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Histochemical Reactions for Lipid Aldehydes and Ketones.*

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Bennett[1,2] published a histochemical method for the demonstration of the keto-steroid hormone of the adrenal cortex. The method is based on the formation of a yellow phenylhydrazone on treatment of frozen sections with phenylhydrazine. The reaction is prevented by extraction of the lipids with alcohol or by pretreatment with semicarbazide. From these facts Bennett draws the conclusion that the reaction is due to the presence of a lipid ketone, probably a keto-steroid, "since no aldehydes with the solubility properties of corticosterones have been detected in the adrenal cortex."

Feulgen and his collaborators[3-5] published a series of papers in which they showed the presence in many tissues of a loosely bound aldehyde which they called plasmal. This aldehyde is of lipid nature, and can be liberated from the compound of which it is a component by mild hydrolysis or oxidation. The best reagent for plasmal is Schiff's reagent, fuchsinesulfurous acid, which stains it in an intense purplish shade. Plasmal will also give very promptly all the typical aldehyde reactions, *e.g.*, it forms a bisulfite addition product, a phenylhydrazone, a semicarbazone and a thiosemicarbazone. Further studies showed that plasmal is a rather complex mixture, its bulk consisting of palmitic and stearic aldehydes.

On the basis of these facts Verne[6] worked out a microtechnical method for the demonstration of plasmal in tissue sections, and gave a description of the pictures obtained. Large amounts of plasmal were found in many tissues containing lipids, especially phospholipids. Verne stresses the intense and widespread aldehyde reaction in the adrenal cortex.

As it seemed very probable that Bennett's stain was nothing but a plasmal reaction, the pictures obtained in adrenal glands by the two reactions under various conditions were compared.

The results were the following: oxidation is an important factor in both procedures. Entirely fresh tissues, or tissues fixed for 6 hr in formalin prepared with freshly boiled distilled water and protected from contact with air, do not react visibly either with Schiff's reagent or with phenylhydrazine for several hours. However, both technics prescribe oxidation of the sections before applying the reagent. With the plasmal technic oxidation (or possibly hydrolysis) is catalyzed by mercury bichloride, with Bennett's technic either iodine or aeration in an oxygen-saturated buffer solution is used. During these procedures the acetal linkages break up, and both reactions become positive. Long exposure of the tissues to solutions such as formaldehyde or distilled water, unprotected from air, will have the same effect as direct oxidation. The extent and intensity of the reaction will increase progressively for about 5 to 6 days. The pictures obtained by the two methods were invariably identical, except for the shade. This applies to all species examined (man, dog, cat, guinea pig). As there is a great individual variation in the pattern of the plasmal reaction in human adrenals, it was especially easy to compare the pictures in a considerable detail.

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1 Bennett, S. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 786.

2 Bennett, S. H., *Am. J. Anat.*, 1940, **67**, 151.

3 Feulgen, R., and Voit, K., *Arch. ges. Physiol.*, 1924, **206**, 389.

4 Imhäuser, K., *Biochem. Z.*, 1927, **186**, 360.

5 Feulgen, R., and Behrens, M., *Z. physiol. Chemie*, 1928, **256**, 15.

6 Verne, J., *Ann. de physiol.*, 1929, **5**, 245.

Moreover, sections from different kinds of necrobiotic tissue (tubercles, infarcts, tumors) which show a very intense plasmal reaction were equally positive with Bennett's stain, and the pattern of distribution shown by the two reactions was again identical. The same applies to myelin sheaths both in the brain and in peripheral nerves, although neither necrotic tissues nor myelin are known to be rich sources of ketosteroid hormones.

While ketosteroids under the conditions of Bennett's reaction do give phenylhydrazones similar to that of plasmal, the method is by no means specific for them, the less so as even a positive ketosteroid reaction would be completely masked by the incomparably more intense plasmal reaction. Only a ketone test which is directly positive without any previous oxidative treatment of the tissues can be accepted as a presumptive evidence for the presence of ketosteroids, if the possibility of a false positive reaction due to hydrolysis of plasmal can be ruled out.

Summary. Bennett's histochemical test for ketosteroids in the adrenal cortex is not a specific reaction since it merely indicates the presence of lipid bodies having aldehyde or keto groups. Lipid aldehydes, not related in any way to ketosteroids, are present in large amounts in the adrenal cortex.

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Absorption of Carbohydrates in Humans.*

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The rate of absorption of glucose from the intestinal tract has been generally considered to be independent of both the concentration of glucose, and the absolute amount of glucose present in the intestine. However, observations on the absorption of various hexoses have revealed that the rate of absorption varies in the following order: galactose>glucose>fructose>mannose>xylose>arabinose.[1] The investigation by Cori was conducted on rats and the technic consisted of the oral administration of various carbohydrates in various dosages. After definite intervals the animals were sacrificed and the carbohydrate remaining in the intestine was determined.

In our own studies on insulin shock treatment, it was observed that the time required for the patient to rouse from coma was dependent on the concentration of glucose administered. In the ordinary procedure for insulin shock treatment, patients are awakened by the oral administration of 600 cc of 30% glucose, and they regain consciousness in about 15 to 30 min. Frequently patients failed to rouse from coma with 30% glucose and required the administration of glucose intravenously. In such patients, almost invariably, the administration of 600 cc of 5% glucose terminated the coma within a reasonable time. It was therefore thought of interest to determine the rate of absorption of glucose solutions varying from 5 to 30% concentrations.

Method. Patients were chosen from the wards of Bellevue Psychiatric Hospital, who were to all intents and purposes physically normal. The diagnoses of these cases included psychopathic personality and recovered reactive depressions in whom the situational defect causing the difficulty had been alleviated while the patient was in the hospital. On the morning of the experiment, patients received no breakfast and after a one-half hour rest in bed, a fasting blood sample was drawn from the antecubital vein. A 30 g dose of glucose in concentrations varying from 5 to 30% was administered by mouth and blood samples were obtained at intervals of 10, 15, 30, and 60 min. Blood sugars were

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1 Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.