remains unknown, although our present preliminary data would suggest that it occurs after 20 weeks of gestation. We are studying more fetal adipose tissues at different gestational ages to determine when the reduction in the activities of the lysosomal enzymes occur in the differentiating adipose cell. If it occurs after the cell had fully differentiated, it may be possible to determine if the reduction in the lysosomal hydrolases is primary or secondary to the process of lipid accumulation in the cell.

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Purification and partial characterization of a human plasma α_1-heteroglycan

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Summary. A heteroglycan was purified from human plasma and partially characterized in terms of its major properties. It is noteworthy that the carbohydrate content of this blood constituent is unusually high (75%).

During our studies on the very soluble macromolecular constituents of human plasma, we have recently discovered a nondialyzable heteroglycan which distinguishes itself by an unusual chemical composition. The present paper describes the isolation, purification and some of the major chemical and physicochemical properties of this blood polysaccharide. *Isolation of the heteroglycan.* Pooled normal plasma (250 l) was fractionated according to Cohn's method 6[2]. After removal of the 5 major protein fractions[2] a supernatant solution[3] (400 l) was obtained which contained, in addition to low mol.wt acidic[4], neutral[5] and basic[6] proteins, the hitherto unknown heteroglycan. For the isolation of this heteropolysaccharide, 200 g of CM-cellulose previously equilibrated against pH 5.5, $\Gamma/2$ 0.05 sodium acetate buffer was mixed with the mentioned supernatant solution, stirred overnight and allowed to settle. Because of the presence of the high concentration of ethanol in this solution (40%), the isolation procedure was carried out at -5 °C. Subsequently, the CM-cellulose with the adsorbed protein was suspended at 4 °C in the above mentioned buffer and centrifuged. This technique which was repeated once, led to the removal of the bulk of ethanol and protein. The washed CM-cellulose was then transferred in the cold into an appropriate column and eluted with the same buffer but employing a NaCl gradient (figure). The first fraction contained primarily albumin and the second, in addition to the latter protein, α_1- and β-globulins. Fraction 3, however, contained essentially a heteroglycan (0.25 g) which on paper electrophoresis stained intensely with PAS and weakly with amidoblack. Fractions 4 and 5 contained albumin and α_1-globulins, respectively.

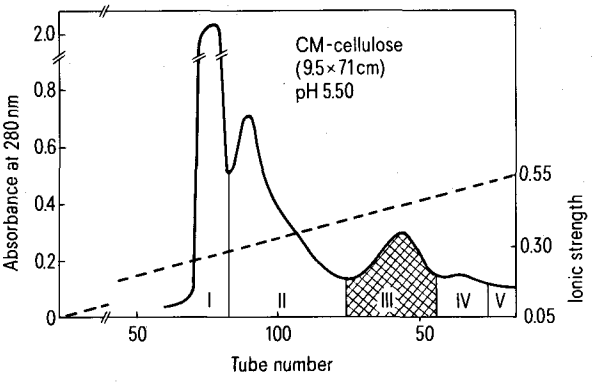
Purification of the plasma heteroglycan. Rechromatography of fraction 3 under the same conditions as described above afforded the removal of a considerable portion of the contaminating proteins at low ionic strength while the carbohydrate was displaced at higher ionic strenght. Other proteins (β_1- and γ-globulins and basic proteins) were eluted at even higher salt concentrations.

Partial characterization of the plasma heteroglycan. Homogeneity of this plasma heteroglycan was established by paper (pH 8.6, $\Gamma/2$ 0.1 citrate-barbiturate buffer) and disc

Composition of human plasma α_1-heteroglycan

Physicochemical properties	
Molecular weight	70,000
Sedimentation constant (S)	3.6
Electrophoretic mobility[a] ($cm^2 \cdot volt^{-1} \cdot sec^{-1}$)	-4.5×10^{-5}
Chemical properties	
Sialic acid (%)	25
Neutral hexoses (%)	25
Hexosamines (%)	25
Total carbohydrate content (%)	75
Peptide moiety (%)	30

[a] At pH 8.6 $\Gamma/2$ 0.1 citrate-barbiturate buffer.



Chromatography on CM-cellulose of a crude heteroglycan preparation isolated from normal human plasma. A flow rate of 200 ml/h was employed and 18-ml fractions were collected. The proteins and the heteropolysaccharide were eluted at a constant pH of 5.5 using an ionic strength gradient (initial $\Gamma/2$ 0.05 as NaAc and final $\Gamma/2$ 0.55 as 0.05 NaAc plus 0.50 M NaCl). Appropriate fractions were pooled as indicated by the roman numerals. Fraction III (cross-hatched) contained the heteroglycan.

(running pH 9.5, $\Gamma/2$ 0.01 Tris-glycine buffer, 7.0% gel, 2 mA per tube for the first 15 min then 5 mA for further 50 min) electrophoresis. Monodispersity was also observed on ultracentrifugation. The mol.wt was determined by sedimentation equilibrium analysis (12,590 rpm, 80 min and 20 °C)[7]. The major physicochemical and chemical properties of this plasma heteroglycan are listed in the table. The mentioned intense staining characteristic of the heteropolysaccharide with PAS is considered to be due to the high sialic acid content, and the lack of staining with amidoblack appears to reflect a low polypeptide content, properties which were confirmed by chemical analyses. It should be noted that the above described heteroglycan distinguishes itself not only by its sialic acid content but also by its total carbohydrate content which exceed those of the well characterized human plasma glycoproteins[8].

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Effects of alloxan on orotic acid and glycogen content in various vertebrate species

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Summary. Alloxan treatment induces a decrease of orotic acid content in various organs of carp, frog, pigeon and rat, parallel to a decrease of liver and muscle glycogen content. Loss of orotic acid and glycogen cannot be prevented by orotic acid and carbamyl phosphate given i.p. Mice, rats and pigeons use up and excrete exogenous orotic acid rapidly, but carps and frogs accumulate it.

It was found by Hurlbert et al.[1,2] that rat and pigeon livers produce UDPG from ^{14}C-orotic acid both in vivo and in vitro. Leloir et al.[3] later established that liver synthesizes glycogen from UDPG with the help of glycogen synthetase, the activity of which is enhanced by insulin in rat heart[4], rat diaphragm[5] and adrenal gland[6] and dog liver[7]. Steiner and King[8,9], and Losert et al.[10] observed the increase of glycogen synthetase activity in insulin-treated diabetic rats. Younathan et al.[11] reported that alloxan inhibited the in vitro synthesis of UDP and UTP, while Methfessel et al.[12] tried to prevent alloxan diabetes with orotic acid.

We suppose that the effect of alloxan might be connected with the structural similarity of the pyrimidine bases and alloxan. Therefore we have studied the change of orotic acid content in various organs of carp, frog, pigeon, mouse and rat as a result of alloxan treatment. Since after a treatment with orotic acid an increased glycogen content was found in rat liver by Sidransky et al.[13], and in mouse and catfish liver by Fekete and Toth[14], the relationship of orotic acid to glycogen synthesis has also been studied in alloxan-treated animals.

Materials and methods. Our experiments have been performed with mice (CFLP strain, weighing 28–30 g), rats (CFY strain, weighing 220 g), frogs (*Rana esculenta*, weighing 90–100 g; time of the experiment: May), carps (*Cyprinus carpio*, weighing 360–380 g; time of the experiment: April), and pigeons (*Columba livia* var. *domestica*). 1 group contained 10 animals of both sexes. 20 mg/kg orotic acid was injected into each animal once a day. The same amount of carbamyl phosphate was administered i.p. in physiological saline, while 150 mg/kg of alloxan was added. Monosodium orotate was obtained from ICN K & K Laboratories, Plainview, N.Y. USA, lithium salt of carbamyl phosphate from Serva Feinbiochemica, Heidelberg, FRG, and alloxan from 'Reanal' Budapest, Hungary.

1 experiment took 7–8 days. Blood sugar of mice, rats and pigeons was determined by means of Hultman's method[15] and that of frogs and fishes according to Ek and Hultman[16]. Orotic acid was estimated by the combined method of Schulzek et al.[17] and Kesner et al.[18]. Since, in accordance with Mordoh et al.[19] it was found that in the case of fishes, frogs, turtles and birds glycogen of 700–1200 sedimentation coefficient can be obtained with the help of the $HgCl_2$ method[20], this extraction method and anthrone reagent[21] was used for glycogen assay.

Results and discussion. After the alloxan treatment, orotic acid content decreased in all the organs investigated. This decrease varied from 30 to 60%, and, as it is shown in figure 1, there was no great difference between the orotic acid content of the pancreas or other organs of rat, pigeon and frog, i.e. the effect of alloxan is not specific to the pancreas. Figure 2, however, shows that the glycogen content of the liver of alloxan-treated animals except pigeon also decreases, which indicates a definite connection between the change of orotic acid and glycogen.

On the 6th or 7th day of alloxan treatment, blood sugar increased slightly in mice and rat. If simultaneously carbamyl phosphate was also injected, 500–600 mg% blood sugar was found. In case of mice and rats, as a result of alloxan plus carbamyl phosphate intoxication, death was more frequent, whereas in pigeon loss of body weight indicated that the toxicity of alloxan is enhanced by carbamyl phosphate.

Exogenous orotic acid does not alleviate the loss of orotic acid and glycogen of alloxan-treated animals. No increase of orotic acid content was found in pigeons 24 h after orotic acid application in rats and mice after orotic acid plus alloxan administration. So exogenous orotic acid is evidently utilized and/or excreted by these animals. On the other hand, 24–36 h after the last administration of orotic acid, or orotic acid plus alloxan, to frogs and carps, 4–10 times more orotic acid was found in their liver, muscle, blood and kidney than in those of the other animals studied. This certainly means that fishes and frogs utilize and metabolize orotic acid in different way than birds, and mammals.

In the liver of alloxan-treated rats, McLean and Novello[22] found an increased activity of the carbamyl phosphate synthetase, while Kirsten et al.[23] observed dramatic decrease of aspartate; and since in our experiment carbamyl phosphate did not moderate the decrease of orotic acid caused by alloxan, it appears that one of the reasons for the inhibition of orotic acid biosynthesis is the damage of the enzyme asparate-transcarbamylase. This is supported by the