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Studies on the Chemistry of Oil Tanning. II. On the Chemical Nature of the Bonds between Peroxidized Fats and Collagen

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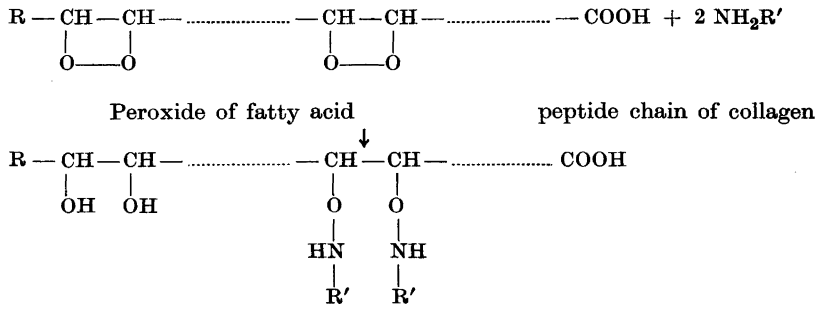
As mentioned in the preceeding paper, the most generally accepted theory of the chemistry of oil tanning was advanced by Fahrion [1], who suggested that peroxide groups in the fats are the reactive groups which combine with the collagen of the skin. In our experiments we were able to confirm Fahrion's views. As to the nature of the bonds between the peroxidized fats and collagen, Fahrion thinks that peroxides react with collagen ($R'NH_2$) in a manner which can be summarized as follows:



On this theory oil tanned leather should be a derivative of hydroxylamine. This seems *a priori* very improbable, and, as has been pointed out by Gnamm [2], has never been proved. Furthermore, it is a well-known fact that the tanning process takes place only at an acid pH. For instance, L. Klenow [3] followed the pH during oil tanning and found that it decreased from 12 to 3.5 and that the tanning did not start until the pH had decreased to 5.5. In the preceeding paper we reported on a similar observation: that it was not until the acid number began to increase rapidly, *i.e.*, the alkali in the skin had been neutralized, that vigorous peroxidation and the tanning process itself commenced. These facts make it appear highly improbable that the peroxide groups of the fats do react at all with the amino-groups of the collagen since, on the acid side of the isoelectric point of collagen (about pH 4.8) only a very small proportion of free amino-groups should be found.

Estimation of the amount of fixed fatty acids. Samples of skin and leather were extracted with petrol ether and ethyl ether to remove free fat. The ash contents of the dried samples were determined by incineration and the nitrogen contents by the Kjeldahl method. The skin contained 3.3 % ash and 17.27 % N, corresponding to 17.80 % N on the basis of the organic matter. The leather contained 3.3 % ash

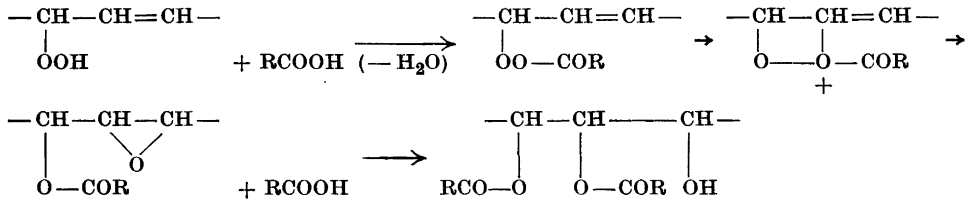
and 16.49 % N, or 17.05 % N in the organic matter. The lower nitrogen content of the leather must be accounted for by the fatty acids bound firmly to the skin, and from the equation $17.05 \times 100 = 17.80\ (100-x)$, the percentage of oil may be calculated to be 4.2. From the saponification value of the oil, 185, it can be calculated, further, that this corresponds to about 0.14 mmol of fatty acids per gram of leather.

Determination of amino-N in skins and leather. If the tanning process involved a reaction with free amino-groups the number of such groups should decrease during the process. We therefore made determinations of amino-N in samples of untanned skin and of leather, using the Van Slyke manometric method. The samples of the skin and leather were treated in exactly the same way. They were dried carefully, then mixed with dry ice and milled three times in a small coffee-mill whereby a fine powder was easily obtained. For the determinations, 0.5 g of powder was suspended in 10 ml of freshly boiled distilled water and introduced into the reaction vessel of the Van Slyke apparatus through the dropping funnel which was afterwards rinsed with 10 ml of water. The reaction vessel was shaken occasionally and the nitrogen evolved during two hours was measured.

In the samples of sheep skin 0.35, 0.40, and 0.39 (average 0.38) mmol of amino-N per g of skin was found. This value is of the same order as those found by earlier investigators [4].

In three corresponding samples of leather 0.35, 0.38, and 0.32 (average 0.35) mmol of amino-N per g of leather was found. The values vary appreciably probably due to the fact that the powders had a great tendency to adhere to the walls of the funnel and form lumps. On the other hand, it is clear that there has been no significant decrease in the number of amino-groups, or at least a much smaller one than the mole fraction of fatty acid fixed.

The considerations mentioned above make it probable that the hydroperoxide groups react with the carboxyl groups of collagen. For the reaction between fat peroxides and an organic acid, Farmer *et al.*[5] have proposed the following mechanism:



An analogous reaction might take place in oil tanning as a result of which a fatty acid molecule would become attached to one or to two peptide chains, in the latter case forming a bridge which would account for the stabilization of the collagen molecule.

Attempts to determine the fixed fatty acids. According to the above scheme, the linkages between the fatty acids and collagen are of an ester character and thus should be saponifiable. An attempt to titrate them was made in the following way. Samples of defatted leather weighing 1—2 g were treated in 100 ml Erlenmeyer flasks equipped with reflux condensers with 20 ml of 0.1 *N* NaOH for two hours at 50—52° C, a temperature well below the shrinkage temperature of collagen. After cooling, 20 ml of 0.1 *N* HCl were added, the flasks were vigorously

shaken for 15 minutes, and the contents were titrated with 0.1 *N* NaOH using phenolphthalein as indicator. An average of 4.49 ml of 0.1 *N* NaOH per g of leather was required, while, without heating, 1,41 ml was used to titrate the acid groups of 1 g leather. From the difference between these two figures it can be calculated that saponification liberates 0.31 mmol carboxyl per g of leather. This figure is of the same order as that expected if one mole of fatty acid combines with two carboxyl groups (2 × 0.14 = 0.28). It should be mentioned that during the alkali treatment the leather lost its characteristic yellow colour, and on drying got the same hard consistency as untanned collagen. Thus it appears that the leather has probably become detanned, in accord with the theory of formation of ester-like bonds during the tanning process.

Conclusion. Our observations point to the conclusion that the essential chemical process in oil tanning is the formation of peroxide groups which then combine with the carboxyl groups of the peptide chains of the collagen molecule. Newer concepts of tanning require the formation of bridges between the peptide chains for the stabilization of the collagen molecule. The following suggests a hypothesis which is in accordance with our observations:

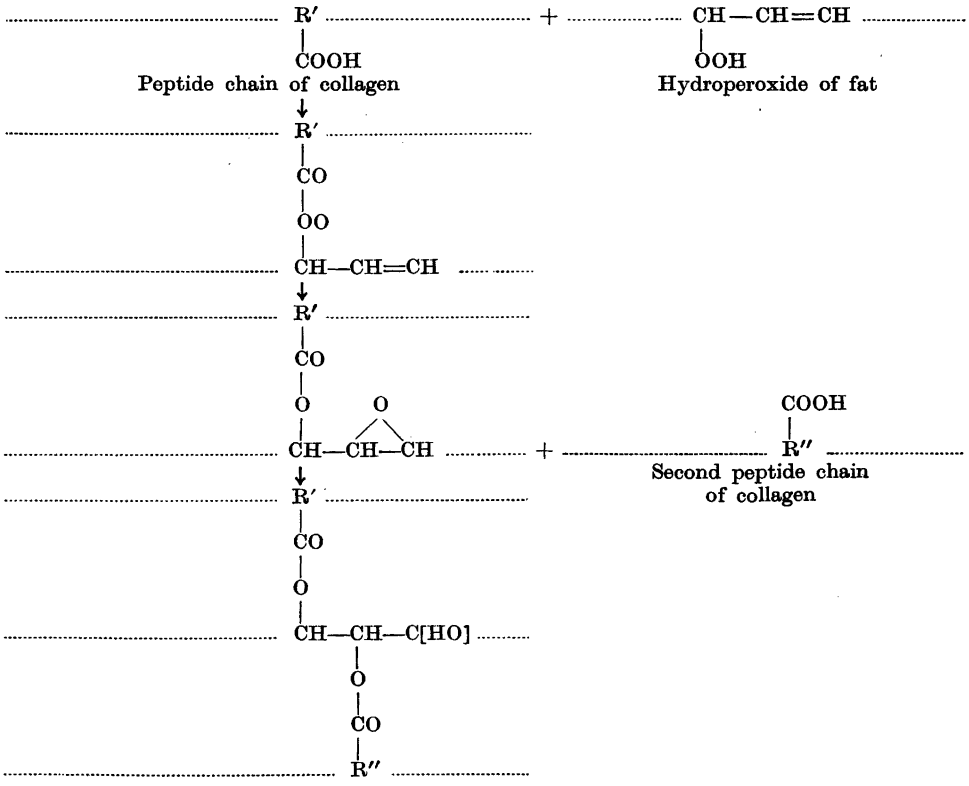


Fig. 1. Tentative structure of oil tanned skin.

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