THE INSULIN CONTENT OF THE Pancreas IN CATTLE OF VARIOUS AGES

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In attempting to estimate the insulin content of pancreas, there are many factors to be considered. In the first place, the amount of insulin may vary with the species of animal from which the pancreas is obtained. For example, it is generally believed that, per gm. of tissue, fish pancreas is much richer in insulin than ox pancreas. Secondly, the insulin content of the pancreas may vary with the season of the year. It has been our experience, in using beef glands, that the best yields of insulin are obtained during the winter months. Thirdly, since insulin is readily destroyed by proteolytic enzymes, fresh pancreas should be a better source of insulin than material which has been kept for some time before extraction. However, one of the most important factors is the method of extraction and purification of the insulin. Jephcott (1) has shown that the concentration of alcohol, the quantity and kind of acid, and the temperature during extraction all have a bearing on the amount of insulin which may be obtained from ox pancreas. Another factor is the age of the animal from which the pancreas is obtained, and it is this problem that we have investigated in the present research. We have experimented with the pancreas of one species only, namely the ox. All the glands were collected at the same season of the year (January 23 to February 7). Further, the time elapsing between the killing of the animals and the extraction of the insulin was the same in all age groups. A standard method of extraction and purification of the insulin was used.

EXPERIMENTAL

In the present investigation an attempt was made to determine the insulin content of the pancreas of cattle of the following ages:
fetal calves under 5 months; fetal calves 5 to 7 months; calves 6 to 8 weeks (still being fed with milk); cattle approximately 2 years old; cows 9 years and older; pregnant cows 7 years and older. In all cases, with the exception of the pancreas of fetal calves, the same procedure was followed in obtaining the pancreas and in the purification of the insulin. The method for each age group was as follows: Within $\frac{3}{4}$ hour after the removal of the glands, or about 1$\frac{1}{2}$ hours after the death of the cattle, the pancreases of twelve to sixteen animals of the same age group were collected, minced, and thoroughly mixed. Three 25 gm. samples from each age group were weighed. To each of these, 100 cc. of extraction liquid were added. This liquid consisted of 750 cc. of absolute ethyl alcohol, 250 cc. of distilled water, and 15.0 cc. of concentrated hydrochloric acid. The insulin in each sample was extracted by shaking the acid-alcohol and pancreas for 2 hours at 37°. The residues were then removed by filtration through a double layer of cheese-cloth and extracted again with 100 cc. of the acidic ethyl alcohol for 1 hour. After the filtration of the second extractions, the first and second filtrates of each sample were combined and concentrated ammonium hydroxide added to each until the reaction was distinctly alkaline to litmus. The precipitates were centrifuged off and the volumes of the supernatant liquids measured. 10.0 cc. aliquots of these solutions were pipetted into three 50 cc. centrifuge tubes and the insulin in each was precipitated by adding 15 cc. of absolute ethyl alcohol and 25 cc. of ether. It was found from control experiments that this procedure precipitated the insulin quantitatively. The mixtures were placed in the refrigerator for 12 hours. They were then centrifuged and the precipitates were dried in vacuo. Each weighed approximately 15 mg. In view of the fact that a large number of samples were prepared in a comparatively short time, it was necessary to store some of the dried precipitates in vacuo for 3 or 4 weeks until each sample could be assayed. Preliminary experiments showed that this caused no loss of potency.

When working with the pancreases of fetal animals, it was necessary to vary the procedure slightly owing to the small size of the glands and the limited number which could be obtained at one time. In view of this, the three samples from each of these age groups were not extracted on one day, as described above for
the older animals. Thus, with the fetal pancreas, it was necessary
to adopt the following method. If, in the course of an hour's
killing, three or more fetal calves of approximately the same age
were obtained, the pancreatic glands were removed. These were
minced and processed as one lot. The minced glands were weighed
and the acid-alcohol added, 4.0 cc. for each gm. of pancreas. The
extraction and purification were then conducted in a manner simi-
lar to that described for pancreas from the older animals. As
occasion afforded, two additional lots of fetal pancreases of the
same age group were collected and processed by the same method.
The insulin precipitates obtained in each of these experiments were
assayed independently, as described below.

Method of Assay

The insulin content of each of the eighteen samples of dried
powder prepared by the process already described was determined
by the mouse method of assay (2) in the following manner. The
powder from each sample was dissolved in 40.0 cc. of isotonic saline
to which sufficient hydrochloric acid had been added to adjust the
reaction of the insulin solution to pH 2.5. An approximate indi-
cation of the activity of this solution was obtained by injecting
0.25 cc. quantities of it into twenty-five mice, and the convulsion
rate compared with that obtained when similar quantities of a
standard insulin solution were injected into the same number of
mice. From the result of this test, the solution of unknown activ-
ity was diluted so that in subsequent tests approximately the same
number of convulsions occurred among the mice injected with the
solution of unknown strength as among those injected with the
standard solution. To determine the potency of any sample, the
solution was assayed four times against a standard insulin. The
total number of mice used in such a test was 200. The average
value of the four assays is recorded in Table I, and is expressed as
units of insulin per gm. of pancreas. The average value of three
samples is taken as the insulin content of the pancreas in each age
group. This value is also incorporated in Table I.

DISCUSSION

The values in Table I show the comparative amounts of insulin
which can be extracted from the pancreas of cattle of various ages.
Since, in carrying out the experimental work, every effort was made to have only one variable, namely age, we feel that the results obtained give a fairly accurate indication of the relative insulin content of the pancreas of the various age groups. It is evident from Table I that fetal pancreas is very rich in insulin. This is particularly true for fetal calves under 5 months, those on which the growth of hair has not commenced. In this group it will be noticed that the yields of the three experiments vary considerably. This is not surprising when it is remembered that in the case of fetal pancreas the glands were collected on three different occasions. Thus an error of a few weeks in the estimation of the age of these calves would result in an inaccurate determination of the insulin content for the age group in question. If the average yield of insulin per gm. of pancreas in the various groups be plotted against age, it will be noticed that the yield of insulin decreases very rapidly with age, until about 7 months after birth. After this time, increasing age causes a much less marked decrease in the yield of insulin. It is interesting to recall that fetal pancreases were used as the source of insulin in the early work on the preparation of insulin by Banting and Best (3).

The large yields of insulin obtained from fetal pancreas are of interest when considered in conjunction with the enzyme content of fetal animals. Ibrahim (4) found, in the case of human embryos, that pepsin and trypsin could be demonstrated in the digestive juices at about the 4th month, and that erepsin was present at the 5th month. Sampson (5) showed that there was

<table>
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<th>Description of cattle</th>
<th>Results of biological assays (international units per gm. pancreas)</th>
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<tr>
<td></td>
<td>Sample 1</td>
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<tr>
<td>Fetal calves, under 5 mos.....</td>
<td>29.2</td>
</tr>
<tr>
<td>&quot;  &quot; 5-7 mos..................</td>
<td>23.2</td>
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<tr>
<td>&quot;  &quot;  &quot; (pregnant).........</td>
<td>2.0</td>
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no evidence of proteolytic activity in fetal pig pancreas until about half the gestation period had elapsed. Since insulin is destroyed by proteolytic enzymes, the low concentration of these enzymes in fetal animals may partially explain the large yields of insulin obtained from this pancreas. There are other explanations which might be advanced. The evidence of the transfer of an insulin-like material from fetal animals to the depancreatized mother is still controversial. Carlson and Drennan (6) found that if a bitch was depancreatized in the later stages of pregnancy, it showed no signs of diabetes. Removal of the pups by cesarean section, however, was followed by symptoms of diabetes. Similar findings were also reported by Aron, Stulz, and Simon (7). On the other hand, pregnancy did not decrease the insulin requirement of the depancreatized dogs observed in Macleod's laboratory (8), and most of the clinical evidence is in line with this finding. However, since there is a possibility of a mechanism providing for the transfer of an insulin-like substance between mother and fetus, we decided to determine whether or not the high insulin content of fetal pancreas was accompanied by a change in the amount of insulin contained in the pancreas of the mother. Our results with the pancreatic glands of pregnant cows over 7 years of age indicate that the insulin content of the pancreas of the mother is not affected by pregnancy. In this group the yields of insulin are slightly greater than for 10 year-old cows. This difference, we believe, is due to the difference in the age groups rather than to pregnancy.

SUMMARY

1. It has been shown that the amount of insulin which can be extracted per gm. of beef pancreas varies greatly with the age of the animal. Fetal beef pancreas is relatively rich in insulin.

2. In cows over 7 years of age, pregnancy would not appear to affect the amount of insulin in the pancreas.

3. An improved method for the preparation of experimental quantities of insulin from bovine pancreas has been described.

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BIBLIOGRAPHY

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