THE CHEMICAL COMPOSITION OF THE EPICUTICLE OF WOOL

III. CARBOHYDRATES PRESENT IN EPICUTICLE PREPARATIONS

by

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INTRODUCTION

As shown in our previous publications[24,25] the main constituent of epicuticle preparations obtained from wool by treatment with sodium sulphide or bromine is protein. This same conclusion was reached by ZAHN[31]. The low nitrogen content of our preparations further seemed to indicate the presence of carbohydrate. In fact LAGERMALM AND GRALÉN[15] claim to have demonstrated the presence of a sugar in epicuticle hydrolysates. Moreover these authors suggest that the carbohydrates are to be considered as true constituents of the epicuticle whereas the protein part of the preparations might for a great part consist of contaminations.

BOLLIGER AND MCDONALD[3] found that hair contains considerable quantities of glycogen, which can be extracted with water. As it was difficult to detect the glycogen histologically[4] in the hair, it was assumed that it is loosely bound to the hair keratin. It seems improbable, however, that epicuticle preparations still contain glycogen, because this substance would have dissolved during the treatment used for their isolation. Another water-extractable carbohydrate found in hair by BOLLIGER AND PETERS[5] consists of pentose, probably ribose.

As far as is known to us, no other investigations have demonstrated the presence of carbohydrates in wool. It is known, however, that especially the medulla of some hairs contains non-proteinaceous constituents, *e.g.* steroids[27]. The elementary composition of scoured wool indicates that no major constituents apart from wool keratin are present. If the whole carbohydrate content is assumed to be concentrated in the epicuticle, it should account for less than 0.2 % of the wool weight. By the methods used to obtain epicuticle preparations an enrichment of the carbohydrate occurs. Further enrichment, although accompanied by some loss in quantity, can be obtained by the use of papain-treated[25] wool.

Several sensitive methods have been developed to detect small amounts of carbohydrate in the presence of protein. In the orcinol method[26] the yellow colour of some hydrolysates is a disturbing factor and the colours developed are very unstable; furthermore the time dependence of the absorption is difficult to interprete with sugar mixtures[17]. Better results were obtained with the anthrone reagent of DREYWOOD[7] using a modification of the method of TREVELYAN AND HARRISON[29]. The present investigation deals with the carbohydrate content not only of epicuticle preparations but also of egg shell membranes, because the latter resemble the epicuticle of wool in many respects.

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A further object of the present investigation was to identify the sugars obtained by hydrolysis of carbohydrate-containing epicuticle preparations. As drastic means are needed to dissolve the epicuticle and most sugars are decomposed by hot concentrated acids, we tried to find conditions by which as much as possible of our epicuticle preparations was dissolved without destruction of the sugars. The latter were investigated by paper chromatography after removal of the amino acids and peptides by the use of ion-exchange resins.

METHODS

Determination of carbohydrate

The anthrone reagent[7] has been successfully used for the determination of carbohydrates by a number of authors *e.g.*[2,9,18,19,23,29]. The colour development is not the same for equimolecular amounts of different sugars. For example galactose gives about half as much colour as glucose[18]. The mechanism of the colour development seems to involve the previous formation of hydroxymethylfurfural from hexoses and furfural from pentoses[2,23]. The velocity of these reactions depends on the structure of the starting sugar. The furfural compound formed reacts with the anthrone rapidly, forming a green colour with a maximum at 625, subs. 608 mμ.

In the following, results are expressed in glucose equivalents, because it was found that the main sugar present in epicuticle hydrolysates was glucose.

Hydrolysis

It is known that sugars are destroyed by the action of concentrated strong acids at high temperature (*e.g.*[16]). High temperatures are also destructive when using diluted acids[20], and may cause some decomposition even under neutral conditions[30]. Pentoses and ketohexoses are more easily destroyed than aldohexoses[14,28]. It seems that the use of 1 *N* acid for not more than 2 hours at 100° C is generally accepted as permissible in the case of the hexoses. However, some carbohydrates, *e.g.* cellulose, are not completely hydrolysed by this treatment and require the use of concentrated acid. Concentrated acids tend to cause esterification and reversion but subsequent dilution and boiling nullifies these effects. Such a combined hydrolysis with sulfuric acid has been extensively used in the cellulose field, *e.g.* in the modification of HÄGGLUND AND BRATT[11], and excellent yields of glucose have been reported. Concentrated hydrochloric acid at 37° C is used in the BERGIUS process[1] for wood saccharification.

LAGERMALM AND GRALÉN[15] used 20 or 36 % hydrochloric acid at 100° C for 10 hours to dissolve their epicuticle preparations for carbohydrate analysis. We found that glucose and galactose are destroyed under these conditions.

However, excellent recoveries of glucose (as estimated by the anthrone method) were obtained with concentrated strong acids at moderate temperatures. Wool was not completely dissolved by the sulphuric acid hydrolysis of HÄGGLUND AND BRATT[11], but complete dissolution was achieved in concentrated hydrochloric or hydrobromic acid at 37° C within 16 hours, provided the mixture was vibrated*. The hydrolysis of wool in 10 *N* hydrochloric acid at 37° C has been studied by GORDON, MARTIN AND SYNGE[8], who found that after 24 hours about a third of the peptide bonds has been hydrolysed. The epicuticle preparations proved somewhat more resistant than wool to the dissolving action of strong acids, for example it was found that about 15 % of the organic matter of sodium sulphide treated preparations was not dissolved by 10 *N* hydrobromic acid at 37° C after 16 hours. It seems improbable, however, that any carbohydrate would not be dissolved under these conditions.

EXPERIMENTAL PART

Carbohydrate present in epicuticle preparations

The wool tops used for the preparation of our epicuticle samples contained 0.05 % carbohydrate (glucose equivalents found by the anthrone method). The slight amounts of cellulose fibres always present in wool are considerably enriched by the procedures used to obtain epicuticle preparations. Especially if wool is used which has previously been treated with papain[25], high carbohydrate contents are found. This can be explained, however, on account of the small yield obtained in this case; under the electron microscope the membranes were very thin and had a damaged appearance.

* We used a Müller "Vibro Mischer".

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A convenient way to remove foreign matter from epicuticle suspensions consists of filtration through coarse sinterglass filters. By this method the carbohydrate content of our epicuticle preparations has been reduced to 0.6–0.7 %. It was found by microscopical examination (staining with zinc chloride-iodine), that at least part of this carbohydrate content was due to the presence of (damaged) cellulose fibres.

It seemed desirable to remove the cellulose contaminations from wool more quantitatively by chemical means. Some samples of technically carbonized wool were still found to contain 0.03 % carbohydrate (anthrone method). We therefore treated wool samples with sulphuric acid of 70 % at 38 °C for 15 minutes as recommended for the determination of wool in fibre blends[22]. As the Allwörden reaction was still positive, no essential parts of the epicuticle are removed by this treatment. The carbohydrate content of epicuticle preparations obtained by dissolving acid treated wool samples in sodium sulphide solution was less than 0.4 %; despite this low carbohydrate content, this preparation was still found to contain a few cellulosic fibres on microscopical examination. Probably these contaminating fibres have entered after the acid treatment of the wool. As the preparing of epicuticle samples had to be accomplished in a laboratory where at the same time research on cellulose is performed, this could hardly have been avoided.

The investigation of the sugars in epicuticle hydrolysates was performed after removal of the very large excess of peptides and amino acids by the use of cation-exchange resin ("Dowex 50"). It was shown by control experiments that all ninhydrin-reacting material is withheld by this resin from moderately acid solution without any substantial loss of sugars. In the case of hydrochloric or hydrobromic acid hydrolysates the excess acid was removed by repeated evaporation *in vacuo*. When sulphuric acid was used, the acid had to be removed with baryta or anion-exchange resin. This procedure needs extreme care, because alkaline conditions favour rearrangements of the sugars, and further because sugars are partly lost by passage through certain types of anion-exchange resins. For these reasons we generally prefer the use of hydrochloric acid for hydrolysis.

It was found by paper chromatography that the hydrolysates of our epicuticle preparations contained glucose in appreciable amounts. As the preparation contained cellulose, this result could be expected. It was of interest, however, whether the hydrolysates contained any other sugar besides glucose, especially whether a sugar behaving chromatographically like galactose was present as described by LAGERMALM AND GRALÉN[15]. No such sugar was discerned by us, but unfortunately glucose and galactose resemble each other very much, differing only in the configuration at C4. For this reason they are not well separated on paper chromatograms[12] and a small amount of galactose might be overlooked in the presence of a large amount of glucose.

Experiments to prove definitely the absence of galactose by other methods[6,10,13,21,26] are still being continued.

Our chromatograms also showed some faint spots of faster moving sugars.

Carbohydrate present in egg-shell membranes (ovokeratin)

Inner and outer egg-shell membranes were collected separately from the environment of the air chamber of unboiled eggs. The dried samples contained 1.7 and 1.8 % carbohydrate (glucose equivalents), respectively, as found by the anthrone method.

20 mg samples were hydrolysed (5 ml HCl 12 *N*, 37 °C, 16 hours) and the protein degradation products removed in the usual manner by treatment with cation-exchange resin "Dowex 50". The sugars were investigated by two-dimensional paper chromatog-

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raphy. Spots showed up at the positions of galactose, glucose and mannose. An unidentified spot suggested the presence of a pentose or methylpentose.

DISCUSSION

From the preceding experiments it may be concluded that the epicuticle of wool can be obtained practically free from carbohydrate, if sufficient precautions are taken to remove previously the small amount of cellulosic material always present in commercial wool samples. Removal of the cellulose fibres by acid treatment does not alter the essential properties of the epicuticle layer, as may be concluded from the unimpaired Allwörden reaction of the treated wool samples.

It is admitted that no method has been found to hydrolyse epicuticle preparations completely and at the same time prevent the decomposition of any sugars present in the hydrolysate. However, the major part of the epicuticle can be brought in solution by treatment with concentrated acids at moderate temperatures, and it seems extremely improbable, that under these conditions, the undissolved residue would contain carbohydrate and the solution none.

It is difficult to explain the results obtained by LAGERMALM AND GRALÉN[15] because the hydrolysis employed by these authors might lead to almost complete destruction of any carbohydrate present. So far we were unable to detect a sugar with the R_F-value of galactose in our hydrolysates.

Thus carbohydrate does not form an essential part of the epicuticle. Neither is this the case with the ashes, which were shown[24] to consist mainly of quartz. Small amounts of waxy material were sometimes encountered in our preparations, but this can be prevented by a thorough solvent extraction of the wool to be used for the epicuticle preparations. It was shown previously[24] that even after an extraction with ether for 96 hours the wool still shows an unimpaired Allwörden reaction, and quite normal yields of epicuticle could be obtained from such wools. The possibility that the sebaceous gland takes part in epicuticle formation has also been ruled out on histochemical grounds[24].

The remaining part of the epicuticle preparations consists of protein which differs definitely from the bulk of the wool protein. The differences can be explained by assuming a higher degree of cross-linking[25]. Lanthionine cross-linkages might be responsible for resistance towards papain; indeed it seems as if the epicuticle protein is partly attacked if the papain treatment precedes the treatment with sodium sulphide, while it is known, that the latter introduces lanthionine residues in cystine-containing proteins. On the other hand only limited resistance towards the dissolving action of sodium sulphide was obtained by treating wool with potassium cyanide, which treatment converts a considerable part of the cystine linkages into lanthionine linkages. We did not succeed, however, in demonstrating definitely that lanthionine residues are absent in the original epicuticle.

It is tentatively assumed that another type of cross-linkages, *e.g.*, peptide, is present in wool epicuticle. In this connection the similar properties of ovokeratin are interesting. Although the egg-shell membranes contain some carbohydrate, we do not think this constituent to be able to exert a great influence on the properties of the protein (unless this carbohydrate is involved in some type of cross-linking). It seems more probable that the amounts of the basic amino acids present in both epicuticle and ovokeratin form a clue for further investigations in this field. Some physical methods may also be helpful in obtaining more information.

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SUMMARY

It was found that the carbohydrate content of epicuticle preparations originates from the presence of contaminating cellulosic fibres. Purer preparations can be obtained by mechanical and chemical means without altering the essential properties of the epicuticle.

In contradistinction to the epicuticle of wool, ovokeratin contains a small percentage of carbohydrate. Among the hydrolysis products, galactose, glucose and mannose were identified by paper chromatography.

RÉSUMÉ

Les glucides que l'on trouve dans les préparations de l'épicuticule de la laine, proviennent des impuretés; à l'aide de l'examen microscopique on a pu constater la présence de fibres cellulosiques. Par certaines méthodes chimiques et mécaniques on a pu obtenir des préparations plus pures sans changer les propriétés essentielles de l'épicuticule.

Contrairement à l'épicuticule de la laine, l'ovokératine contient une certaine quantité de glucide. A l'aide de la chromatographie sur papier on a pu identifier parmi les produits d'hydrolyse le galactose, le glucose et le mannose.

ZUSAMMENFASSUNG

Der Kohlenhydratgehalt von Epikutikulapräparaten stammt aus celluloseartigen Verunreinigungen. Es ist möglich reinere Präparate zu bekommen ohne die wesentlichen Eigenschaften der Epikutikula zu ändern.

Im Gegensatz zur Epikutikula enthält Ovokeratin einen kleinen Prozentsatz Kohlenhydrat. Unter den hydrolytischen Spaltprodukten wurden Galaktose, Glukose und Mannose mittels Papierchromatographie nachgewiesen.

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Received July 30th, 1952