CXXXII. ZINC CONTENT OF BOVINE PANCREAS.

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IN a recent publication from these Laboratories, data were presented concerning the relative quantities of insulin which could be extracted from the pancreas of cows and calves of various age groups [Fisher and Scott, 1934]. It was found that the yield of insulin per kg. of young foetal pancreas was about twenty times greater than that of the pancreas of old cows. It has also been shown that certain metals have a very close association with insulin in that traces of zinc, nickel, cobalt or cadmium facilitated the crystallisation of this hormone [Scott, 1934]. Further, commercial preparations of insulin and also insulin crystals prepared by the brucine or saponin methods gave positive tests for zinc. These facts suggested that certain of the metals might be associated with insulin in the pancreas. If such were the case, it would be of interest to determine the quantity of each of these metals in bovine pancreas of various age groups and to correlate the values, if possible, with the amount of insulin found in the pancreas of similar age groups. An investigation of this problem is reported in the present paper.

Many workers have reported the occurrence of zinc in the pancreas of various species [Lutz, 1926; Delezenne, 1919]. The presence of cobalt and nickel is also the subject of other communications [Bertrand and Macheboeuf, 1926]. None of these researches however was performed with reference to the age of the animals from which the pancreas was obtained.

In the present work it was decided to determine the relative quantities of cobalt and nickel in various samples of pancreas by means of some of the well-known colour tests for these metals. Zinc could be determined either by the fluorescence method of Lutz or gravimetrically in the manner outlined by Delezenne. In view of the difficulty of securing a sufficient quantity of satisfactory stercobilin used in the former method, it was decided to follow the procedure described by Delezenne. Moreover, this method obviates the necessity of dry ashing—a feature which has often been criticised on the ground of the possible loss of zinc by volatilisation.

EXPERIMENTAL.

The pancreatic glands were removed from cattle of the following age groups: hairless foetal calves, under 5 months; haired foetal calves, 5–7 months; calves, 6–8 weeks; and cows, 9 years and over. In the older age groups, the glands from at least 10 animals were minced and an aliquot was removed for the investigation. For the foetal groups, it was necessary to collect the glands from a much larger number in order to obtain samples of sufficient size for the determinations. The glands in each group were minced, weighed and kept *in vacuo* over concentrated sulphuric acid for several days. After the tissue had been thoroughly dried, the glands were weighed again. For the determinations, 15 or 20 g. samples of this material were placed in 500 ml. Erlenmeyer flasks.

The procedure used in the estimation of the metallic content of each of these weighed samples was as follows. A solution of equal parts of concentrated sulphuric and nitric acids was added dropwise to the tissue, using about 1 ml. for each g. of tissue. The flask was shaken and very gently heated until copious fumes were formed. After cessation of fuming, the heating was resumed and concentrated nitric acid added dropwise. Vigorous heating and the addition of nitric acid were continued until a pale yellow solution was obtained. This was poured into a small porcelain evaporating dish and evaporated to dryness. Washings from the original flask were added from time to time. The dry white residue was intimately mixed with about 10 ml. of concentrated hydrochloric acid and then evaporated to dryness. The residue was again stirred with 10–15 ml. of concentrated hydrochloric acid and washed into a 15 ml. centrifuge tube. After centrifuging the supernatant liquid was measured and poured into a 50 ml. beaker. The spot tests described by Feigl [1931] for cobalt and nickel were conducted on this solution. For the former metal we used the various modifications of tests with α -nitroso- β -naphthol. Dimethylglyoxime tests described by Feigl were used for nickel. With all samples of pancreas used in this investigation, tests for cobalt and nickel were negative. As a control, drops of the various reagents were added to one drop of a solution containing 0.015 mg. of nickel per ml. or 0.030 mg. of cobalt per ml. One drop of such a standard solution contained sufficient nickel or cobalt to give a strongly positive test, either when the test was conducted directly upon the standard solution acidified with one drop of concentrated hydrochloric acid or when one drop of the standard was mixed with one drop of the unknown solution.

Upon completion of the spot tests for cobalt and nickel, the procedure for the determination of zinc was resumed. The unknown solution in the beaker was gently boiled until the volume had been decreased to about 5 ml. After adding an equal volume of water, the acid solution was poured slowly into 40 % of its volume of concentrated ammonium hydroxide—sufficient to render the mixture distinctly ammoniacal. About 3 ml. of a suspension of calcium hydroxide and a few drops of "superoxol" were added. After standing for a few hours, the mixture was centrifuged and the supernatant liquid decanted into a 200 ml. flask. The precipitate was dissolved in concentrated hydrochloric acid and reprecipitated with ammonium hydroxide. After centrifuging, the supernatant liquid was added to that from the first precipitation. This solution was boiled and a suspension of calcium hydroxide was added from time to time, until the evolution of ammonia could not be detected by moist litmus paper, even when the solution itself was distinctly alkaline. The mixture was centrifuged in a 50 ml. pointed centrifuge-tube and the precipitate washed with distilled water. The precipitate was then dissolved in hydrochloric acid, using a sufficient quantity to ensure that the final solution contained 5–10 % hydrochloric acid. The solution was saturated with hydrogen sulphide, the tube stoppered and kept overnight. After centrifuging, the supernatant liquid was transferred to a small porcelain dish and evaporated to dryness. The residue was stirred thoroughly with four 10 ml. quantities of a 10 % solution of ammonium acetate acidified with glacial acetic acid. The clear solution was then transferred to a 50 ml. pointed centrifuge-tube and the zinc precipitated by saturation with hydrogen sulphide. The tube was stoppered and kept for 3–4 days. The mixture was then centrifuged and the white precipitate of zinc sulphide dissolved in a few ml. of concentrated sulphuric acid. This solution was slowly evaporated to dryness on a weighed platinum dish. Strong heating was avoided in order to eliminate the possible decomposition of the zinc sulphate noted by Delezenne.

From the weight of the residue—zinc sulphate—the amount of zinc per kg. of pancreas was calculated. Similar determinations were carried out for each age group. The values are recorded in Table I.

Table I. *Zinc content of bovine pancreas.*

Description of animal	Pancreas		Zinc per kg. dry tissue mg.	Zinc per kg. wet tissue mg.	Insulin per kg. wet tissue* i.u.
	Wet weight g.	Dry weight g.			
Cows (9 years and over)	500	130	57	15	1,800
			80	21	
			60	16	
Calves (6–8 weeks)	200	56	90	25	11,400
			75	21	
Foetal calves (haired, 5–7 months)	370	94	55	14	23,000
			70	18	
			75	19	
Foetal calves (hairless, under 5 months)	155	33	153	33	33,200

* Fisher and Scott [1934].

DISCUSSION.

The zinc content of bovine pancreas, reported in Table I, shows no relation between the age of the cattle and the metallic content of the glands. The pancreas was found to contain about 20 mg. of zinc per kg. of fresh tissue. This value is in agreement with that reported by Delezenne. The fact that there is no simple relation between the zinc content and the amount of insulin which can be extracted from the glands is not surprising when it is remembered that crystalline zinc insulinate contains only 0.5 % zinc. There is thus in the pancreas many times the quantity of zinc necessary to account for the available insulin in the pancreas as the zinc salt.

The fact that the presence of nickel and cobalt was not detected in the pancreas of cattle of any age group may mean that only traces of these metals are present and that the amount is beyond the sensitivity of our method. Bertrand and Macheboeuf [1926] reported 0.715 mg. of nickel and 0.357 mg. of cobalt per kg. of dried ox pancreas. Although there is a wide difference between some of the values reported by them for ox pancreas, there is no significant difference between the quantity of these metals in ox pancreas and that found in calves' pancreas. Their values were obtained by comparing the weight or colour of the compounds which these metals form with dimethylglyoxime in their unknown solutions with the weight or colour formed by a known quantity of nickel or cobalt with the same reagent. They used samples of approximately 200 g. In our work it would be rather difficult to obtain sufficient foetal pancreas to conduct duplicate determinations with this size of sample. In view of the fact that, in the present investigation, tests for these metals were positive when a drop of the standard nickel or cobalt solution was added to the unknown solution, our inability to detect these metals in the pancreas was not due to the presence of an interfering substance.

SUMMARY.

Bovine pancreas was found to contain about 20 mg. of zinc per kg. of fresh tissue, irrespective of the age of the animal from which the gland was obtained. The quantity of insulin which can be extracted from bovine pancreas bears no simple relation to the amount of zinc contained in the glands. Cobalt and nickel were not detected in the pancreas.

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