VAGUS CONTROL OF PANCREATIC FUNCTION

EXPERIMENTAL INSULIN RESISTANCE

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INTRODUCTORY

The first contribution to the knowledge of the nervous control of glycemia was made by Claude Bernard who showed that hyperglycemia follows piquer of the floor of the fourth ventricle. It is not improbable that in such a procedure the vagus nerve would be affected due to close proximity of its nucleus to the site of injury. Pflüger (22) found that destruction of the nerves leading to the pancreas produces the same effect as complete pancreatectomy. This author also gave numerous examples of hyperglycemia caused by curare, atropin and other substances affecting the nervous system. Kuntz (17) quotes De Corral and states that in 1918 it was finally proved that a marked hypoglycemic effect is produced in dogs after stimulation of the vagus below the diaphragm, the nerves supplying the liver having been destroyed surgically. While after denervation of the liver alone hyperglycemia results. The vagus control of pancreatic function in carbohydrate utilization has been reported since by numerous investigators (5).

The interdependence between the secretory work of the pancreas and the pancreatic control of carbohydrate metabolism described by one of us (6, 7), has been confirmed by several investigators. Many years ago it was established, mostly by works of Heidenhain and Pavlov (21), that the vagus is the chief regulator of the external secretion of the pancreas.

During the last few years several cases of insulin resistance have been recorded in clinical literature. Those observed by
Lawrence (18), Arany (4) and Root (24) may be mentioned. Root describes a case of bronze diabetes which had the following symptoms: Absence of free HCl in the stomach, blood sugar between 0.230 and 0.300 per cent, marked acidosis and other symptoms common in diabetes. On post-mortem examination he found the following changes in the pancreas: parenchyma replaced chiefly by fat and fibrous tissue and the presence of several "nearly normal" islands of Langerhands. This case is discussed, in some detail, because it demonstrates that despite the presence of islet tissue the administrations of insulin were futile.

As has been stated already by one of us (7), the hypoglycemic property of the three polypeptides, secretin, insulin and peptone, is probably due to their stimulation of the secretory function of the digestive glands, particularly the pancreas. It is probable then that substances which inhibit or suppress the secretion of pancreatic juice may cause a hyperglycemia that would not yield to insulin. Curare has been reported to induce a rise in blood sugar which is not alleviated by insulin (23). It is quite logical to assume that in curare poisoning the secretion of pancreatic juice would be suppressed, because of the paralysis of the motor-nerve endings.

In regard to the effect of atropin, literary data are somewhat confusing. While some authors state that it is strongly antagonistic to the action of insulin (5, 9) on blood sugar and causes insulin-resistant hyperglycemia, others claim that atropin does not affect the hypoglycemic property of insulin.

Eppinger and Hess (11) were the first to emphasize the fact that hypoglycemia is a constant finding in vagotonia. In thyroid over-activity the high blood sugar level, increased cardiac rate and some other symptoms suggest impaired vagus function.

**EXPERIMENTAL**

The following substances were used either alone or in combination for the purpose of anesthesia: Chloroform U. S. P.

1 While pure secretin has not yet been isolated as a chemical entity there are reasons to believe that it is of the nature of a polypeptide.
(Merck and Company); chloretone 40 per cent solution in 40 per cent alcohol (Park Davis and Company); nembutal "844" (Abbott Laboratories). Chloroform was used only in a very few cases and was administered through inhalation. For the others chloretone and nembutal were given intraperitoneally. Chloro-
tone dosages were computed from the potency standard of 1 cc. per 1000 gram body weight; with nembutal the doses were estimated from the standard of 1 cc. per 5 pounds live weight (mammals); cold-blooded animals received 1.7 cc. per 1,000 grams. In a few cases overdosages of these drugs were employed to determine their effect on blood sugar.

Four varieties of insulin, two commercial and two crystalline preparations were used. Commercial products were Lilly's A-183 and Sears' 5140D and 5141D. Dr. John J. Abel of Johns Hopkins University and Dr. Myron Heyn of Frederick Stearns Company kindly supplied the crystalline insulin. It is our pleasant duty to express our gratitude to them. The crystalline insulin was prepared for injections by dissolving the crystals in 0.8 per cent saline acidified with hydrochloric acid to 0.02 per cent titratable acidity. Doses varied from 1.2 to 40 units for single administrations. The maximum amount given to one animal was 201 units. The crystalline insulin was assumed to have a potency of 24 units in 1 mgm. of the solid substance. The mode of administration was varied to suit the individual cases, being given by subcutaneous, intramuscular, intravenous and intracardiac injections (turtles).

Other drugs and chemicals used were atropin sulfate and pilocarpine hydrochloride in 1 per cent solutions, the former in dosages from 0.8 to 5 mgm., the latter in 0.1 to 3 mgm. Also curare (Dr. Theod. Schulhardt, Germany, 1908), the lethal dose of which was found by us to be 1 mgm. for albino rats (40 to 50 grams), death coming in four to five minutes.

Hydrochloric acid 0.2 was administered into the stomach to produce secretin action.

Subjects used in these experiments included both cold- and warm-blooded animals. Among the former were a few fish (Micropterus salmoides) and a large number of turtles of the
following species: Chrysemys marginata, Emys blandingii, Chelydra serpentina and others. Among the warm-blooded animals were pigeons, albino rats and rabbits, guinea pigs and dogs. Two observations were made upon man. In the majority of cases the subjects were fasted for fifteen hours or more. Observations were made for periods of a few hours to several days.

In order to avoid the hyperglycemic effects of anesthetic substances doses were carefully computed for each specific case. The figures were based upon a number of control experiments.

Some blood samples were obtained from the radial vein (dog and man) and others by intracardiac puncture. Usually the specimens were analyzed immediately, sodium fluoride and thymol being added to arrest glycolysis. The estimations of blood sugar were made by the method of Folin-Wu.

Despite the contention of London (Pflueger's Arch. f. d. ges. Physiol., 1931, ccxxviii, 542–547), "peripheral" blood can be satisfactorily employed for the study of carbohydrate metabolism; since the results obtained were parallel with those determined on blood taken from the "central" circulation (heart).

In all sacrificial experiments a careful study of the pancreas and digestive system was made in order to avoid misinterpretation of results in cases presenting pathological changes. Approximately 150 experiments were carried out.

An understanding of glycemia must be based upon a knowledge of the fasting levels and periodical fluctuations which occur in the various individuals and species.

The following are the initial blood sugar estimations: fish (fasting), 0.050 per cent; turtles (fasting), 0.030 to 0.070 per cent; pigeons 0.200 to 0.250 per cent; albino rats, rabbits and guinea pigs, 0.100 to 0.200 per cent; man and dogs (fasting), 0.070 to 0.100 per cent. Our data on pigeons, which seem to be high, are in accordance with previously reported findings of other investigators (8, 25).

Table 1 indicates the uniformity of results obtained in both cold- and warm-blooded animals after insulin administration. In the former class it is obvious that the hypoglycemic action of insulin is slightly delayed though none the less marked. A
TABLE 1  
Action of insulin on glycemia

<table>
<thead>
<tr>
<th>DATE</th>
<th>SUBJECT</th>
<th>WEIGHT</th>
<th>ANESTHESIA</th>
<th>DRUGS USED</th>
<th>INTERVENTION</th>
<th>BLOOD SUGAR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>grams</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/13/31</td>
<td>Chrysemys marginata</td>
<td>350</td>
<td>0.7 cc. chloretone</td>
<td>10 units Lilly's insulin</td>
<td>None</td>
<td>0.045</td>
</tr>
<tr>
<td>11/2/31</td>
<td>Chrysemys marginata</td>
<td>415</td>
<td>0.7 cc. chloretone</td>
<td>5 units Lilly's insulin</td>
<td>None</td>
<td>0.045</td>
</tr>
<tr>
<td>11/2/31</td>
<td>Emys. blandingii</td>
<td>1,500</td>
<td>1.5 cc. chloretone</td>
<td>5 units Lilly's insulin</td>
<td>None</td>
<td>0.048</td>
</tr>
<tr>
<td>12/8/31</td>
<td>Chrysemys marginata</td>
<td>344</td>
<td>0.6 cc. nembutal</td>
<td>10 units Lilly's insulin</td>
<td>None*</td>
<td>0.100</td>
</tr>
<tr>
<td>1/10/32</td>
<td>Chelydra serpentina</td>
<td>2,600</td>
<td>1.4 cc. nembutal</td>
<td>24 units F. S. insulin</td>
<td>T.31°-32°, fasting 3 days</td>
<td>0.227</td>
</tr>
<tr>
<td>2/29/32</td>
<td>Chelydra serpentina</td>
<td>3,000</td>
<td>4.5 cc. chloretone; 2.75 cc. nembutal</td>
<td>6.6 units F. S. insulin</td>
<td>30 cc. 0.2 per cent HCl into stomach†</td>
<td>0.516</td>
</tr>
<tr>
<td>11/25/31</td>
<td>Guinea pig</td>
<td>691</td>
<td>0.2 cc. nembutal</td>
<td>5 units Lilly's insulin</td>
<td>None</td>
<td>0.128</td>
</tr>
<tr>
<td>12/3/31</td>
<td>Albino rabbit</td>
<td>1,800</td>
<td>0.5 cc. nembutal</td>
<td>3.3 units Lilly's insulin</td>
<td>None</td>
<td>0.154</td>
</tr>
<tr>
<td>1/4/32</td>
<td>Albino rat</td>
<td>287</td>
<td>0.05 cc. nembutal</td>
<td>1.2 units Heyn's crystalline insulin</td>
<td>None</td>
<td>0.100</td>
</tr>
<tr>
<td>3/10/32</td>
<td>Dog N 145</td>
<td>15,000</td>
<td>None</td>
<td>8 units Abel's crystalline insulin</td>
<td>None</td>
<td>0.075</td>
</tr>
<tr>
<td>3/8/32</td>
<td>Dog N 150</td>
<td>13,800</td>
<td>None</td>
<td>8 units Abel's crystalline insulin</td>
<td>None</td>
<td>0.073</td>
</tr>
</tbody>
</table>

A few selected experiments.
* Pancreatic pathology.
† Pancreatic pathology and hyperglycemia caused by anesthesia.
remarkably low level of 0.007 per cent was observed in a comatose turtle. Among the warm-blooded subjects, the lowest values obtained were: 0.036 per cent (guinea pigs and dogs), and 0.026 per cent (rabbit, died in coma).

Various means were employed in producing a vagus block; most frequently section on cardia and administration of atropin were used. In all cases the results were parallel. In no subject

| Table 2 |

**Insulin miosis**

Of a series of observations the above show the constricting action of insulin on the pupil of the eye. This effect is of relatively short duration. Atropin prevents insulin miosis. Dog N 148 had a tumor of the thyroid gland.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SUBJECT</th>
<th>DRUG USED</th>
<th>BEFORE</th>
<th>1 hour</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/27/31</td>
<td>Dog N 148</td>
<td>5 units Lilly's insulin</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7.5</td>
</tr>
<tr>
<td>12/3/31</td>
<td>Rabbit</td>
<td>3.3 units, same</td>
<td>6</td>
<td>2.5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/22/31</td>
<td>Pigeon</td>
<td>10 units, same</td>
<td>4</td>
<td>3.5</td>
<td>4</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>2/9/32</td>
<td>Man E. B.</td>
<td>0.44 mgm. Abel's crystalline insulin</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/15/32</td>
<td>Dog &quot;Y&quot;</td>
<td>10 units Stearns' insulin</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3/6/32</td>
<td>Man E. B.</td>
<td>0.5 mgm. Abel's crystalline insulin; 0.8 mgm. atropine sulphate</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/8/32</td>
<td>Dog N 145</td>
<td>0.33 mgm. Abel's crystalline insulin</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Convulsions.*

was insulin able to produce hypoglycemia of any marked degree after blockage of the vagus innervation. The fact that these results were obtained in such a wide range of animal life and occurred with such a degree of uniformity makes them indeed significant.

Constriction of pupils, decrease in heart rate, blood pressure and body temperature, and secretion of gastric juice all follow insulin administration. After adequate dosage of atropin these changes
<table>
<thead>
<tr>
<th>DATE</th>
<th>SUBJECT</th>
<th>WEIGHT</th>
<th>ANESTHESIA</th>
<th>DRUGS USED</th>
<th>INTERVENTION</th>
<th>BEFORE</th>
<th>AFTER 2 hours</th>
<th>AFTER 4 hours</th>
<th>AFTER 6 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/13/31</td>
<td>Chrysemys marginata</td>
<td>420</td>
<td>None</td>
<td>None</td>
<td>Decapitation</td>
<td>0.050</td>
<td>0.169</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/9/31</td>
<td>Chrysemys marginata</td>
<td>700</td>
<td>None</td>
<td>None</td>
<td>Decapitation</td>
<td>0.060</td>
<td>0.100</td>
<td>0.160</td>
<td></td>
</tr>
<tr>
<td>11/2/31</td>
<td>Chrysemys marginata</td>
<td>365</td>
<td>None</td>
<td>5 units Lilly’s insulin</td>
<td>Decapitation</td>
<td>0.052</td>
<td></td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>10/12/31</td>
<td>Emys. blandingii</td>
<td>650</td>
<td>1 cc. chloretone</td>
<td>None</td>
<td>Section of both vagi</td>
<td>0.050</td>
<td>0.097</td>
<td>0.130</td>
<td>0.155</td>
</tr>
<tr>
<td>11/19/31</td>
<td>Chelydra serpentina</td>
<td>2,500</td>
<td>3 cc. chloretone</td>
<td>20 units Lilly’s insulin</td>
<td>Section of both vagi</td>
<td>0.069</td>
<td>0.127</td>
<td>0.155</td>
<td></td>
</tr>
<tr>
<td>12/3/31</td>
<td>Albino rabbit</td>
<td>1,800</td>
<td>8 cc. nembutal</td>
<td>3.3 units Lilly’s insulin</td>
<td>Section of both vagi</td>
<td>0.152</td>
<td>0.168</td>
<td>0.130</td>
<td></td>
</tr>
<tr>
<td>11/25/31</td>
<td>Guinea pig</td>
<td>711</td>
<td>2 cc. nembutal</td>
<td>5 units Lilly’s insulin, 5 mgm. atropin</td>
<td>None</td>
<td>0.130</td>
<td>0.145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/4/32</td>
<td>Albino rat</td>
<td>1 cc. nembutal</td>
<td>1.2 units Heyn’s crystalline insulin, 0.1 mgm. atropin</td>
<td>None</td>
<td>0.196</td>
<td>0.266</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/29/31</td>
<td>Dog N 145</td>
<td>14,000</td>
<td>None</td>
<td>5 units Lilly’s insulin, 2 mgm. atropin</td>
<td>None</td>
<td>0.098</td>
<td>0.200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/8/32</td>
<td>Dog N 145</td>
<td>14,000</td>
<td>None</td>
<td>5.3 units Heyn’s crystalline insulin, 3 mgm. atropin</td>
<td>None</td>
<td>0.086</td>
<td>0.087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/7/32</td>
<td>Dog N 150</td>
<td>13,000</td>
<td>None</td>
<td>6 units Abel’s crystalline insulin, 3 mgm. atropin</td>
<td>None</td>
<td>0.066</td>
<td>0.066</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/8/32</td>
<td>Dog N 150</td>
<td>13,000</td>
<td>None</td>
<td>6.4 units Abel’s crystalline insulin, 4 mgm. atropin</td>
<td>None</td>
<td>0.070</td>
<td>0.065</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3**

*Vagus control of glycemia*

This table shows the varying degrees of hyperglycemia resulting from decapitation, section of vagi or adequate doses of atropin. This hyperglycemia is resistant to insulin due to impaired vagus innervation.
do not occur. It was interesting to note that injection of 0.8 mgm. atropin and 12 units insulin in the human subject produced practically no change in the size of the pupils. Obviously that amount of atropin alone would have resulted in marked mydriasis.

Most investigators agree that insulin has a definitely hypotensive action. This they attribute, however, to impurities and not to the substance itself. Our experiments show that crystalline insulin is as strongly hypotensive as the commercial products used by other observers.

Insulin causes a marked bradycardia, resembling pilocarpine in this respect. Such action was most easily observed in turtles as the heart was exposed to view. In cases of relatively high insulin dosage, complete cessation of cardiac activity was noted.

**Graph 1. Vagotonic Nature of Insulin Hypoglycemia (Dog N 150)**

These data are obtained in the same animal (dog N 150). The first curve (February 5, 1932) demonstrates hypoglycemic action of insulin. Six and four tenths units of Abel's crystalline insulin injected. The blood sugar was reduced from 77 to 46 or 31 mgm. The second curve (April 8, 1932) shows that lowering of blood sugar by insulin depends on the functional activity of vagus. Six and four tenths units of same insulin and 4 mgm. atropin sulfate injected. The blood sugar was reduced from 70 to 62 or 9 mgm. (very slight reduction).
The pulse rate in the human subject also showed undeniable slowing, and was accompanied by a decrease in body temperature of one degree. This change was felt both by the subject and by the observer.

**DISCUSSION**

Physiologists and clinicians alike agree that stimulation of the vagus (clinically known as vagotonia) results in constriction of the pupils, secretion of gastric juice, decrease in heart rate, blood pressure and body temperature. Many observers have also reported hypoglycemia to be a constant finding.

It follows logically therefore that the persistent appearance of this group of symptoms following each administration of insulin should link the two together in the relation of cause and effect. Suppression of these signs by atropin would place it in the category of insulin antagonists.

Other investigators have recorded their observations on insulin miosis (16). Our results are merely confirmatory. The study of the secretion of gastric juice in response to insulin is more fragmentary. Experiments made in connection with this report showed such a secretion to be parallel in all respects to one which would follow direct vagus stimulation. High acidity and pepsin content are indications of its nature. The further evidence that atropin suppresses the secretagogue action of insulin on gastric glands makes the vagus control quite clear.

Some authors have stated that insulin dilates the pupil and increases the cardiac rate and blood pressure. Careful investigation of the subject, as well as the literature pertaining to it, bring us to the conclusion that such contentions are unwarranted. All of the observations we have made are distinctly contrary to such views.

Although denied by some, the existence of fluctuations of the normal blood sugar values has been established by others who have made very extensive studies of this problem. According to Stammers (25), “the amount varies in different individuals and in the same individual at different times” (human subjects). These variations, which are periodic in nature, have been observed
by one of us to occur also in dogs (6, 7). Further evidence in support of this fact is offered by Agren, Wilander and Jorpes (3) who found that cyclic changes in the glycogen content of the liver of rats and mice occur "which are to a large extent independent of the intake of food."

As measured in decrease in glycemia, the response of both cold- and warm-blooded animals to insulin administration was remarkably uniform. The degree of hypoglycemia depended, of course, upon the amount of the substance given. This action, however, was either wholly or nearly completely suppressed by interruption of the vagus innervation. Section of the nerves on the cardia or adequate doses of atropin had identical effects in rendering the subjects insulin resistant.

The foregoing observations are presented as evidence of the vagonotonic nature of insulin. Conceeding this point brings us to the consideration that insulin must stimulate the external secretion of the pancreas. Several of our observations give direct proof for such a statement. The occurrence of appreciable amounts of pancreatic juice in the gastro-intestinal tracts of both cold- and warm-blooded animals in hypoglycemic coma cannot be ignored. Also the disappearance or marked reduction in the number of zymogen granules in pancreatic acini after insulin administration lends further support to this view.

Doubtless the secretion of gastric juice always causes an increased alkalinity of the blood and thus facilitates the utilization of carbohydrates. This must be especially true following the secretion of the highly acid gastric juice evoked by insulin. Two factors in the stimulation of pancreatic juice are secretin action and direct stimulation of the secretory nerve. The constant occurrence of a lowered blood sugar level after such action links them closely together. This sequence of events explains logically the time elements apparent in the production of the hypoglycemia following insulin administration.

These studies were undertaken in the hope that some contribution might be made to the knowledge of insulin action and the nature of insulin resistance.
SUMMARY

1. The hypoglycemic effect of insulin occurs uniformly in a wide variety of vertebrates. Its appearance is evident although delayed in cold-blooded animals. This is in accord with the observations previously reported by other investigators.

2. Crystalline insulin has strong vagatonic properties.

3. Symptoms of vagus stimulation by insulin are: increased respiratory rate, diminished cardiac rate, blood pressure and body temperature.

4. Pure crystalline insulin, as shown by Abel and co-workers, is a monophasic hypoglycemic drug. It is probable, however, that some commercial insulins contain as an impurity a substance with hyperglycemic properties.

5. Cases having pancreatic pathology show a diminished response to the hypoglycemic action of insulin.

6. Vagus blockage by surgery or adequate dosage of atropin renders the subjects unreactive to insulin.

7. Confirming observations of Jorns it was found that atropin and insulin exert antagonistic actions on the pupil of the eye.

8. Insulin stimulates the secretory function of digestive glands.

REFERENCES