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THE INFLUENCE OF CABBAGE EXTRACTS UPON THE BLOOD SUGAR LEVEL

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The occurrence of a hypoglycaemic substance in cabbage, and its concentration and ultimate purification as the hydrochloride of an unidentified compound, was reported by Dubin and Corbitt in 1923. Ederer (1927) also obtained concentrates from cabbage, which had the added advantage of being active when administered by mouth, and in 1938 MacDonald and Wislicki, using methods of extraction based upon those of Dubin and Corbitt (1923), obtained concentrates from cabbage which, when administered orally to fasting rabbits, caused a marked fall in the level of the blood sugar, and which relieved the diabetic symptoms of a depancreatized dog. Attempts to confirm the conclusions of Dubin and Corbitt were made, without success, by Braun and Rees (1935), Jorgensen and Lynn (1935), and Lewis (1950). Similarly, Lewis (1950) failed to confirm the findings of MacDonald and Wislicki and Ederer.

Since the isolation of a substance similar in its physiological properties to insulin but with the added advantage of being active when administered orally would be of considerable significance, it was decided that the possibility of isolating such a substance from cabbage was worthy of further investigation. It was felt, moreover, that the experimental data given by Dubin and Corbitt, Ederer, and MacDonald and Wislicki were incomplete and permitted of a certain breadth of interpretation. For example, no mention was made of the age of the cabbage when harvested, nor was the length of time which had elapsed between its harvesting and use noted. Furthermore the conditions under which extraction was made were not fully given. Mention was not made of the *p*H of extraction, the temperature during extraction, or possible contamination and inactivation of the "hypoglycaemic" factor by metallic ions, or enzymes.

An attempt has therefore been made to repeat the work of Dubin and Corbitt, and MacDonald and Wislicki, under more strictly controlled conditions, since it is known that factors such as those cited influence the chemical content of plant tissues. In addition, isolation of active material has been attempted using other methods of extraction, and the effects of extracts prepared according to the methods of MacDonald and Wislicki and Dubin and Corbitt upon the morning blood sugar levels of rabbits fed with them, and upon the blood sugar levels of rabbits with an artificially induced hyperglycaemia, have been studied.

*A. Modifications of the methods of Dubin and Corbitt and MacDonald and Wislicki**

(*a*) Seedling cabbage: two batches were extracted, one by D. and C.'s method and one by M. and W.'s method.

(*b*) Spring cabbage: two batches were extracted by D. and C.'s method. One batch was harvested immediately before extraction.

(*c*) Mature cabbage harvested immediately before extraction: two batches were extracted by M. and W.'s method.

(*d*) Cabbage allowed to stand in a minced state exposed to the atmosphere for 24 hours before extraction: one batch was extracted by M. and W.'s method.

(*e*) The extraction of cabbage at different levels of *p*H: minced cabbage was extracted with (i) 70 per cent (v/v) ethanol adjusted to neutrality by the addition of N-ammonia; (ii) 70 per cent ethanol adjusted to neutrality by the addition of 0.5 N-sodium hydroxide; (iii) 70 per cent ethanol to which was added sufficient 0.5 N-sodium hydroxide to bring the *p*H to 7.2; and (iv) 70 per cent ethanol to which was added sufficient 0.02 N-citric acid to adjust the *p*H to 5.4. In all other respects M. and W.'s method was followed in these extractions.

(*f*) Evaporation at ordinary pressures: one batch was extracted by D. and C.'s method and one by M. and W.'s method, except that in each extraction evaporation under ordinary pressure was substituted for evaporation under reduced pressure.

(*g*) Evaporation at 50° C.: two batches extracted as in (*f*) except that evaporation under reduced pressure was so controlled that the temperature of distillation was maintained at 50° C.

(*h*) Comminution of the cabbage at a low temperature: in order to minimize enzymic activity during mincing, the cabbage was comminuted by immersion in 70 per cent (v/v) ethanol to which solid carbon dioxide had been added. The frozen material was then crushed to a coarse powder by means of a pestle and mortar, and from then onwards M. and W.'s method of extraction was followed. A further batch of cabbage was treated in this manner and then extracted by D. and C.'s method.

(*i*) Extraction in the absence of metals: two batches were extracted, one by D. and C.'s method and one by M. and W.'s method. The cabbage was comminuted by pounding in porcelain mortars with porcelain pestles until a contused mash was formed. After maceration the liquid was expressed from the marc by means of a porcelain screw press.

(*j*) Control of the temperature of the water bath: two batches were extracted, one by D. and C.'s method and one by M. and W.'s method, but in order to avoid the risk of local overheating during vacuum distillation the temperature of the water bath in which the distillation flask was immersed was not allowed to exceed 45° C.

(*k*) Combination of modifications (*h*), (*i*), and (*j*): two batches were extracted, one by D. and C.'s and one by M. and W.'s method, with modifications (*h*), (*i*), and (*j*).

(*l*) Precipitation of the "hypoglycaemic" fraction by means of *n*-butanol: a large batch was extracted by M. and W.'s method except that the final precipitation of the "hypoglycaemic" fraction was performed by the addition of *n*-butanol. This extract is referred to hereafter as *Al.*

B. The use of other methods of extraction

(*a*) The extraction of freeze-dried cabbage juice: The cabbage was minced by means of an iron roller mincer in the presence of solid carbon dioxide; solid carbon dioxide was added to the minced cabbage and the juice expressed from the latter by means of an iron or porcelain screw press The expressed juice was chilled by the addition of a further small amount of solid carbon dioxide, and was freeze-dried by means of an oil-mercury vapour pump high vacuum system. The residue was powdered and extracted

* These will be referred to as D. and C.'s method and M. and W.'s method respectively.

with 80 per cent (v/v) ethanol for 24 hours. The insoluble matter was removed by filtration and re-extracted for a further period of 24 hours with 80 per cent ethanol. The combined filtrates were adjusted by addition of absolute ethanol to an alcoholic content of 93 per cent (v/v). When precipitation was complete, the precipitate was separated by filtration and after removal of traces of alcohol administered orally or parenterally to the experimental animal.

In essence, this method consisted of extracting the residue obtained from the cabbage juice after freeze drying by D. and C.'s method. It assumed that the " hypoglycaemic " principle was present in the expressed juice and dispensed with the initial 24 hour maceration with 70 per cent ethanol during which time some decomposition might occur. Three batches were treated as described in the foregoing account.

(*b*) The preparation of a hydrochloride from cabbage juice: the expressed juice from minced cabbage was filtered and treated with a saturated aqueous solution of picric acid until there was no further precipitation. The precipitate was removed by centrifugation, and treated with excess of hydrochloric acid-ethanol. After several hours (occasional shaking) the insoluble material was removed by filtration and the filtrate poured into five times its volume of acetone. The precipitated hydrochloride was removed from the supernatant liquid by centrifugation and dried *in vacuo*.

(*c*) The fractionation of cabbage juice by half-saturation with ammonium sulphate: the juice expressed from minced cabbage was filtered and treated with an equal volume of saturated ammonium sulphate solution. After several hours, the precipitate was removed by centrifugation, suspended in water, and dialysed in cellophane sacs against running water until no further reaction for sulphate could be obtained. The contents of the dialysis sacs were then separated by centrifugation into soluble and insoluble fractions. The insoluble matter was tested as it stood, the other fraction was evaporated under reduced pressure to approximately one-third of its volume before administration to the experimental animal.

(*d*) The fractionation of cabbage juice by saturation with ammonium sulphate: the method was the same as that described in (*c*) except that sufficient ammonium sulphate was added to the expressed juice to saturate it.

C. Biological experiments

(*a*) The administration of cabbage extracts to rabbits with artificially elevated blood sugar levels: Four healthy adult rabbits were selected and fasted for 24 hours. A.R. Dextrose (1 g./kg.) was then administered either by subcutaneous injection (Rabbits 1 and 2) or by means of the stomach tube (Rabbits 3 and 4) and samples of blood for sugar determination taken at intervals of one half, one, two, three, four, and five hours after dosage. This experiment was repeated three times with Rabbit 1, twice with Rabbit 2, and once each with Rabbits 3 and 4. In similarly conducted experiments the same amount of A.R. Dextrose was administered to each member of the group, together with extract equivalent to 4 kg. of cabbage originally extracted. In Rabbits 1 and 2 the dose was injected subcutaneously, in Rabbits 2 and 3 it was given by stomach tube. The extract administered to Rabbits 1 and 2 was prepared by D. and C.'s method, that administered to Rabbits 3 and 4 by M. and W.'s method. Two repeat experiments were carried out upon Rabbit 1, three upon Rabbit 2, and one each upon Rabbits 3 and 4. The results were subjected to statistical analysis.

(*b*) Effect upon the morning blood sugar levels of fasting rabbits: These experiments were carried out in order to ascertain the effect, if any, of the cabbage extract upon the fasting blood sugar level of normal rabbits over a period of several days.

A group of six healthy rabbits were selected and each animal fasted for 24 hours. Samples of blood, for blood sugar estimation, were then taken. Three of the animals were given a dose of 5 g. extract *Al* per kg. body weight and the other three dosed

similarly with 2.5 g./kg. of the same extract. Both groups were fed, and after feeding subjected to a further period of starvation for 24 hours, when the fasting blood sugar level was again determined. This process was repeated a further three times. A parallel group of five animals which did not receive a dose of the extract but which received identical treatment in all other respects was used as a control.

(*c*) The effect of repeated dosage at two-hourly intervals: In these experiments the extract *Al* was used. Two healthy rabbits were selected and each fasted for 24 hours before experimentation. Samples for blood sugar estimation were then taken and 2.9 g./kg. body weight of the extract *Al* were administered orally to each animal. This dosage was repeated twice in one animal and three times in the other. The interval between the administration of doses was about two hours.

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 no food during the 24 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 discontinued. Dosage was calculated in grams or fractions of a gram of extract per kilogram of body weight of the rabbit or in terms of the weight of cabbage originally extracted.

Before dosage the fasting level of the blood sugar in the animal under experiment was determined. Blood samples were taken at intervals of $\frac{1}{2}$, 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 doses of the extracts were usually dissolved ; on fasting rabbits after administration of graded doses of insulin, and as indicated under *C* (*a*) and (*b*).

Results

A. None of the modifications of the processes of Dubin and Corbitt and MacDonald and Wislicki, outlined in section *A* (*a*) to (*l*), gave extracts which produced symptoms of hypoglycaemia. Two experiments were usually carried out for each modification. The maximal falls in blood sugar in mg. per 100 ml. blood were: (*a*) 13, (*b*) 31, (*c*) 21, (*d*) 23, (*e* iii) 30, (*e* iv) 9, (*f*) no fall, (*g*) 21, (*h*) 6, (*i*) 7, (*j*) 11, and (*k*) 4. The extract prepared as described in section *A* (*d*) was also administered intravenously when it caused a transient but quite marked hyperglycaemia (two rabbits).

The extract prepared by butanol precipitation (section *Al*) was available in large quantity, and it was tested more extensively than those obtained by extracting smaller batches of cabbage in the laboratory. Doses of 50 g. of the extract by mouth did not produce any hypoglycaemia ; in one case a profound hyperglycaemia was observed. Dosage with 5 g./kg. body weight of the rabbit did not produce hypoglycaemia, a mild hyperglycaemia being recorded. Doses of 2.5 g./kg. produced no symptoms of hypoglycaemia. In these experiments falls in the level of the blood

sugar of 19, 29, and 22 mg. per 100 ml. respectively were recorded. In the first two of these experiments an initial phase of mild hyperglycaemia was also observed. In the remaining three experiments in which a dose of 2.5 g./kg. body weight was given, one showed neither hypoglycaemic nor hyperglycaemic trends, whilst in the other two experiments a mild degree of hyperglycaemia was recorded. Administration of a dose of 1 g./kg. body weight to two rabbits produced no symptoms of hypoglycaemia. In one of these experiments a fall in the level of the blood sugar of 19 mg. was observed, in the other no fall was recorded.

B. None of the other methods of extraction described in section *B* (*a*) to (*d*) gave extracts producing symptoms of hypoglycaemia. The maximum falls of blood sugar in mg. per 100 ml. were (*a*) freeze dried cabbage juice, 6 mg.; (*b*) hydrochloride, 12 mg.; (*c*) insoluble fraction, 14 mg.; soluble but non-dialysable fraction, 16 mg.; (*d*) insoluble fraction, 20 mg.; soluble but non-dialysable fraction, 5 mg.

C. (*a*) Analysis of variance of the blood sugar levels between zero time and half an hour after treatment in rabbits given dextrose produced no evidence that, after this period of time had elapsed, the extract had any significant effect upon the glucose tolerance curve. There was, however, a significant difference between the blood sugar level at zero time and one hour after treatment when the results obtained from administration of dextrose solution and of dextrose solution plus cabbage extract were compared.

(*b*) In one rabbit there was a fall of 17 mg./100 ml. in the fasting level of the morning blood sugar after a period of 24 hours had elapsed, and in another a fall of 5 mg./100 ml. after 48 hours. There appears to be no evidence pointing to the extract producing any significant effect upon the morning blood sugar levels of fasting rabbits.

(*c*) Two experiments were carried out. Repeated dosage with extract *Al* at intervals of two hours did not produce any symptoms of hypoglycaemia. The maximum fall in blood sugar level recorded was 7 mg./100 ml.

Discussion

Statistical analysis of the results obtained has shown that the blood sugar level after one hour is significantly lower when cabbage extract is administered mixed with solution of dextrose than when solution of dextrose is administered alone. This difference was of the same order whether administration was by the parenteral route or by mouth. Thus there appears to be no doubt that the rate of absorption of dextrose is altered by the extract. This effect is probably not of an insulin-like nature since extracts administered alone do not appear to lower significantly the fasting level of the blood sugar in rabbits. Further, the extract appears to slow down and prolong dextrose absorption. This is not, of course, an insulin-like effect. To what this influence upon dextrose administration is due is not clear. It is suggested that gastric secretion may be impaired, or that the permeability of the gut wall may be lessened. Further investigation of these points is necessary, but since the extract is soluble and forms only an insignificant fraction of the dry weight of the administered mixture, these factors are not likely to be responsible for the observed effects.

Little can be said regarding the results obtained from the other methods of procedure, etc., adopted in this work. No evidence was obtained from them indicating that they were overcoming adverse factors in the extraction process, etc., since none yielded positive results.

It cannot be said with certainty that the existence of an insulin-like substance in cabbage has been ruled out, since the factors and combinations of factors which may be influencing its successful extraction are very numerous and consideration is being given to further experimental work.

Summary

1. Attempts to modify the extraction processes of Dubin and Corbitt (1923) and of MacDonald and Wislicki (1938) have failed to produce hypoglycaemic extracts of cabbage.

2. The use of alternative methods of extraction has similarly failed to produce active extracts.

3. Administration of cabbage extracts to rabbits with artifically elevated blood sugar levels slowed down and prolonged the absorption of glucose.

4. Cabbage extracts did not significantly lower the morning blood sugar levels of fasted rabbits.

5. Repeated administration at two-hourly intervals of an extract prepared by precipitation with *n*-butanol was ineffective in producing an insulin-like response.

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