INSULIN CONTENT OF PANCREAS FROM LAHORE SLAUGHTER HOUSES

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THE insulin content of pancreas of animals slaughtered in local slaughter houses has been studied with a view to examine the possibility of producing insulin locally—because of the difficulty in the availability of this essential product. It is well known that a number of factors such as the species of the animal, health, age, climate and season, affect the insulin content of the pancreas. No studies of the insulin content of locally available pancreas have, hitherto, been made in this country. These are of interest, even if the industrial production of insulin may not be economically feasible, for conditions of scarcity of this drug continue to occur periodically.

In Lahore there are two slaughter houses, one for beef and the other for mutton. In the beef slaughter house 80 to 100 animals are slaughtered five times a week, while in the mutton slaughter house 800 to 1200 animals are slaughtered in the same period. Cows, oxen, bulls and buffaloes are slaughtered in the beef slaughter house, while different species of goats and sheep are slaughtered in the mutton slaughter house.

The animals slaughtered in the beef slaughter house are such as have become otherwise uneconomical to maintain and are often old and in a poor state of nutrition. These are required by law to be over ten years of age or otherwise declared unfit for any other purpose. The animals slaughtered in the mutton slaughter house are generally young but often quite lean since they are driven from long distances to the city for slaughter. This long walk of a week or ten days with scarcity of food on the way make them physically frail at the time of slaughtering.

Pancreas of animals were collected under suitable conditions immediately after slaughtering and examined for insulin content. A number of relatively pure preparations of insulin were made from the extracts of the pancreas during the course of the work, but these were found to lose some of the insulin activity during the process. It was, therefore, found more convenient to assay suitable extracts directly without any elaborate treatment for purification. These were assayed using rabbits as experimental animals. The yield of insulin has been expressed in units per kilogram of pancreatic tissue.

Extract Preparation

Insulin extracts were prepared by the method of Hinkel and Maxwell.[1] The processing was done within an hour of the slaughter of the animals as otherwise the yield of insulin was found to be lowered even when the pancreas were rapidly chilled. This is in conformity with the earlier work.[2,3]

Five hundred grams of the defatted, minced pancreas were added to 1,500 ml. of 85% alcohol containing 25 ml. of phosphoric acid to give a pH 3.0. This was vigorously shaken mechanically for three hours, centrifuged and re-extracted under similar conditions. The combined extracts were made alkaline to pH 8.2 with NH_4OH, filtered and reacidified to pH 3.5 with H_2SO_4. After concentration in vacuo at 20 °C., to a volume of 500-600 ml. the residue was filtered off and the clear solution used for assay of insulin.

Methods for the standardization of the insulin extracts generally make use of mice or rabbits as experimental animals.[4] The mouse is easier to handle and has, therefore, replaced the rabbit in most laboratories but due to the non-availability of a suitable strain of white mice we used rabbits in this study. The method we followed is described by Marks.[5] For each test, eight rabbits were used. These were maintained on a diet of chick pea, green vegetables, and grass. Before the actual tests, the animals were made to fast for 20-24 hours. After each test they were given a rest of three to six days before the cross-over test. Blood was collected from the ear veins in oxalated capsules.

Reference Standard

Unfortunately at the time of these experiments we did not have a standard insulin preparation. We used for comparison a preparation of insulin manufactured by Eli Lilly and Co, Indianapolis, marked as containing 40 units per ml. We hoped to standardise this preparation against a proper standard at a later stage. This solution was diluted with

distilled water containing 0.3% cresol and 0.85% NaCl and pH adjusted to 2.5 so that the diluted solution contained one unit of insulin per ml. This was used as a standard for comparison.

The method of Hagedorn-Jensen[6] as adopted by Marks[4] was used for the estimation of blood sugar. Marks[4] had used 0.005 N sodium thiosulphate solution for titration, but we found it more convenient to use a solution of 0.001 N strength, which resulted in the greater accuracy of results in our hands. The method was standardised using a glucose solution of known strength.

Conclusions

A number of extracts were prepared in the manner described above both from beef and mutton pancreas and assayed. The results of ten representative samples are shown in the table I.

The results summarised in the above table show that insulin content of beef pancreas examined, range from 892-1002 units per kg. and that of mutton pancreas from 585-695 units per kg. These yields are much lower than those reported in the literature for the pancreas of normal animals of these species.

TABLE I

INSULIN CONTENT OF BEEF PANCREAS

No.	Weight of minced pancreas (g.)	Vol. of the final extract (ml.)	Pancreas extract: Dose per animal (ml.)	Pancreas extract: Total reduction in blood sugar (%)	Standard preparation: Dose per animal (units)	Standard preparation: Total reduction in blood sugar (%)	Response ratio	Potency of the sample with respect to standard (%)	Calculated potency of the extract (units/ml.)	Insulin content of the pancreas (units/kg.)
1.	500	535	1.0	66.90	0.5	246.10	27.2	33.07	0.1654	179
2.	500	630	1.0	297.71	0.5	242.26	122.88	141.58	0.7079	892
3.	500	675	0.5	202.08	0.5	261.05	77.4	70.9756	0.710	959
4.	500	480	0.5	256.12	0.5	248.98	102.86	104.4415	1.044	1002
5.	500	500	0.5	230.06	0.5	248.45	92.598	89.362	0.8936	894

INSULIN CONTENT OF MUTTON PANCREAS

1.	500	800	1.0	212.37	0.5	234.06	90.73	86.86	0.4343	695
2.	337	500	1.0	111.82	0.5	233.01	47.989	45.366	0.2268	337
3.	500	300	0.5	250.27	0.5	254.02	98.87	98.2965	0.983	590
4.	500	580	1.0	248.39	0.5	240.33	103.35	105.22	0.5261	610
5.	500	600	1.0	252.88	0.5	257.30	97.42	97.42	0.4871	585

Inspite of our best efforts it was not possible for us to process the pancreas in less than one hour after the slaughter. It is possible that some loss of insulin occurred during this time on account of enzyme action. The insulin content of mutton pancreas is found to be much lower as compared with that of beef pancreas. Even the values of beef pancreas are low as compared to data reported by Fischer and Scott,[7] given below in table II.

TABLE II

Animal	Age	Units of insulin per kg. of pancreas
Foetal calves ..	5 months	29,000—38,800
Foetal calves ..	5-7 months	21,100—24,900
Calves ..	6-8 weeks	10,400—12,800
Cattle ..	2 years	3,900— 6,100
Cows ..	9 years	1,700— 2,000

It has been estimated that the slaughter houses of Lahore which provide about 30-40 kg. weight of the pancreas per day can yield about 30,000 units of insulin per day. This figure is too low to justify the production of insulin on a commercial basis. However, since the total number of animals slaughtered in the country in different slaughter houses is very large, there still remains the possibility that if the slaughter houses are scientifically organised, and freezing facilities provided, the production of insulin and even of other glandular products may come within the range of feasibility.

References

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