(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].

1 This study was supported by grants from the National Institutes of Health (GM-10374), US Public Health Service.
2 E.J. Cohn, L.E. Strong, W.L. Hughes, Jr, D.L. Mulford, Jr, J.N. Ashworth, M. Melin and H.L. Taylor, J. Am. chem. Soc. *68*, 459 (1946).
3 The authors are very much obliged to Mr L. Larsen, Institute of Laboratories, Massachusetts Department of Health, Jamaica Plain, MA 02130, for the generous gift of supernatant solution of Cohn fraction V.
4 K. Schmid, in: The Plasma Proteins, vol. 1 2nd ed., p. 183. Ed. F.W. Putnam. Academic Press, New York 1975.
5 T. Ikenaka, D. Gitlin and K. Schmid, J. biol. Chem. *240*, 2868 (1965).
6 T. Iwasaki and K. Schmid, J. biol. Chem. *242*, 5247 (1967).
7 D.A. Yphantis, Ann. N.Y. Acad. Sci. *88*, 586 (1960).
8 H.G. Schwick, K. Heide and H. Haupt, in: The Glycoconjugates, vol. I, p. 262. Ed. M.I. Horowitz and W. Pigman. Academic Press, New York 1977.

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

I. Fekete

Department of Zoology and Anthropology, Kossuth University, H-4010 Debrecen (Hungary), 7 November 1977

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

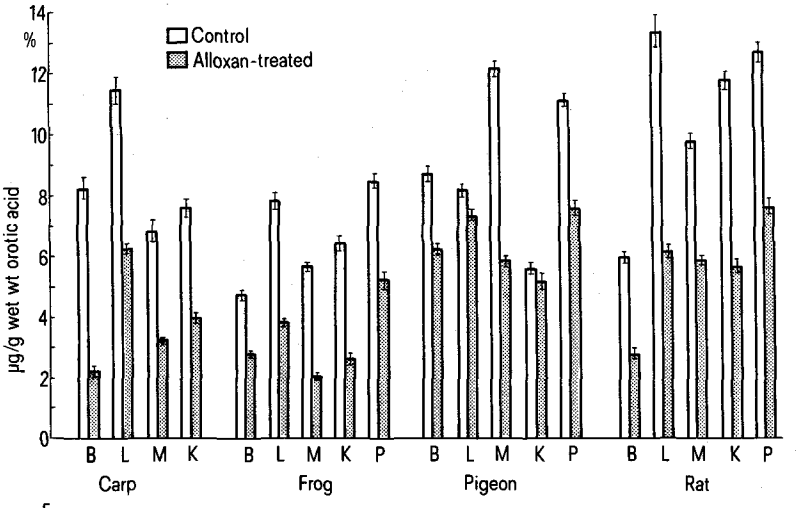

Fig. 1. Orotic acid content of the different organs of various vertebrate animals, B, blood; L, liver; M, muscle; K, kidney; P, pancreas.

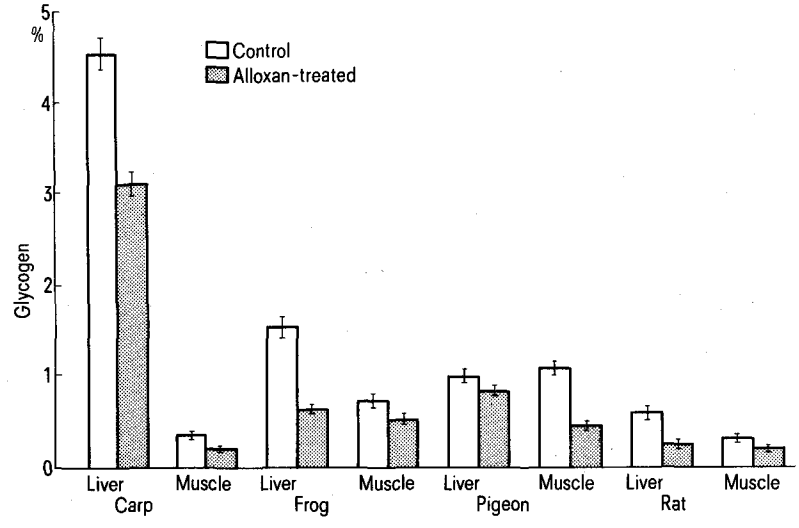

Fig. 2. Glycogen content of the liver and muscle of various vertebrate animals.

observation of Bresnick[24], who described the inhibition of aspartate-transcarbamylase by pyrimidine analogs in rat liver and hepatoma. It seems that the enzyme system catalyzing the biosynthesis of orotic acid is very sensitive to different substrates, intermediates and pyrimidine analogs. This is also corroborated by Kesner[25], who observed a considerable excretion of orotic acid in rats as a result of NH_4Cl plus ureidosuccinate administration. Large quantity of orotic acid was found by Milner and Visek[26] in the urine of rats on arginine-deficient diet. Cardoso et al.[27] found that azauridine-treated leukemia patients excrete 8 g (!) of orotic acid per day.

Our experiments indicate that, by inhibiting orotic acid biosynthesis, short alloxan treatments impair the glycogen forming system, viz. orotic acid → pyrimidine nucleotide (UTP) → UDPG. Long alloxan treatments, however, induce also diabetes as a result of the inhibition of RNA, protein and insulin biosynthesis in mammalian pancreas.

1 R.B. Hurlbert and V.R. Potter, J. biol. Chem. *209*, 1 (1954).
2 R.B. Hurlbert and P. Reichard, Acta chim. scand. *8*, 701 (1954).
3 L.F. Leloir and C.E. Cardini, J. Am. chem. Soc. *79*, 6340 (1957).
4 B.J. Williams and S.E. Mayer, Molec. Pharmac. *2*, 454 (1966).
5 G. Villar-Palasi and J. Larner, Biochim. biophys. Acta *39*, 171 (1960).
6 M.M. Piras, E. Bindstein and R. Piras, Archs Biochem. Biophys. *154*, 263 (1973).
7 J.S. Bishop and J. Larner, J. biol. Chem. *242*, 1354 (1967).
8 D.F. Steiner and J. King, J. biol. Chem. *239*, 1292 (1964).
9 D.F. Steiner and J. King, Biochim. biophys. Acta *119*, 510 (1966).
10 W. Losert, G. Senft, R. Sitt, G. Schultz and H. Kaess, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *253*, 388 (1966).
11 E.S. Younathan, J.E. Stone and T.S. Harris, J. biol. Chem. *239*, 290 (1964).
12 J. Methfessel, R. Schmidt and S. Keil, Z. ges. exp. Med. *140*, 149 (1966).
13 H. Sigransky, E. Verney and D.S. Wagle, Proc. Soc. exp. Biol. Med. *120*, 408 (1965).
14 I. Fekete and Gy. Toth, Experientia *32*, 332 (1976).
15 E. Hultman, Nature *183*, 108 (1959).
16 J. Ek and E. Hultman, Nature *181*, 780 (1958).
17 S. Schulzek, S. Schmidt, W. Pohle and N. Popov, Z. med. Labortechn. *12*, 193 (1971).
18 L. Kesner, F.L. Aronson, M. Silverman and P.C. Chan, Clin. Chem. *21*, 353 (1975).
19 J. Mordoh, C.R. Krisman and L.F. Leloir, Archs Biochem. Biophys. *113*, 265 (1966).
20 M. Szabolcs, I. Francia and I. Fekete, Acta biol. Debrecina *13*, 17 (1976).
21 S. Seifter, S. Dayton, B. Novic and E. Muntwyler, Archs Biochem. Biophys. *25*, 191 (1950).
22 P. McLean and F. Novello, Biochem. J. *94*, 410 (1965).
23 E. Kirsten, R. Kirsten, H.J. Hohorst and T. Bücher, Biochem. biophys. Res. Commun. *4*, 169 (1961).
24 E. Bresnick, Cancer Res. *22*, 1246 (1962).
25 L. Kesner, J. biol. Chem. *240*, 1722 (1965).
26 J.A. Milner and W.J. Wisek, Nature *245*, 211 (1973).
27 S.S. Cardoso, P. Calabresi and R.E. Handschumacher, Cancer Res. *21*, 1551 (1961).