XXIX. THE EFFECT OF CEREAL EXTRACTS ON BLOOD-CALCIUM.

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THE concentration of calcium in the blood is remarkably constant in man and in the laboratory animals so far investigated. Apart from such procedures as dilution of the blood, or administration of salts of calcium or phosphorus, the blood-calcium has not been found to vary to any appreciable extent, except under the influence of certain internal secretions. Collip [1925] has established the rôle of the parathyroid in raising the blood-calcium, and Mirvish and Bosman [1927] have shown that extracts of bovine ovaries are able to bring about a fall of blood-calcium in a characteristic manner in both rabbits and man. A very similar action was found for extracts of suprarenal cortex [Mirvish and Bosman, 1929, 1], and probably also for the testis [Mirvish and Bosman, 1929, 2]. Bearing in mind the necessity for correlation of animal and plant physiology, I proceeded to investigate extracts of plant organs, and chose oatmeal as a convenient possible source of this "ovarian" substance.

Oatmeal has been shown to be associated in some way with a pathological phase of calcium metabolism, *i.e.* rickets. The chief evidence in this connection has been brought forward by E. Mellanby [1922], who showed that puppies on a bare sufficiency of the antirachitic vitamin D could be made to develop rickets by feeding on a diet of cereals, the most potent rickets-producing of which was oatmeal. This work was later extended by Green and Mellanby [1928], who showed that the same interfering action of cereals on the calcification of bones could be demonstrated in rats, and an ingenious method was evolved by these observers to determine quantitatively the degree of anti-calcifying activity of different cereals. They also showed that this anti-calcifying power could be counteracted by simultaneous administration of more vitamin D or of calcium carbonate. They could, however, destroy the anti-calcifying factor by boiling the oatmeal with 1 % HCl. Holst [1927] has obtained similar results. He was able to produce rickets in rats by feeding exclusively on cereals and yellow peas, but not by feeding with rice-starch alone. If oatmeal is extracted with dilute hydrochloric acid, and the extract is given in addition to the starch, the animals develop rickets. It seems, therefore, that here is a positive factor, which Holst calls a "toxic substance," the mode of action of which cannot be accounted for by the vitamin-deficiency

theory of rickets. Holst finds that the production of rickets by feeding with cereals can be prevented by the administration of calcium salts, whereas phosphates have no such effect. M. Mellanby [1923], working on puppies, found that the greater the amount of cereal eaten, other things being equal, the greater the tendency to the production of badly calcified teeth.

Another series of observations of a different kind points to the presence in certain plants of an "ovarian" substance. There is evidence that some plants contain a substance of the same nature as the "female sex hormone" or "oestrin," as seen by the partially positive vaginal smear reactions obtained with extracts of flax and hemp seeds [Kountz, 1927], and by the positive oestrus obtained by Loewe and his associates [1927], using willow catkins and other plants.

The purpose of the present experiments was to attempt to obtain from oatmeal a substance similar to that extracted from bovine ovaries in its action in lowering the blood-calcium.

EXPERIMENTAL.

The cereals employed for extraction were oatmeal, wheat germ, and maize meal, but the majority of the observations were made on finely ground, untreated oatmeal. In the earlier part of my work rectified spirit was used to extract the cereal meal directly, either by percolation in the cold or by extracting on the water-bath and purifying as in the case of the ovary. Most of these extracts gave positive results when tested on the blood-calcium, as did simple aqueous extracts. But these methods of extraction were not used further, as I wanted to ascertain whether the extract of oatmeal with dilute hydrochloric acid, which Holst found to be rachitogenic, possessed also the calcium-reducing property. The method eventually adopted for the preparation of extracts, of which a preliminary account has already been given [Mirvish, 1929], was as follows.

The meal was put in canvas bags, which were suspended in a bath containing the solution for extraction, usually 0.5 % to 1 % HCl in water, and left to soak for a day or two. The bath was then placed in a larger water-bath and heated for 2-4 hours. The fluid extract was then poured off, and the bags of meal drained without being squeezed. The bags of meal were then extracted a second time either with plain water or again with 0.5 % HCl in water exactly as before, and the two extracts combined. On an average 6 litres of water were used to extract 1 kg. of meal, yielding a yellowish solution containing a minimum of starch.

The solution was next evaporated to small bulk. During all evaporations, sodium hydroxide was added from time to time, in order to keep the solution constantly weakly acid to litmus. When the bulk of the solution was reduced to about a quarter of the original volume, it was filtered through glass-wool and the evaporation continued until the final volume was about 100 cc. It was then dialysed against distilled water for 24-48 hours. The inner liquid was

discarded and the yellow dialysate evaporated to small volume. Several crops of crystals (mostly NaCl) could be removed by cooling on ice and further evaporation. Finally one obtained a brownish-coloured paste, and for a time a neutralised aqueous solution of this was used for injection. Later, however, this material was extracted with boiling 95 % alcohol, and the oily yellowish-brown liquid remaining after evaporation of the alcohol was dissolved in water and used for injection.

The technique for estimating the effect of the extracts on the blood-calcium level was that employed for studying the effect of the ovarian extract [Mirvish and Bosman, 1927]. Rabbits, kept under standard conditions, were injected with varying amounts of the extracts, and blood was drawn before injection and at various intervals after injection. The previous work on ovary showed that the blood-calcium falls most frequently 24-48 hours after injection. Control injections of other tissues (muscle, brain, pancreas, spleen, kidney), and of cholesterol and olive oil produce no such fall in blood-calcium, and one must therefore attribute it to the specific substance present in the extract. Some of the results obtained with various types of extract are given in Table I.

Table I. *Injection of various extracts of oatmeal compared with injection of ovarian extract.*

Rabbit	Injected subcutaneously	Serum-calcium in mg. per 100 cc.			
		Normal	After 24 hrs.	After 48 hrs.	After 72 hrs.
	Oatmeal extracted with 95 % alcohol.				
11	Extract representing 40 g. oatmeal	17.5	18.0	17.5	16.9
12	" " 60 g. "	17.5	14.5	14.5	16.5
39	" " 150 g. "	15.0	10.0	12.1	—
40	" " 375 g. "	14.0	11.3	13.8	—
41	" " 375 g. "	15.0	12.2	11.0	—
	Oatmeal extracted with water.				
R 1	Extract W representing 150 g. oatmeal	13.5	12.0	12.5	11.0
R 2	" " 300 g. "	14.0	11.0	11.5	10.0
R 5	" " 200 g. "	12.8	10.5	—	—
R 6	" " 600 g. "	11.5	6.6	—	—
	Oatmeal extracted with 0.5 % HCl.				
Y 4	Extract A representing 200 g. oatmeal	13.1	13.1	9.3	14.3
Y 5	" " 250 g. "	13.6	13.1	9.8	—
Y 6	" " 650 g. "	12.1	8.8	10.7	13.6
16	" E " 180 g. "	15.3	13.55	14.5	11.5
17	" " 270 g. "	16.7	14.0	11.8	—
20	" G " 400 g. "	14.0	15.0	11.5	—
21	" " 800 g. "	11.5	12.0	7.0	—
	Alcoholic extracts of bovine ovary.				
A	Representing 25 g. of ovary	14.5	10.5	15.0	—
B	" 40 g. "	15.0	13.0	11.5	—
C	" 25 g. "	17.0	14.5	14.0	—

These results are very similar to those obtained with ovarian extract, the only difference being that the minimal effective dose is much larger than in the case of the ovary. I next tested the dialysate of this material, as Holst found

that his rachitogenic fraction was dialysable, and this fraction, as well as an alcoholic solution of it, was also found to be calcium-reducing. The method of extraction is of course by no means quantitative, nevertheless it is apparent that each process of refinement of the extract progressively lowers its potency (Table II).

Table II. *Injection of dialysed extracts of oatmeal.*

Rabbit	Injected subcutaneously	Serum-calcium in mg. per 100 cc.				
		Normal	After 24 hrs.	After 48 hrs.	After 72 hrs.	After 96 hrs.
	Oatmeal extracted with 0.5 % HCl and dialysed. Dialysate employed.					
35	Representing 150 g. oatmeal	16.0	17.0	17.5	16.0	—
37	" 225 g. "	12.5	13.8	13.3	14.0	14.0
38	" 350 g. "	14.0	14.5	14.7	15.0	14.7
39	" * 350 g. "	14.0	12.3	11.2	13.7	13.7
40	" 1000 g. "	15.0	12.5	10.2	10.3	13.8
	Oatmeal extracted with 0.5 % HCl, dialysate then extracted with 95 % alcohol.					
14	Representing 600 g. oatmeal	15.0	15.5	15.0	15.0	15.0
15	" 600 g. "	16.0	17.0	16.0	15.0	16.5
16	" 960 g. "	16.0	11.0	15.0	15.0	16.0
17	" 1200 g. "	15.0	14.0	12.5	15.0	15.0
19	" 1800 g. "	17.5	13.5	15.0	13.0	15.0
20	" 1920 g. "	17.5	12.0	16.0	15.5	17.0
21	" 2160 g. "	18.0	16.0	13.0	16.0	16.0
9	" 960 g. "	17.5	14.5	16.0	16.0	15.0
10	" 1600 g. "	18.0	15.0	13.5	15.0	18.0
11	" 2400 g. "	16.0	10.0	14.9	16.0	17.0

* Injected intraperitoneally.

DISCUSSION.

In general the results show that oatmeal extracts are able to reduce the blood-calcium of rabbits, both male and female, the maximum depression occurring on an average at the end of 24 hours, with a return to normal usually within 72 hours after administration. The more effective the dose the earlier is the point of maximum depression, so that it may occur as early as 10 hours after injection. Intraperitoneal injection has a more rapid action than subcutaneous. It has been found that some rabbits do not react at all to the injection, although other animals of the same batch may react to a similar dose of the extract employed. The same experience was also encountered with extracts of ovary, testis and suprarenal cortex, though the cause is not clear. The calcium-reducing substance is present in oatmeal in a concentration of about 1/10 to 1/5 of that of the bovine whole ovary. The experiments demonstrate that oatmeal has the same effect as ovary on the blood-calcium, attributable to a substance which I called "calcovarin" in previous work.

It has been found by various observers that the oral administration of oatmeal produces rickets. This effect has been traced to a dialysable substance which can be extracted from oatmeal by dilute HCl. An extract obtained in the same manner, injected in large doses into animals, produces a noticeable

fall of blood-calcium of the same order as that produced by extracts of bovine ovary. It has not been determined whether this oatmeal extract given by mouth will reduce the blood-calcium, but this is the case with the ovarian extract. It would seem then that the rachitogenic and the blood-calcium-reducing actions are both a function of the same extract, and one must attribute the development of rickets in these cases to an over-supply of "calcovarin."

Another condition where the calcium metabolism is profoundly disturbed is osteomalacia, in which the bones are much softened, the blood-calcium is low (6-8 mg. per 100 cc.), and tetany is frequent. It has been found that the diet of women thus affected contains excess of cereals, and administration of cod-liver oil or exposure to sunlight cures or prevents the disease and restores the normal blood-calcium level. There is considerable evidence of a relationship of the ovarian function to osteomalacia, as it occurs almost exclusively in women, is usually associated with puberty or pregnancy, and removal of the ovaries frequently cures the condition. Recent investigations have disclosed that its occurrence at puberty before pregnancy is at least as common as its association with pregnancy, so that the bony softening cannot be explained away as due to a drain on the maternal calcium for the requirements of the foetus. In spite of the different types of bony defects, there is no doubt that osteomalacia and rickets are closely related diseases occurring at different ages. Bearing in mind that the ovarian secretion has the same influence on calcium metabolism as the rachitogenic element in oatmeal, one can postulate that osteomalacia is due to excessive "calcovarin" supplied to the body partly from an exogenous source, the cereal diet, and partly from an endogenous source, namely that elaborated by the hyperactivity of the ovary, which occurs at puberty and pregnancy.

We can only put forward a working theory at present on the connection between "calcovarin" and vitamin D. Rickets and osteomalacia are both recognised as being primarily diseases of calcium metabolism, and all our clinical and metabolic studies emphasise the fact that calcium metabolism is controlled by an endocrine mechanism, in which the parathyroid plays the leading part. A disturbed state of calcium metabolism, short of gross diminution of calcium intake, should lead us to search for an underlying endocrine disturbance. There is evidence that the ovary produces its effect on the blood-calcium through the parathyroid, and that leads one to suggest the possibility that rickets and osteomalacia are caused by a functional hypo-parathyroidism. It may be that vitamin D is necessary for the proper function of the parathyroid, and, as the body cannot synthesise vitamin D in the absence of sunlight, parathyroid secretion is not elaborated sufficiently and a hypo-parathyroidism or some disturbance of the parathyroid apparatus results. "Calcovarin" also produces a functional hypo-parathyroidism by reacting with or antagonising the parathyroid secretion. Thus a deficiency of vitamin D, or an over-supply of "calcovarin"—endogenous as in osteomalacia, or exogenous as in rickets—

will lead to parathyroid disturbance. This hypothesis may not be warranted by the facts at our disposal, but we know that vitamins have some relation to the endocrine secretions, and it may be that vitamins are necessary for the proper elaboration of the various internal secretions. The production of rickets by a positive factor challenges our vitamin theory and demands explanation.

SUMMARY.

A method has been found for extracting oatmeal with 0.5 % hydrochloric acid, yielding an extract which is free from starch, thermostable, dialysable, and soluble in alcohol. This extract, when injected into rabbits, produces a fall of blood-calcium. The extent of this fall is about 30 % of the normal. It occurs 24–48 hours after injection, the figure returning to normal again within 48–72 hours. The type of calcium depression is the same as was found previously to follow the injection of bovine ovarian extract. From this it is concluded that “calcovarin,” the substance producing the characteristic calcium depression response, is present in oatmeal as well as in ovary. The oatmeal extract which Mellanby and Holst found to be rachitogenic in puppies and rats was prepared in the same way as the extract here tested, and the rachitogenic effect of this extract is attributed to its calcium-reducing property. A suggestion is put forward that rickets and osteomalacia are diseases which may be due to functional disturbances of the parathyroid secretion.

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