XII. THE EXAMINATION OF THE FAECES OF RABBITS FED ON A DIET OF CABBAGE FOR THE OCCURRENCE OF A PHYTOSTEROL, INCLUDING A NOTE ON THE PHYTOSTEROL IN CABBAGE SEEDS AND THAT IN GRASS FRUITS.

By MARY TAYLOR ELLIS.

From the Physiological Laboratory, University of London, South Kensington.

(Received March 4th, 1918)

PREVIOUS experiments by Dorée and Gardner [1908] on the excretion of cholesterol in the animal organism, led to the belief that the phytosterols and similar bodies occurring in the plant given as food appear in the faeces in an unchanged form.

The examination of the product when grass was given as food has already been carried out by these authors. The chief body in the faeces under these conditions is chortosterol, an alcohol having the formula $C_{27}H_{54}O$, or $C_{27}H_{56}O$, which does not give the cholesterol colour reactions, and this substance was also found in grass leaves. Phytosterol itself was either absent, or present in the oily matter of the unsaponifiable ether extract in too small amount to separate. When bran was given in the food, the phytosterol of bran was found in the faeces. Further, from results obtained by Fraser and Gardner [1909] it appeared that when phytosterol was given in the food of rabbits, the greater part appeared in the faeces. Further, experiments carried out later [Fraser and Gardner 1910] by the same authors showed that although phytosterol given in the food increased the cholesterol content of the blood, it did not appear in the blood as such, and must have been altered in form or excreted. In continuation of this work, an examination of the faeces of rabbits fed on a diet of cabbage was made by the author, to determine whether a phytosterol was present, and to investigate the change, if any, in this substance in its passage through the digestive organs.

Rabbits in the laboratory were fed on cabbages, some on the outer leaves and others on the inner stem, including the apical bud—40 kilos of leaves, 30 kilos of stalk were used. Their faeces were collected and extracted separately. The account will thus be divided into results obtained with leaves and with stalks.

The faeces were dried, and then ground up as finely as possible. The dried material was subjected to prolonged extraction with ether in a Soxhlet apparatus. The ether extract thus obtained was saponified with a large excess of alcoholic sodium ethylate according to the method of Kossel and Obermüller. In order to insure complete saponification the saponified liquid was always allowed to stand over-night. The precipitate of soap was separated by filtration, and washed with ether to remove all the ether-soluble substances. The filtrate was shaken up with water several times to free it from excess of alkali. Finally the washed ether extract was evaporated to dryness, the residue dried and kept for examination.

The Leaves.

On saponification it was observed that very little soap was present. The dried ether extract consisted of solid matter, not obviously crystalline, in a brown oil. This matter proved exceedingly difficult to separate, as the brown oil came out of solution with the solid from ethyl alcohol, acetone, and ethyl acetate. The solution in acetone was therefore boiled with charcoal, and afterwards filtered hot. The charcoal was subsequently extracted several times with boiling acetone to remove the substances as completely as possible. From this solution a pure white substance was obtained in small crystals; in granular lumps from acetone and ethyl acetate. It was finally recrystallised from ether, from which it separates in beautiful rosettes of lustrous crystals.

(1) The first substance to come out of solution in ether melts at 78°–79°. It separates from alcohol as a jelly, in a more granular form from acetone and ethyl acetate; from ether and chloroform in much larger rosettes of crystals. It does not give the cholesterol reactions and does not form an acetate. The similarity in properties made it probable that it might be chortosterol, but as the acetate could not be obtained this could not be proved. The amount of the substance was too small for further investigation, but it was evidently not a phytosterol.

(2) The mother liquors deposited a crystalline solid melting between 60°–75°; this was evidently a mixture and proved very difficult to separate.

Finally a substance of m.p. 63° – 64° was isolated which gave a constant melting point after several recrystallisations. It is very light, and separates in a crystalline form from ethyl acetate, acetone, ether and chloroform. From acetone, when the substance is pure, the crystals under the microscope show the form of 4-sided plates. Salkowski's and Liebermann's tests are negative. It has no specific rotation.

From its properties it evidently does not belong to the sterol group, and is probably one of the hydrocarbons or alcohols occurring in plant waxes.

(3) The mother liquors consist of a heavy brown oil, containing a trace of crystalline matter, too small for identification. This gives the cholesterol colour reactions.

The Stalks.

The unsaponifiable residue from the faeces of rabbits fed on cabbage stalks was very similar to that from the leaves in appearance. The solid crystallising out was difficult to separate from the brown oil present, and the residue was dissolved in acetone and boiled up with charcoal.

Two substances were isolated.

(1) The less easily soluble substance which came out of solution almost completely in the cold from most solvents melted between 60° and 70° . After repeated crystallisation from ether, a substance melting at 63° – 64° was separated in small amount. It had the same crystalline properties as that from the leaves, and the m.p. was not altered by mixing the substances from the stalks and leaves in equal proportions.

(2) The more soluble substance remained behind in the mother liquors. On evaporation to small bulk a distinctly crystalline body separated out from the oily solution in rosettes of long needles. The substance was finally purified from absolute alcohol. The melting point was 102° , and the substance gave the cholesterol colour reactions. Only a very small amount of this substance was present, so that it was difficult to identify it further.

THE TISSUES OF THE CABBAGE.

An examination of the dried leaves and stalks of the cabbage itself showed that the unsaponifiable ether extract was very small in amount. It consisted mainly of the same substances as appeared in the faeces, and although the mother liquors gave the phytosterol colour reactions, no crystalline substance like phytosterol was isolated. It appeared from these results that a typical phytosterol, if present, only occurred in the cabbage plant to a very small

extent, and as a comparative experiment, the seeds were examined to find out if they contained this substance.

The Seeds. Two kilos. of cabbage seeds were ground up very finely and extracted with ether. The extract was saponified in the usual way, and the unsaponifiable matter, after drying, weighed 14.9274 g.

It consisted of a mixture of crystalline matter with a brown oil. The residue was recrystallised from alcohol, and finally purified from solution in acetone. The crystals from alcohol were glistening plates, from acetone smaller. They gave the cholesterol colour reactions; the melting point of the pure substance was 142° . A substance with the same melting point was described by Bömer [1899, 1901] and later by Windaus and Welsch [1909] in rape oil. The latter authors were able to show that it was a mixture of two substances: (1) brassicasterol, $C_{28}H_{46}O$, m.p. 148° , and (2) a phytosterol $C_{27}H_{46}O$, m.p. 142° . They obtained the bromide of the acetate, and found that brassicasterol formed a tetrabromide insoluble in glacial acetic acid, whereas the other substance formed a dibromide which remained in solution.

This phytosterol resembles the sitosterol described by Burian [1897] and later by Ritter [1902] in wheat and rye embryos.

In order to determine if the substance isolated from cabbage seeds was the same as that in rape oil the following experiments were carried out.

Preparation of the acetate. 0.05 g. of the substance was mixed with 0.1 g. of sodium acetate, and a few cc. of acetic anhydride added. The mixture was boiled under a reflux condenser for $\frac{3}{4}$ hour and then poured into much water and allowed to stand over-night. The precipitate was then filtered, washed free from acetic acid, and dissolved in alcohol, from which it was twice recrystallised. The crystals melted at 137° . This seemed so similar to the mixtures of substances in rape oil, that it was interesting to observe if a tetrabromide would be formed.

Preparation of the bromide of the acetate. A further preparation of the acetate was carried out, and about 0.25 g. was dissolved in 2 cc. of ether, and 3 cc. of a 5 % solution of bromine in glacial acetic acid were added. After some time crystals separated out. To determine if these were identical with those of the tetrabromide of the acetate of brassicasterol, they were purified by recrystallisation from absolute alcohol, but the results were not very conclusive. The tetrabromide of the acetate of brassicasterol described by Windaus and Welsch, melts at 209° . The melting point of the substance just described was lower. It is hoped later to carry out the experiments on the same scale as was done by Windaus and Welsch, who used much larger quantities.

The results obtained from these experiments: that the cabbage seeds contain a relatively large amount of a mixture of phytosterols, whereas the vegetative organs seem to contain a very small amount, made it interesting to compare the investigation of grass described by Dorée and Gardner [1908] with an examination of the fruit.

GRASS FRUITS.

515 g. of grass fruits were extracted, giving an unsaponifiable residue weighing 2.05 g. From this residue on crystallisation from acetone, the first substance which separates out in small quantity, resembles chortosterol in its solubility, but it was too small in amount to be purified satisfactorily. It is probably derived from the leaf-structures surrounding the grass fruit, and inseparable from it. From the mother liquors, 0.07 g. of a crystalline substance resembling phytosterol was obtained. This melted at 135° – 137° and gave the cholesterol colour reactions.

0.04 g. was converted into the acetate, which after recrystallisation melted at 119° – 120° . This remained unaltered on subsequent recrystallisation.

It would seem, from the data available, that this is a mixture of sitosterol and some similar body, but the quantity was too small to attempt separation of the constituents.

SUMMARY AND CONCLUSIONS.

(1) The chief constituent of the unsaponifiable ether extract of the faeces of rabbits fed on a diet of cabbage, was not phytosterol, but a mixture of substances of lower melting point, probably hydrocarbons or alcohols such as are known to occur in plant waxes. A typical phytosterol was not isolated either from the faeces or from the vegetative organs of the cabbage plant itself, although a small amount of a substance melting at 102° and giving the cholesterol colour reactions was separated from the faeces of rabbits fed on cabbage stalks. This might be coprosterol, although that substance has never been found in the faeces of the herbivora, but the amount was too small for complete identification.

(2) Cabbage seeds, on the other hand, contain relatively a large amount of crystalline matter, which seemed similar to the mixture of phytosterols present in rape oil. This is interesting as rape and cabbage are different species of the same genus—*Brassica*.

(3) Grass fruits also contain phytosterol, although the chief constituent of the unsaponifiable ether extract of the leaves is chortosterol, which does not give the cholesterol colour reactions.

(4) These results seem to indicate that whereas phytosterol is present in the seeds, in the vegetative organs it is absent or present in relatively small amount.

Such a conclusion, however, would hardly be justified unless some more accurate method of separation were employed, permitting of the separation of phytosterol which may be present in the oily residues, as they give the phytosterol colour reactions.

Further experiments are in progress, and the details will be given in a later communication.

In conclusion the author would like to express her thanks to Professor Waller and to Mr Gardner for the facilities afforded in the Physiological Laboratory.

The work was carried out partly with the aid of a grant from the Government Grant Committee of the Royal Society, to whom thanks are due.

REFERENCES.

- Bömer (1899), *Zeit. Nahr. Genuss.*, **2**, 705.
— (1901), *Zeit. Nahr. Genuss.*, **4**, 865.
Burian (1897), *Monatsh.*, **18**, 551.
Dorée and Gardner (1908), *Proc. Roy. Soc.*, B, **80**, 212.
Fraser and Gardner (1909), *Proc. Roy. Soc.*, B, **81**, 230.
— (1910), *Proc. Roy. Soc.*, B, **82**, 559.
Ritter (1902), *Zeit. physiol. Chem.*, **34**, 461.
Windaus and Welsch (1909), *Ber.*, **42**, 612.