LXXXVIII. INSULIN FROM THE COD FISH. THE DIRECT APPLICATION OF PICRIC ACID TO THE ISLET TISSUE.

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By the courtesy of Professor J. J. R. Macleod we were given advance information with regard to the interesting results, since published by McCormick and Noble [1924], of experiments on the insulin content of the principal islets which occur in the cod, halibut and other common fishes. This possible source of insulin was consequently explored in connection with the fisheries of this country, and although there is no clear prospect of its assuming practical importance in the commercial supply of the hormone in this country, the results obtained are worth recording.

The Toronto workers have shown that there is a large principal islet in the cod occurring as a cap over the top of the gall bladder. This principal islet is very easily detached and the method of preservation adopted by McCormick and Noble is to drop the islets, as soon as they are collected, into 70 % alcohol, containing about 0.3 % hydrochloric acid. Their results lead them to suspect that the amount of insulin in tissue so preserved diminishes according to the time elapsing before the material is worked up, and an experiment made in this laboratory suggests the same thing.

In considering alternative methods of collecting the tissue it appeared probable that use might be made of the facts that insulin is precipitated by picric acid and that the picrate obtained is soluble in watery alcohol and watery acetone. These properties were observed in previous work on the general nature of insulin, and the solubility of the picrate in mixtures of water and alcohol was mentioned in a recent paper published in conjunction with Starling [1924]. Dodds and Dickens independently discovered the solubility of the picrate in watery acetone and made use of this property in extracting insulin from mammalian pancreas [1924, 1].

Accordingly the following experiments were arranged.

Four 8 oz. medicine bottles were taken; in the first were placed 6 oz. saturated aqueous picric acid solution, in the second 4 oz. 5 % picric acid in absolute alcohol, in the third 4 oz. 5 % picric acid in acetone, in the fourth 6 oz. 0.3 % hydrochloric acid in absolute alcohol. These were sent on January

15th to Dr E. S. Russell, of the Ministry of Agriculture and Fisheries, with the request that islet tissue from freshly caught cod-fish should be added to each until the contents of the bottle reached the 8 oz. mark.

The tissue would thus be immediately fixed by picric acid in the case of the aqueous solution, and the insulin precipitated *in situ*, and it was thought possible that in bottles 3 and 4 the insulin might be converted into picrate and this automatically extracted by the approximately 60 % alcohol-water and acetone-water mixtures respectively, which would be obtained when 4 oz. islet tissue had been added to each bottle.

The fourth bottle was to be used for an extraction according to the method of McCormick and Noble.

Mr Michael Graham, Assistant Naturalist on the staff of the Ministry of Agriculture and Fisheries, spent a week at sea on a trawler in the early part of February, collecting islet tissue from absolutely fresh fish, but unfortunately the weather was very severe and the amounts of tissue which he was able to secure were so small that they could not be worked up satisfactorily.

A second similar series of collecting bottles was therefore supplied to him, and cod islets from the Yorkshire shore fisheries were collected. This material was not so fresh as that collected on the trawler, varying periods, up to 24 hours, elapsing between the catching of the fish and the removal of the islet tissue, but, nevertheless, relatively large amounts of insulin were found in it.

The material was collected on Feb. 21st, 26th, and 27th, 1924, and was worked up on March 5th.

The collection in alcoholic and acetone solutions of picric acid was only partially successful. Its proper trial involved a certain proportion between islet tissue and fixing fluid, which could not be observed. The preservation by McCormick and Noble's method also resulted in a very poor yield. It is probable that the tissue was not sufficiently fresh for success by this method. It is the more significant that, even with material of less than perfect freshness, preservation in watery picric acid was successful. Only the method of working up the material collected in this manner will be described in detail.

EXTRACTION OF INSULIN FROM ISLET TISSUE FIXED IN AQUEOUS PICRIC ACID.

The aqueous picric acid solution was poured off and the actual pieces of tissue, which varied considerably in size, were counted and then weighed in the moist state. 108, corresponding to the same number of fish, weighed 9.6 g. They were then cut up with scissors and the tissue appeared to be stained uniformly throughout with picric acid. It was ground in a mortar with sand and returned to the picric acid solution. After standing for a day it was filtered off and the picric acid solution was discarded. The tissue was then ground repeatedly in a mortar with small amounts of 75 % acetone, each extract being filtered off. In all 150 cc. of the watery acetone were used. The combined

filtrates were evaporated *in vacuo*. After the acetone had been removed the picrate, with some fatty material, separated out, adhering mostly to the sides of the flask. The precipitate suspended in the watery layer was collected by centrifuging. It was then dissolved in about 5 cc. of a mixture of 75 parts of absolute alcohol and 25 parts of 3 *N* aqueous HCl. The solution was transferred to the flask and the remainder of the picrate and fatty material dissolved in it. The solution, which was rather turbid, was transferred to a centrifuge tube and the flask washed out with a further 5 cc. of the alcoholic HCl. The solution was centrifuged to remove the suspended matter and was then poured into 200 cc. acetone. The hydrochloride thus precipitated was filtered off, washed with acetone and ether and dried in the usual way [Dudley and Starling, 1924]. 0.126 g. hydrochloride was obtained. Its activity, expressed in terms of the original Toronto rabbit unit, proved to be 1 mg. per rabbit unit. Hence we obtained a yield of 1.17 rabbit unit per fish and 13.12 (or practically 40 clinical units) per gram of wet tissue. This yield is slightly greater than the highest recorded by McCormick and Noble, who in one instance got 35 clinical units per gram of islets from the pollack. The insulin content of this islet tissue is therefore of the order of ten times that of mammalian pancreas. It seems likely that the application of this same method of preservation would be even more successful if the islet tissue could be fixed while still perfectly fresh.

While the collection of our material was in progress we heard from Drs Dodds and Dickens of a process, which they had independently worked out for the preparation of insulin from mammalian pancreas and which involved the purification of a picrate, obtained from an original watery extract, by solution of the insulin-containing fraction in wet acetone [1924, 1]. We informed them of the above method, which we already had under trial for fish insulin, in which picric acid was applied directly to the original tissue. They have now tried an essentially similar process for extracting insulin from the mammalian pancreas, and have obtained very good yields [1924, 2]. It is possible that this method will prove applicable to the commercial production of insulin from slaughter-house material, but in any case we believe that the collection of fish islets in aqueous picric acid, and subsequent extraction of the insulin by the above method, will be found to have advantages wherever the circumstances make this source of insulin worth exploitation. A watery solution of picric acid can be transmitted to the site of collection without raising excise difficulties. It appears further, that, when once the fresh islet tissue has been dropped into an adequate quantity of this fixative, it can be kept for any time determined by convenience, transmitted without refrigeration to the factory where the extraction of the insulin is to be carried out, and still give a yield at least as good as that obtained with the greatest precautions as to speed and temperature, when the alcohol process of preservation is used.

Our best thanks are due to Dr E. S. Russell for his assistance in this investigation and particularly to Mr Graham for his careful collection of material under extremely trying weather conditions. I am greatly indebted to Dr H. H. Dale for very helpful suggestions in connection with this work and to Mr H. P. Marks for carrying out the physiological tests.

SUMMARY.

(1) A convenient method of extracting insulin from the islet tissue of fish is described. It consists in applying picric acid directly to the tissue, extracting the insulin-containing picrate fraction with watery acetone, and converting the picrate into a soluble hydrochloride as described in a previous paper.

(2) From the islet tissue of the cod, although collected from fish which had been caught at varying intervals up to 24 hours previously, a yield of 13·12 rabbit units per gram of tissue was obtained. Probably more would be obtained from absolutely fresh islets. The islet tissue of the cod contains, weight for weight, apparently about ten times as much insulin as mammalian pancreas.

REFERENCES.

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