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CABBAGE EXTRACTS AND INSULIN-LIKE ACTIVITY

BY

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In 1922 the preparation of suitably active extracts of the islet tissue of the pancreas by Banting and Best laid the foundation of modern diabetic therapy; disadvantages in insulin administration were, however, soon evident. These led to numerous attempts being made to administer insulin or insulin preparations by mouth and to search being made for other substances having an insulin-like activity, but which were active when administered orally. In this search attention was paid to the plant kingdom, especially to those plant organs in which active carbohydrate metabolism was known to occur. Collip (1923) obtained extracts from many plant sources which when injected into experimental animals caused hypoglycaemia; similar work was performed by Perri (1942) with similar results. Dubin and Corbitt (1923) claimed to have isolated from an alcoholic extract of cabbage a substance which when injected into fasting rabbits caused a marked lowering of the blood sugar. Ederer (1927) found that cruder extracts of cabbage when administered by mouth had a similar effect, and Macdonald and Wislicki (1938), using methods of extraction and purification not unlike those of Dubin and Corbitt, demonstrated the presence in cabbage of a hypoglycaemic substance orally active in dogs and rabbits. On experimental grounds, the validity of the conclusions drawn by Collip and by Dubin and Corbitt were questioned by Braun and Rees (1935) and by Jorgensen and Lynn (1938). Luntz (1940) on clinical grounds did not consider the findings of Macdonald and Wislicki more than mildly encouraging.

In view of the conflicting evidence and of the potential importance of an insulin-like substance which could be administered by mouth, an attempt has been made to repeat the work of Dubin and Corbitt, Ederer, and Macdonald and Wislicki.

METHODS

Method of extraction of Dubin and Corbitt (1923).—The cabbage was cut finely with a sharp knife and thoroughly minced. The minced cabbage was extracted for 24 hours by macerating with sufficient 95 per cent (v/v) ethanol to give a 70 per cent (v/v) alcoholic solution. The supernatant liquid was poured off, the marc pressed, the liquids mixed and filtered. The filtrate was evaporated under reduced pressure to a volume of 75 ml./kg. of cabbage extracted. The concentrate was extracted with sufficient ether to remove the chlorophyll and the chlorophyll free extract evaporated under reduced pressure to a volume of 60 ml./kg. of cabbage extracted. Adjustment was then made with 95 per cent (v/v) ethanol to give an 80 per cent (v/v) alcoholic solution.

This was allowed to stand for twenty-four hours in a cool place and the supernatant liquid decanted from the precipitate. The former was then adjusted with 90 per cent (v/v) ethanol to give a 93 per cent (v/v) alcoholic solution and allowed to stand for twenty-four hours in a cool place. The precipitate was separated by filtration or centrifugation and dried with alcohol and ether; it was used either at once or stored in vacuum desiccators over a suitable desiccant. When necessary, further purification was effected by dissolving the concentrate in water, treating the solution with a saturated aqueous solution of picric acid, decomposing the precipitated picrate with hydrochloric acid-alcohol, and precipitating the hydrochloride with ether or acetone.

Method of extraction of Macdonald and Wislicki (1938).*—The cabbage was cut finely with a sharp knife and thoroughly minced. The minced cabbage was extracted by macerating with 70 per cent (v/v) ethanol (giving an alcoholic solution of 47–50 per cent (v/v)) for twenty-four hours. The supernatant liquid was then poured off, the marc pressed, the liquids mixed and filtered. The filtrate was concentrated under reduced pressure to a small bulk. The aqueous concentrate was extracted with sufficient ether to remove the chlorophyll and to the chlorophyll-free liquid was added four times its own volume of 98 per cent (v/v) ethanol. After standing in a cool place for twenty-four hours the supernatant liquid was decanted from the precipitate and the former treated with three times its own volume of absolute ethanol. The resulting precipitate was separated by filtration or centrifugation and dried with alcohol and ether; it was used either at once or stored in vacuum desiccators over a suitable desiccant. Further purification was effected as described in the method of Dubin and Corbitt.

Method of extraction of Ederer (1927).—Active preparations were claimed to have been obtained by treating fresh green cabbage leaves as follows:—(1) By simple decoction. (2) By expressing the juice. (3) By evaporating an aqueous extract to a syrup and then to dryness.

Purification of the extract was brought about by treatment with kaolin followed by filtration or centrifugation. No further details were given in the original paper.

The author prepared the decoction by the method of the British Pharmaceutical Codex (1934). The dry extract was prepared by evaporating the filtered, freshly prepared decoction to dryness in a porcelain dish. The press juice was obtained by mincing the cabbage finely and then pressing in an iron screw press.

Methods of administration and estimation of activity.—The extracts were administered in filtered aqueous solution, by stomach tube or by subcutaneous injection, to fasting rabbits, which had received water but not food during the twenty-four hours before the commencement of the experiments. The normal diet of these animals consisted of a morning feed of oats, greens, and water.

Samples of blood for estimation of the sugar content were obtained from the marginal ear veins. Blood sugar content was estimated by the method of Hagedorn and Jensen (1923).

When administration was by stomach tube, great care was taken to avoid injuring or unduly exciting the rabbits. Experiments in which injury had occurred or was suspected or in which there was undue struggling were not continued.

Dosage was calculated where possible in grammes or fractions of a gramme of extract per kilogramme of body weight of the rabbit, or in terms of the weight of cabbage originally extracted.

*I am indebted to Dr. Wislicki for the details of this method.

Before administration of the dose the fasting level of the blood sugar in the animal under experiment was determined. Blood samples were taken at intervals of ½, 1, 2, 3, 4, and 5 hours after the administration of the dose or as indicated in the text.

Control experiments were performed by carrying out parallel experiments, viz:— On fasting rabbits without dosage of any kind ; on fasting rabbits after simple insertion of the stomach tube ; on fasting rabbits after administration through the stomach tube of volumes of distilled water equivalent to those in which the doses of extracts were usually dissolved ; on fasting rabbits after administration by subcutaneous injection of graded doses of insulin.

RESULTS

Extracts prepared by the method of Dubin and Corbitt

Thirteen experiments were performed ; in eight of these administration of the extract was by subcutaneous injection ; in the remainder administration was by means of the stomach tube. Six batches of the extract were used. In four experiments no fall in blood sugar level below the fasting value was recorded, in seven experiments the maximum fall did not exceed 10 mg., and in one experiment the maximum fall recorded was 31 mg. In the latter experiment the lowest level of the blood sugar recorded was 104 mg./100 ml. No symptoms of hypoglycaemia were observed.

Extracts prepared by the method of Macdonald and Wislicki

Fifteen experiments were performed ; in five of these administration of the extract was by subcutaneous injection ; in the remainder administration was by means of the stomach tube. Six batches of the extract were used. In six experiments no fall in the level of the blood sugar was recorded ; in a further six experiments the maximum fall did not exceed 20 mg. In three experiments the maximum fall recorded lay between 21 and 38 mg. (21, 26, and 38 mg.). In this group no symptoms of hypoglycaemia were observed.

It was observed that large doses of the extract (Batch No. 6) when injected subcutaneously gave rise to a profound hyperglycaemia.

Extracts, etc., prepared by the methods of Ederer

From the observations of Ederer (1927) the feeding of raw cabbage as well as the administration of raw cabbage juice would be expected to produce symptoms of hypoglycaemia. Accordingly three fasting rabbits were allowed to eat raw cabbage and the weight eaten noted. No symptoms of hypoglycaemia were observed. Similarly none was observed to follow the parenteral or oral administration of raw cabbage juice.

Administration of the other extracts prepared as described by Ederer did not produce a fall in the level of the blood sugar or any hypoglycaemic symptoms.

In order to investigate the possibility of contaminants masking the "hypoglycaemic" action of the extracts described above, purification was taken a stage further in one instance and a hydrochloride prepared according to the method of Macdonald and Wislicki (1938). No evidence was obtained that this possessed any hypoglycaemic properties.

Discussion

Careful repetition of the work of Dubin and Corbitt, Ederer, and Macdonald and Wislicki has not indicated the presence of an insulin-like substance in cabbage. In no instance was an insulin-like reaction obtained. It is, however, not possible to rule out completely the existence of such a substance in plant tissues, since its presence and its extraction, if present, may be subject to many factors or combinations of factors unknown. No information as to the type of cabbage, its age, its condition, its source, the soil upon which it was grown, the season during which it was harvested, etc., is given by the authors mentioned above, and since these factors are known to influence the chemical content of plant tissues, it may well be that they are operative here. It may also be that the method of extraction, again imperfectly described, is at fault, and here again many unknown factors may come into play. Careful consideration has been given to the factors mentioned above as being the possible causes of failure to obtain active extracts and attempts are being made to overcome them.

Summary

1. The work of Dubin and Corbitt, Ederer, and Macdonald and Wislicki on the preparation of "hypoglycaemic" extracts of cabbage has been repeated.

2. It has not been found possible to reproduce the findings of these workers.

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Addendum

by A. D. Macdonald

In the prolonged absence abroad of Wislicki, I can only express the disappointment both he and I feel at Lewis's failure to repeat our results. That we found something in our extracts which could on oral administration lower the blood-sugar of the rabbit, and keep a pancreatectomized dog in reasonable health, I am confident. We were also supplied with active extracts prepared by our friends at the Wellcome Laboratories. None of these extracts was stable—by the end of a month they were practically inactive. Several attempts to test the extract in human diabetes were made but were unfortunate, and with the coming of the war the work was discontinued, as our extracts only provided about an equivalent of a unit of insulin from a kilogramme of cabbage. We had hoped of course to pass this aspect of the problem into the hands of the chemist.

Whether the presence of a hypoglycaemic fraction is seasonal or whether its instability is increased by some manipulation or contact which we avoided ten years ago is uncertain. Recently Wislicki has tried and failed to produce an active extract, but like myself he is still confident that there was "something there."