A HYPOGLYCÆMIC SUBSTANCE FROM THE ROOTS OF THE DEVIL'S CLUB (*FATSIA HORRIDA*)

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THIS paper presents data which show that from the roots of the Devil's Club (*Fatsia horrida*) an extract can be obtained that exhibits marked hypoglycæmic properties. The material is active when fed by the mouth and apparently has no marked toxic effects. Although the active material has not been isolated in the pure state certain observations lead the writers to believe that it is not identical with any insulin-like material of plant origin so far reported in the literature.

A large number of insulin-like materials, both synthetic and of plant origin, has been described in recent papers. For a review of these the work of Hill and Howitt[1] and of Braun and Rees[2] may be referred to. The most active of these substances appear to be derivatives of guanidine, and synthetic preparations such as synthalin (decamethylene diguanidine) have been made available commercially. A material of plant origin, but of unknown constitution, has also been marketed under the trade name "myrtillin". Three commercial preparations derived from guanidine and intended for the oral treatment of mild cases of diabetes have been favourably reported on by Frank, Nothmann and Wagner (synthalin),[3] von Beznak and Hariss (anticoman),[4] and by Hirsch (omalkan).[5] The toxicity of guanidine derivatives appears to be the subject of some controversy,[1] but the data would suggest that up to the present no guanidine derivative has been prepared that does not show some toxic effects, particularly in regard to the liver and kidneys.

The extract investigated by the writers was prepared from the fresh or dried bark from the roots of the Devil's Club, a shrub that grows wild in great abundance along the coast of British Columbia. The extract is made by infusion with hot water. Such a preparation has long been used by Pacific Coast Indians, though for what specific purpose is not clear. Our attention was brought to this material through the examination by one of us of a surgical patient, who, on hospitalization, developed marked symptoms of diabetes. This person, it was learned, had kept in apparent good health for several years by oral doses of an infusion of this root bark, and is in fact still leading a normal life with the aid of this infusion.

EXPERIMENTAL

Biological assays were carried out on white Belgian hares. In preliminary work a starvation period of twenty-four hours was allowed. Later this was extended to forty-eight hours, and in all experiments reported in this paper the animals have been starved for that period. Blood sugars were determined by the micro method of Folin.[6] In control animals starved for forty-eight hours the blood sugar never varied more than ± 5 mg. per 100 c.c. from the initial starvation level for a period of six hours. The average variation was considerably less than this. However, only changes greater than 10 mg. have been taken as significant.

The work was done on a colony of 24 animals, 6 of which never received any extract and were used as controls in a pathological examination for toxicity. The remaining 18 were all used regularly for assay work, and, whilst considerable variation in individual response was noted, all the animals reacted in the same general way towards the extract. One circumstance, however, deserves to be mentioned here. During the earlier part of the work the animals were fed a diet which included a certain proportion of greens, together with oats and alfalfa. During the winter months the greens were left out of the diet, which for some 5 or 6 months consisted chiefly of oats and bran with water *ad lib.* After several months on this acid diet it was noticed that it was practically impossible to produce hypoglycæmia with extracts that had previously proved to be very active. Three days after the greens had been restored to the diet the extracts showed their original hypoglycæmic action. This observation corroborates that of Page.[7]

The following technique was finally adopted for preparing an extract suitable for investigation.

The bark was stripped from the roots either by hand or by means of an improvised ball mill, boiled three times with twice its volume of water, and the extract filtered off. Tannins were removed with neutral lead acetate and excess lead by means of hydrogen sulphide. The precipitated tannins, which came down in a gelatinous form, were separated by means of a Sharples super-centrifuge. The excess hydrogen sulphide was removed by boiling, and the extract finally evaporated, so that the number of c.c. of final volume was one-third the number of grams of the original bark. The extract was made slightly alkaline with magnesium oxide, filtered, and stored for use. Unless otherwise stated this extract was used throughout the investigation.

No systematic investigation has yet been made regarding the yield of potent extract that can be obtained from a given quantity of roots. Seasonal variations and the parts of the roots selected will undoubtedly be important factors. The following data may however give some idea of the yields we have obtained. The bark comprises from 10 to 20 per cent of the weight of the green roots, the larger roots giving the smaller yield. The moisture content of the bark averages about 70 per cent. In general we have found that an extract made from dried bark when made up to a volume in c.c. equal to the number of grams of the dried bark is of the same potency as one made from the green bark when made up to a volume in c.c. equal to one-third the number of grams of the original green bark. Over two hundred assays have been made with this extract. These data are too numerous to report in detail. Typical data have therefore been given in the form of charts.

Effect on alimentary hyperglycæmia.—Two starved rabbits of similar weight were fed 1.0 c.c. of 50 per cent glucose per pound of body weight and the rise in blood sugar was followed every half-hour. This was repeated once a week for five weeks. In every case a curve similar to those given in Chart 2 was obtained. The same rabbits were then fed the same amount of glucose and in addition an oral administration of a simple water extract of dried Devil's Club root bark in amount equivalent to 0.4 gram of the dried bark. Three repetitions of this experiment gave blood sugar curves similar to those shown in Chart 2.

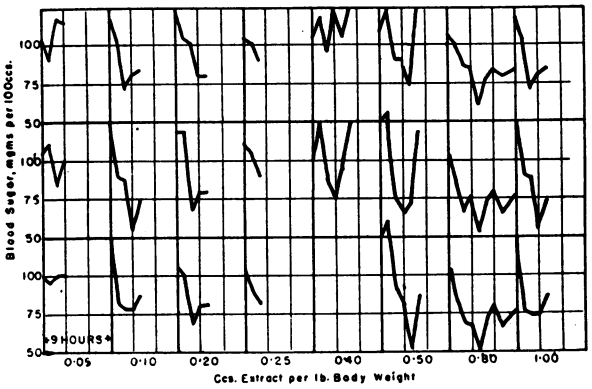

Chart 1.—The hypoglycæmic effect of graded doses.

With glucose alone a maximum rise of about 45 mg. glucose per 100 c.c. of blood was reached at the end of the first hour. At the end of the second hour the original blood sugar level had not been reached. When the extract was fed with the glucose the maximum rise was but 25 mg. and this was reached at the end of the first half-hour. Thereafter the blood sugar fell rapidly, and at the end of an hour and a half was more than 25 mg. below the initial starvation level. Usually the initial starvation

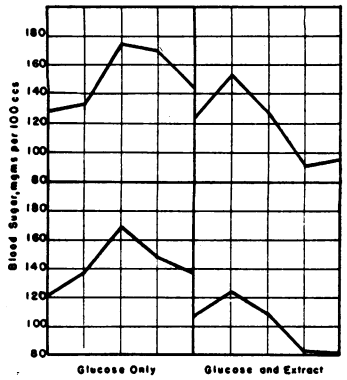

Chart 2.—Effect on alimentary hyperglycæmia.

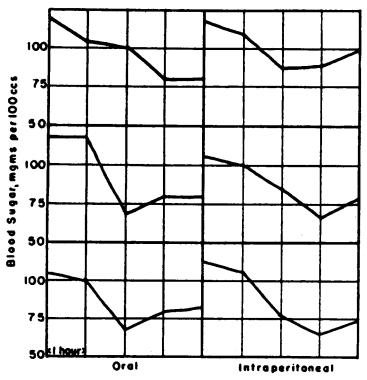

Chart 3.—Oral *vs.* intraperitoneal administration.

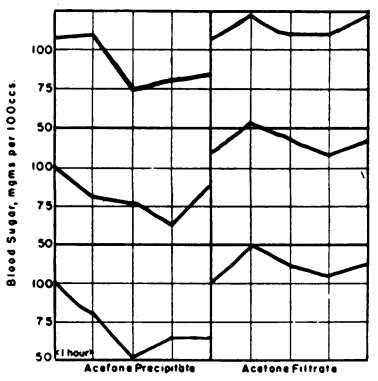

Chart 4.—Separation of hypoglycæmic from hyperglycæmic principle by acetone.

level was not reached again until after the third hour.

These data are conclusive in showing that the extract contains a substance very active in reducing alimentary hyperglycæmia.

The hypoglycæmic effect.—The tannin-free concentrated extract was then fed to starved rabbits without administration of glucose. The extract was given orally by means of a graduated pipette in amounts based on the body weight of the starved rabbit. Blood sugars were determined immediately before giving the extract and at hourly intervals afterwards. A few typical data are given in Chart 1. At a level of 0.05 c.c. per lb. of body weight the results were not conclusive. In some cases a noticeable drop in blood sugar occurred, but in others there was either no effect or a slight initial rise. At levels of 0.1, 0.2 and 0.25 c.c. per lb. of body weight a rapid hypoglycæmia was produced, with a reduction of the blood sugar of 35 to 70 mg. per 100 c.c. At levels of 0.4 and 0.5 c.c. per lb. of body weight some peculiar results were obtained. In nearly every case an initial rise in blood sugar was noted, followed in most cases by a very rapid drop. At these higher doses however there was nearly always a decided fluctuation in the blood sugar values which would lead one to suspect that in the extract a substance was present that had a hyperglycæmic effect which only became noticeable above a certain level. Even at these high doses we have not yet succeeded in producing coma, although rabbits with blood sugars below 60 mg. per 100 c.c. were always extremely lethargic. With doses of 0.8 and 1.0 c.c. per lb. of body weight the erratic behaviour of the blood sugar was more pronounced, but in general the gross hypoglycæmic effect was lengthened.

It might be mentioned here that although a standard starvation period of forty-eight hours was used, the oral feeding of the extract sometimes produced results that differed markedly in identical rabbits used at various times on the same dose. These variations, however, were not individual variations but occurred in groups. For instance a group of three rabbits might give an average lowering of the blood sugar of 50 mg. in three hours, taking five hours to return to the initial starvation level. A week later the same three rabbits, on the same diet and starved for identical periods, when fed the same dose of exactly the same extract might give a lowering of 65 mg. within two hours but return to the original starvation level in three hours. The room in which the rabbits were kept varied in temperature within very wide limits, especially during the winter time, and we are inclined to think that this variation may have been an important factor in this peculiar behaviour.

The toxic effect of the extract.—Twelve rabbits were selected for a toxicity test. Six of these 12 had never been given the extract. They were fed the colony diet and were used as controls. Six other rabbits were fed 1 c.c. of the concentrated extract daily for five months. At the end of this time the twelve rabbits were killed and the livers, kidneys, and adrenals were sent to Dr. H. H. Pitts, pathologist at the Vancouver General Hospital. Dr. Pitts kindly examined these organs for us and has given us permission to quote the following from his report:

> "We have made a great many sections of each block and have stained the liver sections for fat, and, while I believe there is no question that there is definitely more fatty degeneration in the livers of those animals who have had the extract, I do not consider that it is of any significant amount, for in some of the sections of the controls there is almost as much and in sections of different blocks from the same specimen of the experimental ones there is practically no degeneration, so that it is apparently a very patchy type and apparently not of any particular pathological significance."

Since the livers of some of the control animals showed as much fatty degeneration as those from the animals which received the extract the above report would appear to indicate that the extract was without any marked toxic effects. However, the extract used in these experiments was not pure and contained besides the active material some gums, sugars (0.6 per cent as glucose), magnesium acetate, a small amount of magnesium hydroxide and possibly other natural contaminants. Among these contaminants must be included a hyperglycæmic principle. A single isolated example which we have never been able to duplicate will illustrate the activity of this latter substance.

Three starved rabbits were given 1.0 c.c. per lb. of body weight of the standard tannin-free concentrated extract. Blood sugars were then determined hourly with the peculiar results shown in Table I.

TABLE I.

HYPERGLYCÆMIA PRODUCED BY DEVIL'S CLUB ROOT EXTRACT

Blood sugar, mg. per 100 c.c.

Rabbit No.	*Wt., lb.*	*Starvation*	*1st hour*	*2nd hour*	*3rd hour*	*4th hour*	*7th hour*	*8th hour*	*9th hour*	*24th hour*
13	11	133	278	182	250	256	250	172	244	87
14	11.3	118	222	200	244	256	244	182	227	111
19	8	111	250	196	222	244	250	182	200	95

The same three rabbits have since responded normally to smaller doses of the extract. Other rabbits when fed this high dose have given results similar to those shown in Chart 1. So far no explanation has been found for these results, for even when the hyperglycæmic factor (see below) is separated from the hypoglycæmic factor the rise in blood sugar is not so pronounced as in the present case.

Oral vs. intraperitoneal administration.—Oral and intraperitoneal doses have been compared at levels of 0.2 c.c. of concentrated extract per lb. of body weight. Some comparative data are plotted in Chart 3. There is not a great deal of difference between the results of the two methods of administration. The average lowering is approximately 40 mg. from the starvation level for both methods. The rate of fall is also about the same for each method.

Separation of the hypoglycæmic from the hyperglycæmic factor.—In an effort to purify the concentrated extract attempts were made to precipitate the active constituents. It was found, eventually, that the hypoglycæmic substance was less soluble in dilute (30 to 40 per cent) acetone than in water. By diluting the extract with water and then adding acetone to bring the concentration up to about 40 per cent, an amorphous precipitate was thrown down, whilst the sugars, gums and organic salts were retained in solution. The precipitate and filtrate were freed from acetone by vacuum evaporation and made up with water to the original volume of the undiluted extract. These two extracts were then biologically assayed at a level of 0.2 c.c. per lb. of body weight. The results given in Chart 4 indicate that the precipitate undoubtedly contained the hypoglycæmic factor, depletions in the blood sugar of from 35 to 50 mg. being obtained. After four hours the blood sugars were still much below the original fasting level. In the case of the filtrate a hyperglycæmia of 20 to 25 mg. was produced within the first hour and, although this hyperglycæmia slowly disappeared during the next two hours, no hypoglycæmia was ever produced. The sugar present in the extract is too small to account for this rise in blood sugar, and it has been assumed that the hyperglycæmic factor remains in the filtrate.

The precipitate thrown down by the dilute acetone is insoluble in neutral or slightly alkaline media, although in the original extract it appears to remain in solution. It is soluble, however, on slight acidification, a fact which suggests that the unknown material is basic in nature. Another fact which bears out the above observations is that on long standing the concentrated alkaline extract gradually deposited a small amount of an amorphous precipitate which on biological assay appeared to have a greater hypoglycæmic effect than the solution itself. The amount of precipitate so far obtained has been too small to do much with in the way of identification. It is planned to obtain larger amounts of it and to subject it to a systematic chemical examination. Clinical trials of the extract on diabetic patients under adequate control are also to be undertaken in the immediate future.

The writers wish to acknowledge their gratitude to their technician, Miss Norma Rogers, whose assistance has been invaluable in accomplishing the large amount of routine laboratory work. They are also deeply indebted to Dr. H. H. Pitts, of the Pathological Department of the Vancouver General Hospital, for his generous cooperation in preparing and examining sections for the toxicity tests. Finally, their thanks are extended to the Fisheries Research Board of Canada for the use of an unused room in the Fisheries Experimental Station in which a large proportion of the experimental work was carried out; and also to Mr. Kitagawa for supplying the first information regarding the raw material.

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