity of urine is 55 cc. with traces of sugar.

From the experimental evidence above stated it would seem permissible to conclude with a high degree of probability:

1. that together with the blood, a substance is secreted through the pancreatic vein, which, in rabbits, is capable of neutra-

lising to some extent the action of levorotory suprarenin on the sugar-content in the blood, but that, on the other hand, this does not apply to the glucosuria, which, judging from the values above mentioned, has a tendency to be relatively higher with a reduced than with an increased amount of blood sugar.

2. that injection of secretin seems to stimulate the activity of the blood from the pancreatic vein.

3. that, to judge by some experiments, heretofore presented, (others were made which shall be dealt with in a later publication) the substance producing this action is thermostabile and is soluble in 75 % alcohol.

Third Series (C.).

In connection with the preceding experiments the question suggested itself as to whether the antagonism between suprarenin and the blood from the pancreatic vein was to be looked upon as the outcome of the action of a substance, which exalts the glycogenesis or of one inhibiting the glycogenolysis.

Concerning this point I purpose to publish some data afterwards. For the present I feel called upon to bring forward two experiments demonstrating the action of the blood from the pancreas on the glycogen in the liver. It may be taken for granted that with a dog in nitrogenous equilibrium every derangement in the process of carbohydrate metabolism will also affect the N.metabolism in one way or other. Two successful experiments are available (others were performed, which, however, did not always yield conclusive evidence) actually pointing to a change in the N-metabolism. It was also evident from all these tests that glucosuria is likely to appear, and that in some cases traces of sugar occurred after the injection of blood from the pancreatic vein

We present the data of the tests reported here in the following table: (see table page 9)

N°. 36. The urine of a dog in N. equilibrium and weighing $5^1/_2$ KG. was tested for sugar every day, invariably with a negative result. On the 23d of Dec. 30 cc. of blood from the pancreatic vein was injected. Within 24 hrs two portions of urine were collected (80 cc. reduced FEHLING and yielded a positive fermentation test; 60 cc. reduced and yielded a positive fermentation test).

N°. 37. 30 cc. of blood from the pancreatic vein, liberated from protein by 96 cc. of alcohol and evaporated down to its original

TABLE V. (Dog 7 KG.)

Number	Date	Diet	N. per 24 hrs	Urine	Remarks
34	11 June '12	100 gr. horse-flesh 100 „ milk 50 „ bread	12 a.m. / 11 p.m. 1.695	30	
			11 p.m. / 12 a.m. 3.729	75	
			Total 5.424	105	
	12 „ „	150 gr. horse-flesh 100 „ milk 50 „ bread	12 a.m. / 11 p.m. 2.31	33	
			11 p.m. / 12 a.m. 4.22	110	
			Total 6.53	143	
	13 „ „	same diet	12 a.m. / 11 p.m. 4.068	60	
			11 p.m. / 12 a.m. 2.214	49	
			samen 6.282	109	
	14 „ „	„ „	12 a.m. / 11 p.m. 4.013	48	At 8 p.m. a subcutaneous injection of 20 c.c. of blood from the pancreatic vein under the influence of secretin.
			11 p.m. / 12 a.m. 4.208	78	
			Total 8.221	126	
	15 „ „	„ „	12 a.m. / 11 p.m. 3.706	44	
			11 p.m. / 12 a.m. 4.407	75	
			Total 8.113	119	
	16 „ „	„ „	12 a.m. / 11 p.m. 1.864	20	
			11 p.m. / 22 a.m. 3.254	50	
			Total 5.118	70	
	17 „ „	„ „	12 a.m. / 11 p.m. 2.612	34	At 11 p.m. the first hour of a new period of 24 hrs 20 c.c. of dog's blood from the jugular vein is used subcutaneously. Two weeks before part of the pancreas had been extirpated without evoking diabetes.
			11 p.m. / 12 a.m. 1.85	21	
			Total 4.462	55	
	18 „ „	„ „	12 a.m. / 11 p.m. 3.823	47	
			p.m. / 12 a.m. 3.084	78	
			Total 6.907	125	

TABLE VI. Dog 11 K.G.

Nº.	Date	Diet	N. per 24 uur	Urine	Remarks
35	30 Aug.	300 gr. horseflesh 100 » milk 50 » bread	1st portion 4.236 gr. 2d » 5.92 » Total 10.156 gr	117 150 267	
	31 »	same diet	1st portion 4.143 gr. 2d » 7.167 » Total 11.31 gr.	138 210 348	At 9 p. m. an injection of 20 cc. of blood from the jugular vein of a normal dog.
	1 Sept.	»	1st portion 6.7 gr. 2d » 3.791 » Total 10.491 gr.	204 112 316	
	2 »	»	1st port. } urine 2d » } only once 10.27 gr.	 262	
	3 »	»	1st port. } urine 2d » } only once Total 7.494 gr.	 202	
	4 »	»	1st port. 11.289 gr. 2d » 4.845 » Total 16.134 gr.	280 114 394	At 8 p. m. a subc. inj. of 28 cc. of blood from the pancreatic vein under the influence of secretin. *The urine of the 2d portion reduces* FEHLINGS *solution.*
	5 »	»	1st port. 2d » 4.173 gr. 4.173 gr.	 94 94	Outside circumstances are responsible for the fact that the N-determination occurred only once in 24 hrs, so that the average of the two values should be taken.
		average = 9.7	1st portion 9.18 gr. 2d » 6.283 » Total 15.463 gr.	225 154 379	
	6				
	7		in 24 hrs 9.395 gr.	238	

volume, was injected into a dog, weighing 4 KG. Next day the 1st portion of urine gave a positive reduction with FEHLING, fermentation test positive. The third day no reduction.

These experiments show, that the blood from the pancreatic vein at first exalts the N. metabolism and then lowers it again. Under these circumstances it would not be proper to dwell any longer on these contrary effects, as we are unable to account for them satisfactorily.

It also appears that the blood favours the permeability of the kidney tubules for sugar.

This experience throws light on the contrary results in our preceding series of experiments in which the glucosuria was disproportionate to the sugar content in the blood. It would seem then that the secretum of the pancreas subserves the function of the kidney as well as the glycogenesis of the liver. Indeed, this has also been admitted by DE MEYER and others, however, on a different basis and just the other way about. DE MEYER held that the internal secretum of the pancreas *prevented* sugar from passing through the kidney. It must be borne in mind, however, that DE MEYER experimented with artificial renal circulations, which readily lead to paradoxical phenomena.

Summary.

1. Secretin decreases the amount of sugar in the blood.

2. The blood that has passed through the pancreas, is capable of neutralising the action of levorotatory suprarenin on the sugar content in the blood. In this study no effort has been made to detect whether this action is due to a diminished splitting of the glycogen in the liver or perhaps to an increase in the formation of glycogen. Presumably the activity of this blood (internal secretum) is furthered by the injection of secretin. The secretum is thermostable and is soluble in alcohol. These results are perfectly concordant with DE MEIJER's experience.

3. Our experience that the secretum favours the permeability of the kidney for glucose instead of lessening it, clashes with the results of DE MEIJER's investigations.

Rotterdam, Dec. 1912.

Botany. — "*On the nucleolus and karyokinesis in Zygnema*". By Prof. C. VAN WISSELINGH. (Communicated by Prof. J. W. MOLL).

(Communicated in the meeting of April 25, 1913).

Whilst *Spirogyra* has very often been used for the investigation of the nucleus and nuclear division, *Zygnema* has so far as I know, up to the present only been studied for this purpose by two investigators. It should be no cause for surprise that the latter alga has