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BLOCKING OF THE FEULGEN REACTION

(A preliminary report)

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It has been shown in a preliminary report [3] that the reductivity of plasmalogens and periodate-peracetate-Schiff-positive lipids can easily be eliminated by reduction of the carbonyl group. In those studies the reduction of the oxo-group was carried out at room temperature with $NaBH_4$ (sodium borohydride) dissolved in borate buffer pH 7.6. This procedure has never failed to yield reliable results.

The investigations were later extended to the oxidation products of poly- and mucopolysaccharides which exhibit reducing properties. A detailed account of the results will be published in the near future [4].

The present studies were devoted to the selective blocking of the Schiff—Feulgen positive apurinic acid arising upon acid hydrolysis of the nucleic acids in cell nuclei. According to present knowledge, the reaction of acid-treated DNA with leucofuchsin is due to the aldehyde carbonyl group formed at the first C-atom of deoxyribose simultaneously with the splitting of purine rings [7]. The blocking reactions of the addition and condensation type [6], and the reduction of the aldehyde group with aluminium-isopropoxide, as proposed by Lhotka and Davenport [5], did not become widespread because of technical difficulties. Therefore, attempts were made to work out a new method for the reduction of the hypothetical aldehyde group of deoxyribose and thereby for the blocking of the Feulgen reaction.

Of the complex metal hydrides widely used in recent years for different purposes, $NaBH_4$, because of its selectivity, water solubility and high reactivity, seemed promising for the reduction of oxo-groups under histochemical conditions [1]. $NaBH_4$, under mild experimental conditions, transforms the aldehyde and oxo groups into primary and secondary alcohols and thus the reducing power of the original molecules is lost.

The optimal conditions for blocking of the Feulgen reaction were studied on different organs of the rat and on malignant tumours of various origin. The materials were treated with alcohol or formaldehyde and embedded in paraffin. The formation of the oxo group of deoxyribose was induced by acid hydrolysis with n HCl at 56° C for 10 minutes. The sections were then treated

with $NaBH_4$ (1 mg/ml) dissolved in borate buffer pH 7.6 at room temperature for 30 minutes. After washing with water the leucofuchsin reaction was carried out with treated (reduced) and control sections. The treatment of both normal and malignant tissues resulted in a complete inhibition of the Schiff—Feulgen reaction.

On the basis of the well known selectivity of $NaBH_4$ towards oxo-groups it seems safe to conclude from the results presented that the aldehyde group of the deoxyribose molecule plays a major role in the Feulgen reaction.

It should be stressed that under the experimental conditions referred to above the submicroscopic structure of apurinic acid formed after treatment with acid underwent no marked changes due to the blockage. This was indicated by the identical rivanol anistropy of the $NaBH_4$-treated samples and authentic apurinic acid [2].

Further studies are in progress in connection with the possible application in histochemical studies of other complex metal-hydrides exhibiting a wider spectrum.

SUMMARY

$NaBH_4$ has been found selectively to block the oxo-groups responsible for the Feulgen—Schiff positivity of tissue nucleic acids.

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