HISTOCHEMISTRY

Variation in Lyoglycogen in Starved and Normal Guinea Pigs

IT has been shown that the histochemical staining reaction for tissue glycogen depends on the amount of lyoglycogen present in the tissues[1]. The present communication is a preliminary report of the nature of lyoglycogen. Lyoglycogen was obtained from the liver of six guinea pigs, two of which had been starved for 72 hr. and others that had had a normal diet. Hydrolysis of the resultant samples of lyoglycogen was carried out by refluxing with $N/1$ sulphuric acid at 100° C. for 8 hr. The acid solution was neutralized with barium carbonate, filtered and concentrated *in vacuo*. The solutions were applied to Whatman 3 *MM* papers which were developed in *n*-propanol–ethylacetate–water for 15–18 hr. Locating-reagents used were aniline-diphenylamine and silver nitrate.

Glycogen will yield glucose and small quantities of isomaltose on hydrolysis; sometimes other oligosaccharides are present due to acid reversion. The presence of isomaltose in hydrolysates of glycogen is taken to indicate 1–6 linkages in that molecule.

Fig. 1 shows a typical chromatogram. Samples of glycogen from the animals that were well fed yielded glucose and isomaltose in the hydrolysate. Although no quantitative estimates of the amount of isomaltose present in the glycogen hydrolysates from the well-fed animals were made, some indication of the amount of isomaltose present compared with the amount of glucose present may be given by the depth of staining on the chromatogram. The yield of isomaltose from these samples of glycogen seems to be far in excess of the expected quantity or of any that could be produced by acid reversion, consequently the number of 1–6 linkages in the parent glycogen would be greatly increased and may be identifiable with that part of the glycogen molecule or separate polysaccharide that Rosenfeld[2] described as having 33–47 per cent of 1–6 linkages.

The glycogen from the starved animals yielded only glucose. The absence of isomaltose in the hydrolysate obtained from the starved animals suggests a basic difference in the structure of the glycogen molecule in these conditions in that 1–6 linkages are absent.

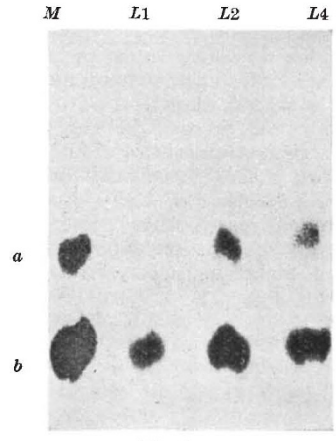

Fig. 1

Fig. 1. *M*, marker; *a*, isomaltose; *b*, glucose; *L1*, glycogen hydrolysate from starved animal (liver glycogen 242 μgm./100 mgm. liver wet weight). *L2*, *L4*, glycogen hydrolysate from fully fed animals (*L2*, liver glycogen 1,130 μgm.; *L4*, liver glycogen 1,012 μgm./100 mgm. liver wet weight). Note absence of isomaltose in *L1* and excessive amounts of isomaltose in *L2* and *L4*

From this preliminary work on glycogen that can be detected histochemically, it seems that the nutritional state of the animal has some influence on the structure of the glycogen molecule.

We thank Dr. W. J. Wheelan for his valuable suggestions and his gift of the disaccharide isomaltose.

W. J. C. WILKINSON
J. H. KUGLER

Department of Anatomy,
The University,
Sheffield, 10.

[1] Kugler, J. H., and Wilkinson, W. J. C., *J. Histochem. Cytochem.*, **8**, 195 (1960).
[2] Rosenfeld, E. L., *Biokhimiya*, **23**, 877 (1958).

HÆMATOLOGY

Specific Antigens of Human Leucocytes and Thrombocytes

ATTEMPTS have been made to classify the specific antigens of human white blood cells and platelets, by using immune sera prepared in rabbits[1,2], sera from pregnant multiparous women[3,4] or from patients receiving multiple transfusions[5,6]. The different methods used by the various authors led often to inconsistent results.

In the present work immune sera from rabbits, monkeys and multiparous pregnant women have been used and compared using different serological methods.

Results obtained with rabbit anti-human leucocyte and thrombocyte sera showed the presence of common antigenic constituents of human erythrocytes, leucocytes and thrombocytes. These species specific antibodies of low titre (1/20–1/80) could easily be removed through appropriate absorptions. Common antigenic properties of thrombocytes and leucocytes of various species could also be observed. These antibodies, which were cell-specific for man, rabbit, mouse, sheep, guinea pig and rat, could be absorbed easily.

The titres obtained in rabbits against human leucocytes and thrombocytes were 1/640–1/1,280. The anti-leucocyte sera prepared against the leucocytes of a single donor reacted with leucocyte samples from every other human donor although with varying intensity, which may be due to differences in the agglutinability of the cells, with no correlation to their antigenic structure. The absorption of the serum with cells of any donor abolished completely the titre against all other donors. Similar results were obtained with thrombocytes.

The immune rabbit sera reacted strongly using the direct agglutination, agar-gel precipitation and tanned cell hæmagglutination tests. No correlation has been found between the intensity of agglutination of the leucocytes and the thrombocytes of the same donor.

The reactions obtained with the monkey anti-human leucocyte and anti-thrombocyte sera were similar to those obtained with the rabbit sera using the direct agglutination technique. The monkey anti-sera gave weaker reactions with the agar precipitation and the tanned cell hæmagglutination techniques.

The results obtained with sera of multiparous pregnant women differed significantly from those obtained with the animal anti-sera. Only direct