INSULIN SECRETION BY THE PANCREAS OF THE CHICK EMBRYO

L. G. Leibson, Z. P. Zheludkova, and L. I. Chilingaryan

Laboratory for the Study of Endocrine Function and Metabolism
(Head, Doctor of Biological Sciences L. G. Leibson)
I. M. Sechenov Institute of Evolutionary Physiology, AN SSSR, Leningrad
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A number of morphological studies have been made of the development of the islets of Langerhans in the pancreas of chick embryos [4,7,8,10,13], which however give no clear idea of its secretory function. Also, as with other endocrine glands, to determine the part played by the islets in regulating the metabolism of the embryo, accurate information is required as to when the onset of their hormonal activity occurs. The most convincing evidence of secretory function of any gland is the identification of its hormone in the blood. Practically no observations of this kind have been made on the endocrine glands of embryos. In particular, no information is available on the time at which insulin occurs in the blood of chick embryos.

METHOD

For the detection of insulin we used a modern method—that of the isolated rat diaphragm [11]. Although the reliability for a quantitative estimation is somewhat doubtful, it was completely satisfactory for our purpose.

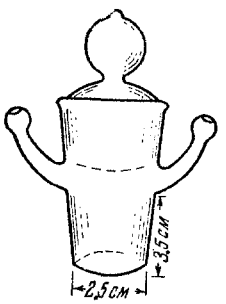

Fig. 1. Vessels for testing the absorption of glucose by the isolated rat diaphragm.

We used the method of Vallance-Owen and Hurlock [12]. Rats, which were mostly males weighing 120-150 g, were starved for 16-18 hours, and then decapitated. The diaphragm was then excised with a small scalpel, and immediately placed in saline cooled on ice [5], containing no glucose, and prepared with twice-distilled water. The diaphragm was lightly rinsed in the solution, and then laid out on filter paper placed on a glass plate and moistened with the same cooled solution. The posterior part of the diaphragm and its fibrous portion were removed, and the remainder was cut as nearly as possible into two equal portions. Both halves were immersed in cooled saline and left on ice for 20-25 minutes. They were then removed, lightly dried with filter paper, and placed in two special vessels (Fig. 1), one of which contained saline mixed with 300 mg% glucose (2 ml), and the other the same solution to which insulin or plasma had been added (see below).

In the control experiments, both vessels were filled with saline and glucose. The vessels were plugged with a ground stopper; rubber tubes were connected to their outlets, and one served to connect the vessels to each other. They were then fixed to a stand made from the wooden portion of a Warburg manometer. The stand was fixed to a support, and a gaseous mixture of 95% oxygen with 5% carbon dioxide was passed through both vessels for 5 minutes (Fig. 2). The vessels were then closed by plugging the rubber tubes with glass stoppers, and were placed in the water bath of Warburg's apparatus at a temperature of 37.3-37.4°, where they were shaken at a rate of 110-130 strokes per minute. After 90 minutes, the vessels were removed from the water bath, dried with filter paper, and their contents estimated for sugar by the Hagedorn-Jensen method. Measurements were also made of the sugar content of the original solutions. The determinations were made in three parallel tests. After each experiment, the vessels were boiled in strong alkali, and treated with a chromic acid mixture.

Blood was taken from the embryos by a method described previously [3]. Heparin was added, and in order to maintain conditions constant, it was added in each case, irrespective of the age of the embryo and of the ability

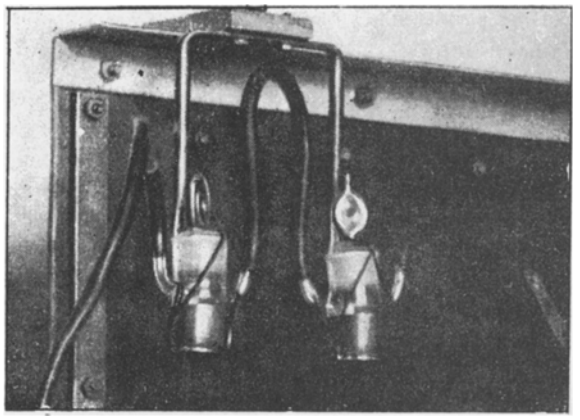

Fig. 2. Vessels fixed to a holder while the gaseous mixture is passed through. The gaseous mixture from the container passes successively through both vessels. The outlet tube is taken to a vessel containing water, which serves to verify that the gas is passing.

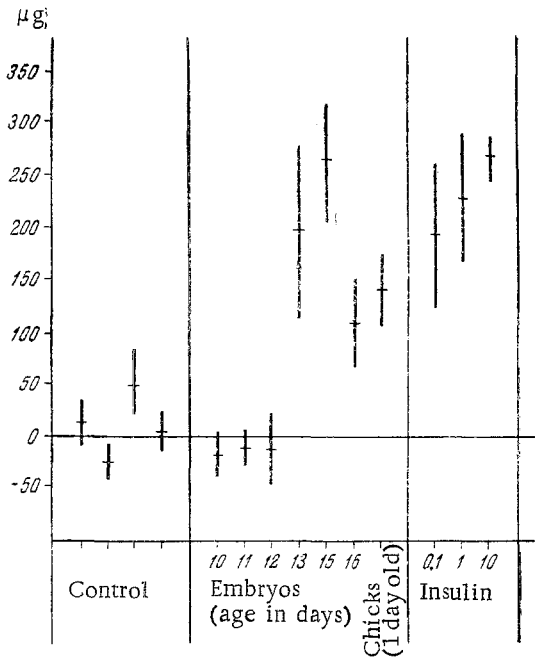

Fig. 3. Increased absorption of glucose by the diaphragm caused by insulin. Each vertical line represents the result of one experiment on 5-6 rats. The cross strokes in between the vertical lines represent the difference, in μg per 10 mg dry weight of diaphragm, occurring in 90 min between the amount of glucose absorbed by the half-diaphragm placed in the test solution and the other half placed in a pure saline solution of glucose. In the control experiments, both halves of the diaphragm were placed in the pure saline-glucose solution. The age of the embryos in days is indicated. One-day-old chicks were used. The insulin concentration is expressed in microunits per ml of the solution.

of the blood to clot. Blood samples collected from embryos of a given day of incubation were mixed; the plasma was separated by centrifugation, and diluted 10 times in saline containing 300 mg% of glucose.

In the experiments with insulin, we used a dry purified preparation, prepared from the insulin produced by the meat plant, and which contained 21 units per mg. It was diluted in a 0.6% solution of acetic acid, so that each ml contained 20 units. It was kept in this state in a refrigerator for two to three weeks. Before the experiment, this solution was diluted with saline containing 300 mg% of glucose; it was first diluted 20 times, and then, according to the concentration required, the dilution was made 10^5-10^8 times. Thus, the ultimate solution contained from 0.01 to 10 microunits per ml.

All the results were expressed as μg of glucose absorbed in 90 minutes per 10 mg of dry weight of diaphragm. Drying was continued at 105° until the weight remained constant, for which two hours was usually required. To determine the effect of plasma or insulin, the absorption in the solution under test was compared with the control solution, i. e., a comparison was made of the absorption of glucose by the two halves of the diaphragm. From the results obtained the mean difference and the standard error were calculated.

RESULTS

Of the large number of experiments which we performed we used only those of the last series, in which simultaneous measurements were made on six rats, or exceptionally on five.

The results are shown in Fig. 3.

They indicate that there is no insulin activity in the plasma of embryos less than 13 days old, and that it can be detected in embryos more than 13 days old. Evidently the pancreas begins to secrete hormone into the blood on the 13th day.

It is difficult to make a quantitative measurement of the insulin activity. Despite precautions, in the control experiments both halves of the diaphragm did not absorb the same amount of glucose. Although, in most cases, the divergence was insignificant, it was sometimes quite large. This effect would explain the quite large value of the standard error of the mean difference of the two absorptions.

In our experiments, we were unable to ensure that a single insulin or plasma solution should have the same effect in different rats. Consequently, in such experiments, the standard deviation of the mean was very high. The reason may have been the large variation in sensitivity of the rat to insulin. Unlike most other authors, we carried out the experiments on genetically inhomogeneous material. Possibly also, we may have failed to take account of some

undetermined experimental conditions which prevented us from obtaining uniform results. Therefore, at the present time, we have only an approximate knowledge of the activity of the plasma of chick embryos. When the plasma is diluted 10 times, its activity is approximately equal to 0.1-10 μunits/ml.

It should be noted that the activity of the plasma taken from 13-and 15-day-old embryos was greater than that obtained on later days. However, there is no reason to suppose that the effect is genuine, and not due to chance. A more accurate quantitative estimation of the insulin activity of chick embryos at different days of incubation must be made, and a great number of observations will be required.

We must also draw attention to the very great sensitivity of the rat diaphragm to insulin, as shown in our experiments. The average value of the excess absorption of glucose was much greater than that which has been reported by other workers. Thus, Wright [15], using an insulin concentration of 10 μ units/ml, found an excess absorption of glucose of 34 μg /10 mg/90 minutes; Vallance-Owen and Hurlock [12] obtained a value of 100 μg, and in our experiments the corresponding figure was 270 μ g/10 mg/90 minutes. The authors quoted give no figures for the effect of lower concentrations of insulin, and apparently there was no effect. However, we found a marked effect even at insulin concentrations as low as 0.1μ units/ml, and the effect did not disappear until the solution was diluted a further 10 times. It is not possible to explain the high sensitivity of the diaphragm, but the condition is favorable to the identification of insulin in blood.

Thus, our results indicate that in chick embryos insulin is secreted into the blood by the pancreas from the 13th day onwards.

This result is in line with the morphological descriptions of the islets of Langerhans [4,8], and agrees with the data on carbohydrate metabolism of the chick embryo. It is at the 13th day that appreciable amounts of glycogen begin to accumulate in the liver [1,6]. Long ago, Potvin and Aron [10] and later Needham [9] attempted to associate the formation of glycogen in the liver with the onset of pancreatic secretion, although later work showed that glycogen appears long before the islets of Langerhans begin to function. Possibly the onset of function is not associated with the appearance of glycogen in the liver, but with its rapid accumulation. This idea has been proposed by L. G. Leibson [1,2], Willier [14], and M. S. Mitskevich [4]. However, until now there were no facts to support it. The statement by Needham [9], based on a personal communication from Pecher and Hannon, that insulin may be detected in the embryo from the 11th day onwards, is contradicted by the fact that on the 11th day there is a reduction and not an increase of liver glycogen. Our results completely explain the sharp change in carbohydrate metabolism observed in chick embryos on the 13th day; we have been able to show that it is precisely at this stage that the pancreas begins to secrete insulin into the blood.

SUMMARY

Insulin activity of chick embryo plasma was determined by tests on an isolated rat diaphragm. The blood from several embryos was mixed; the plasma was separated by centrifugation, and was diluted 10 times with salt solution containing 300 mg% of glucose. The glucose absorption was expressed as micrograms per 10 mg (dry weight) of the diaphragm per 90 minutes. The insulin effect was measured by the difference between the amount of glucose absorbed by one of the halves of the diaphragm, immersed in a test solution, and the amount absorbed by the other half immersed in pure glucose solution. It was found that insulin did not appear in the plasma of the chick embryo until the 13th day. The insulin activity of plasma diluted 10 times was approximately 0.1-10 μ units per ml.

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