XLI. THE EXTRACTION OF THE FAT-SOLUBLE FACTOR OF CABBAGE AND CARROT BY SOLVENTS.

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THE study of the fat-soluble accessory factor in fats has so far not contributed anything very definite to our knowledge of the chemistry of this principle. Observations so far made on active fats favour the view that the activity is not due to the fat itself but to some substance or substances which are in association with it. The fat-soluble factor is however not easily separated from it by differential solvents. All other conceivable methods of fractionation are mostly of a drastic nature not suitable for the manipulation of a physiologically active principle of doubtful stability.

It is now well established that some vegetables form a rich source for a substance physiologically resembling the fat-soluble accessory factor in fats but as in the case of the fats little is known of its chemical character. We are even yet unaware whether it is present in the vegetable cell in solution or in suspension. According to McCollum, Simmonds and Pitz [1916, 1] the fat-soluble factor is not extracted from plants with the fats by ether, chloroform, benzene or acetone. Hot alcohol however according to these authors [1916, 2] removes the factor from maize kernel.

This communication describes some experiments in which active fractions were obtained from fresh vegetables by extraction with absolute alcohol and eventually with ether.

Originally it was intended to study the fat-soluble factor in cabbage but owing to some technical difficulties the investigation was extended to carrots. It has been found by various workers that cabbage is rich in the fat-soluble factor. Preliminary experiments carried out in connection with this research have shown that small quantities of this vegetable in fresh condition were sufficient to promote growth in rats fed on a diet lacking the factor. The aim was to treat the minced cabbage at first with a solvent which would fulfil the dual function of disorganising the plant cells and of extracting the accessory factor. Absolute alcohol was chosen and found to be suitable for the purpose. However the extract thus obtained was not relished by the rats who mostly either refused the food containing it or consumed insufficient

of it. It was therefore found necessary to apply this method of extraction to another vegetable, namely carrots, the extract of which was likely to prove more palatable to the animals. This vegetable although not very rich in the fat-soluble accessory factor was shown by Denton and Kohlman [1918] and Steenbock and Gross [1919] to contain appreciable quantities of it. As anticipated it was found possible to extract by means of alcohol from the carrots an active fraction which the animals took well. This extract afforded the opportunity of further fractionation, and it was found that ether extracted the active principle from it leaving the best part of the extraneous matter behind. As the carrot also contains the antineuritic and antiscorbutic factors it was conjectured from theoretical considerations that the alcoholic extraction would also remove these two factors. Experiments instituted with that purpose have indeed shown that the extract contained the antineuritic and to a smaller extent the antiscorbutic factor. These two principles were however not present in the extract in such high proportions as the fat-soluble factor.

EXPERIMENTAL.

Fresh green cabbage was thoroughly minced and triturated with sand. Absolute alcohol was then added (500 cc. of absolute alcohol for every 100 g. of cabbage) and the mixture allowed to digest in a cool dark place for about 12-18 hours. It was then filtered through a fluted filter and the residue on the filter paper pressed out and filtered. The combined filtrates were green and transparent and possessed the usual appearance of an alcoholic solution of chlorophyll. The alcoholic extract was then evaporated *in vacuo* at 35°. As the solution became concentrated chlorophyll separated out leaving behind a light brown liquid. The addition of alcohol to this solution which was practically free from alcohol produced a small white precipitate which consisted most probably of protein. On further concentration a greenish brown sweet syrup containing reducing sugars was obtained. This syrup possessed the characteristic taste of boiled cabbage. An equivalent of 25 g. of fresh cabbage was added daily to a diet which was complete in every respect but which lacked the fat-soluble factor. The composition of this BC diet was described in a previous communication [Zilva, 1919]. Of the various rats fed on this diet only few consumed the food well enough to demonstrate the growth-promoting power of the extract. Fig. 1 gives the weight curves of two representative rats. The male animal consumed the best part of his food, on the average about 18 g. per day, with the result that it grew well and reached a satisfactory weight within the 11 weeks during which it was kept on the diet. The female animal consumed only about 10-12 g. of the food per day. Such an amount is hardly quantitatively adequate to promote normal growth even if the diet were qualitatively well balanced. It will be seen that although the animal grew, the rate of growth was below the normal. The experiments with the cabbage extract, however, served as a

preliminary indication and demonstrated that it was possible to extract the fat-soluble factor by the method described.

In order to obviate the irregularity in the food intake of the rats carrots were next employed as a source for the accessory factor. These vegetables were treated in the same way as the cabbage. The filtrate obtained in this case was brilliantly clear and yellow in colour. On concentration *in vacuo* at 35° a very sweet and pleasant-tasting syrup was obtained. The syrup could be dissolved in water producing a turbid reddish yellow solution. An equivalent of 25 g. of fresh carrots was given to the animals in their daily diet at first. This preparation the animals consumed very well and as will be seen from Figs. 2 and 3 they developed well on it. Fig. 2 represents the

Fig. 1. Weight curve of rats fed on a diet containing the fat-soluble factor in the form of an alcoholic extract of cabbage.

weight curves of three animals which received the extract after they had subsisted for three weeks on a diet lacking the fat-soluble factor. They immediately resumed normal growth instead of ceasing to grow as was the case with the control animal. Fig. 3 shows how, on administration of an equivalent of 25 g. of fresh carrots to an animal which had existed on a diet free from the fat-soluble accessory factor for ten weeks and which was rapidly losing in weight, normal growth was induced.

It was next desirable to ascertain the minimum dose of the extract that would induce normal growth. Doses equivalent to 15 g., 5 g., 1 g. of fresh

Fig. 2. Weight curve of rats fed on a diet containing the fat-soluble factor in the form of an alcoholic extract from carrots. The perpendicular dotted lines denote the commencement of the treatment.

Fig. 3. The perpendicular dotted line denotes the commencement of the treatment.

carrots were given to three rats. In every case growth was induced (Fig. 4). The addition of the equivalent of 15 g. of carrots produced almost normal growth. The extracts from 5 g. and 1 g. produced less intense growth. An animal which had ceased growing for several months owing to the deficiency of the fat-soluble factor also resumed growth on receiving a dose equivalent to 1 g. of carrot. The growth curve in this case commenced flattening out after about ten days. The diet containing the 1 g. was consumed entirely. About 80 %-90 % of the other two doses was consumed daily by the rats. It may be pointed out in conjunction with the above figures that Steenbock and Gross [1919] found that 5 % of dried carrot was insufficient to produce normal growth while a diet containing 15 % sufficed for the purpose.

Fig. 4. The perpendicular dotted lines denote the commencement of the treatment.

Absolute alcohol extracts in the form of the syrup about 7.5 % of dry matter from fresh carrots which comprises about one-half of the total solids. On drying the syrup *in vacuo* at a low temperature and keeping the residue in a desiccator it was found that after a fortnight an equivalent of 25 g. of carrots was active both in inducing good growth and in curing xerophthalmia in rats. Any further particulars of the keeping properties of the carrot extract in dry condition have not yet been worked out.

The antineuritic factor was tested out on rats. Equivalents of 25 g. per day were added to an AC diet [Zilva, 1919]. It will be seen from Fig. 5 that this dose was sufficient to induce development a little below the normal rate. As these animals only consumed about 70 % of their daily diet it may be assumed that the dose was about the minimum necessary for normal growth.

Fig. 5 also shows the weight curve of a rat which was deteriorating on account of an antineuritic deficiency but which on receiving the extract equivalent to 25 g. of fresh carrots resumed growth.

The tests for the antiscorbutic factor showed that the content of this principle in the carrot extract was very low. Three guinea-pigs kept on a diet of oats and bran and a daily ration of 40 cc. of autoclaved whole milk received after the tenth day a daily dose of the extract equivalent to 25 g., 10 g. and 5 g. of fresh carrots respectively. The animals which received the 10 g. and 5 g. doses developed scurvy, declined in weight and eventually died in

Fig. 5. The perpendicular dotted line denotes the commencement of the treatment.

the ordinary way as they would have done on a purely scorbutic diet (Fig. 6). No delay in the onset of the symptoms or in the fatal termination could be recorded. The guinea-pig which received daily the extract from 25 g. of fresh carrots showed a decided delay in the onset of the scorbutic symptoms. Unfortunately this animal succumbed to some other complaint on the 23rd day. At the post mortem examination mild signs of scurvy were observed which were not acute enough to be responsible for the death of the animal. The extract from 25 g. of carrots contains therefore enough antiscorbutic to delay the onset of the disease¹.

¹ [Note added on 9th of June.] A daily administration of an alcoholic extract equivalent to 50 g. of fresh carrots protected a guinea-pig subsisting on a scorbutic diet for 42 days.

The alcoholic extract from carrots was next concentrated until all the alcohol was driven off and the aqueous solution became concentrated. This solution was extracted by shaking it with ether in a separating funnel. The ethereal solution at first was yellow but on repeated extraction no more colouring matter could be removed from the solution which nevertheless still retained a fairly intense yellow colour. A solution equivalent to 250 g. of fresh carrots extracted with ether as described yielded a residue weighing 0.4935 g. The residue, which was dark brown, on drying for two hours at 110° remained of oily consistency. On extracting it with sodium carbonate and acidifying the solution no precipitate was obtained which would indicate that it did not contain any free higher fatty acids. The residue was next saponified by heating with alcoholic potash under a reflux condenser for two hours, the alcohol driven off, and the aqueous solution filtered. On adding acid to the filtrate a solid substance resembling the higher fatty acids separated out and gradually accumulated on the surface of the liquid. There is little doubt then that the ethereal extract contained some oil.

Fig. 6. The perpendicular dotted line denotes the commencement of the treatment.

In order to test the activity of this fraction autoclaved olive oil was added to the ethereal solution and the ether evaporated. A highly coloured and opaque oil was left behind. Both the colour and the opacity were dependent on the strength of the extract. Through the medium of the oil the ethereal extract, equivalent to 25 g. of carrots, was incorporated in the daily diet which was free from the fat-soluble factor and as will be seen from Fig. 7, which represents the weight curve of two rats treated with this fraction, growth was promoted immediately the treatment was commenced.

While these experiments were in progress Osborne and Mendel [1920] published a short note in which they state that they have extracted with ether from spinach leaves and young clover dried at 60° an oily residue which was active in small quantities. Although they have obtained their fraction from different plants and directly from the dried vegetable their work corroborates the fact that the fat-soluble factor in plants is soluble in ether. Further

experiments with various solvents carried out preferably on the alcoholic fraction ought to throw some light on the subject whether the activity of the ethereal fraction is due to the small amount of the oil or to some other substance associated with it.

Fig. 7. The perpendicular dotted lines denote the commencement of the treatment.

SUMMARY.

1. Alcohol extracts the fat-soluble factor from cabbage and carrots.
2. The alcoholic extract equivalent to 10–12 g. of fresh carrots given daily is sufficient to promote normal growth in rats subsisting on a diet lacking the fat-soluble factor.
3. The alcoholic extract from carrots also contains the antineuritic and to a smaller extent the antiscorbutic factors.
4. An ethereal extract from the alcoholic fraction equivalent to 25 g. of fresh carrots has been found to promote recovery and renew growth in rats declining in weight on account of a fat-soluble factor deficiency.

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