EFFECT OF PYROGENAL ON THE LIVER OF RATS EXPOSED TO CARBON TETRACHLORIDE

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Pyrogenal stimulates regeneration of liver tissue in rats with hepatosis due to injections of CCl_4. The normal pattern of serum protein fractions is restored, with an increase in the albumin content and a decrease in the β-globulin content. After administration of pyrogenal to rats poisoned with CCl_4, the toxic effects of the hepatotropic poison from the liver tissue are reduced in severity, as shown by the more rapid normalization of the serum albumin and β-globulin content during spontaneous recovery.

Much attention has recently been paid to the stimulation of regeneration of hepatic tissue as a method of treatment of cirrhosis of the liver.

Accordingly, in the present investigation a study was made of the effects of high-molecular weight compounds such as lipopolysaccharides of bacterial origin (pyrogenal) [1, 2] on regeneration of liver tissue. According to some reports, lipopolysaccharides of higher plants are capable of stimulating liver regeneration [8].

EXPERIMENTAL METHOD

Experiments were carried out on 80 female albino rats initially weighing 180 g. Liver damage was produced by subcutaneous injection of carbon tetrachloride (CCl_4) in a dose of 0.1 ml/100 g body weight, 3 times a week for 3 months. The experimental animals were divided into two groups: group 1 (44 rats) received CCl_4 only; group 2 (36 rats) received pyrogenal by intramuscular injection in a dose of 20 m.p.d. (minimal pyrogenic dose) per animal 30-40 min before injection of the hepatotropic poison, as a measure of protection. The control group consisted of 20 healthy rats kept under the same conditions. After the end of the course of injections of CCl_4, the rats of group 1 were divided into two subgroups: A) control (14 rats), which were left to recover spontaneously, and B) experimental (14 rats), which received pyrogenal. The rats of group 2 were also divided into two subgroups: C) control (14 rats), allowed to recover spontaneously, and D) experimental (15 rats), which received pyrogenal. Pyrogenal was injected intramuscularly three times a week in doses of 20 m.p.d. per animal. Two courses of treatment were given, the first lasting 1.5 months, and then after an interval of ten days, the second course was given, lasting two months.

The total serum protein content was determined refractometrically, and the protein fractions by paper electrophoresis. The serum cholinesterase activity was determined by Pokrovskii's method [7], and histidase activity by Tabor and Mehler's method as modified by Mardashev and Burobin [5].

Pieces of liver tissue were fixed in Lillie's fluid and embedded in paraffin wax, while some pieces were cut into sections on a freezing microtome. Sections were stained with hematoxylin-eosin, picro-fuchsin-fuchselin, toluidine blue, and Sudan III-IV. The reactions of Brachet, Einarson, and Feulgen and the PAS reaction were carried out. Glycogen was determined by the methods of Shabadash (using a salivary amylase control) and Best.

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TABLE 1. Serum Protein Fractions of Rats with Experimental CCl_4 Hepatosis

Groups of animals	Content of: total protein, %	albumin, %	globulins, % α_1	α_2	β	γ
	After end of course of CCl_4 injections					
1	7,16±0,20	40,3±2,64	13,3±1,17	7,5±0,46	20,9±1,65	18,0±0,78
P	>0,1	<0,01	>0,1	>0,05	<0,01	>0,5
2	7,19±0,22	40,6±2,45	12,3±0,89	7,8±0,99	19,5±1,26	19,9±0,96
P	>0,2	<0,01	>0,2	>0,1	<0,02	>0,1
Healthy	7,46±0,08	48,0±0,55	11,2±0,51	6,4±0,34	16,3±0,45	18,1±0,48
Subgroup	First course of pyrogenal injections					
A—control	7,34±0,23	41,9±1,96	14,4±0,93	6,1±0,59	19,3±1,09	18,3±0,88
P	>0,2	<0,01	<0,01	>0,5	<0,02	>0,5
B—injection of pyrogenal	7,20±0,22	47,4±0,80	12,3±0,53	5,1±0,43	17,1±0,48	18,2±0,81
P	>0,2	>0,5	>0,1	<0,02	>0,2	>0,5
C—control	7,43±0,08	47,5±1,23	13,1±0,59	5,7±0,34	17,6±0,94	16,3±0,79
P	>0,2	>0,5	<0,05	>0,1	>0,2	>0,05
D—injection of pyrogenal	7,25±0,26	47,1±0,98	11,9±0,45	5,7 ±0,21	17,1±0,29	18,2±1,28
P	>0,2	>0,5	>0,2	>0,1	>0,1	>0,5
	Second course of pyrogenal injections					
A—control	7,23±0,15	40,6±0,70	13,7±0,73	7,3±0,59	19,0±1,01	19,4±1,16
P	>0,2	<0,001	<0,01	>0,1	<0,02	>0,2
B—injection	7,30±0,09	46,3±1,93	13,4±0,95	5,7±0,41	16,8±1,33	17,8±0,87
P	>0,1	>0,2	>0,05	>0,1	>0,5	>0,5
C—control	7,33±0,25	45,4±1,73	13,3±0,67	5,9±0,49	17,3±0,93	18,1±1,09
P	>0,2	>0,1	<0,02	>0,2	>0,2	>0,5
D—injection of pyrogenal	7,46±0,16	46,1±1,88	12,9±0,69	5,7±0,44	16,9±0,74	18,4±1,24
P	>0,2	>0,2	>0,5	>0,2	>0,2	>0,5

Note. P denotes significance of difference from group of healthy rats.

EXPERIMENTAL RESULTS

At the end of the course of CCl_4 injections, the animals of groups 1 and 2 developed hepatosis. At autopsy the surface of the liver was covered with large and small nodules. The mass of the liver was increased. Its relative weight in the rats of group 1 was 4.5%, in the animals of group 2, which received pyrogenal during the period of CCl_4 injections, the same pattern of hepatosis developed, but the relative weight of the liver in the rats of this group was slightly less (4%). The relative weight of the liver of the healthy rats was 3%.

Morphological investigation revealed a picture of widespread fatty infiltration of the cytoplasm of the hepatocytes, with atrophy, necrobiosis, and necrosis of the hepatocytes, a sharp decrease in the glycogen content in these cells, and thickening of the interlobular connective-tissue septa.

In the animals of both groups, hypoalbuminemia and an increase in the content of β-globulins were observed (Table 1). In the animals of group 1, receiving CCl_4 only, a sharp decrease in cholinesterase activity was observed both in the serum (2.9±0.24, compared with a normal 7.04±0.29; $P < 0.001$), and in

liver tissue homogenate (197±26.32; normal 293±25.49; $P < 0.01$). The serum histidase activity was increased several times over (3.35±0.39; normal 0.74±0.04; $P < 0.001$). The level of histidase activity in liver tissue homogenate was unchanged (383±56.93; normal 375±44.5).

As a result of pyrogenal treatment, a gradual disappearance of the fatty degeneration, an increase in the number of amitotically dividing cells, and thinning of the connective-tissue bands between the liver lobules were observed in the animals of subgroups B and D. Large hepatocytes, with a strong reaction of the cytoplasm when stained by Brachet and Einarson's methods and a strong reaction of the nucleus when stained by Feulgen's method, appeared. Large quantities of glycogen accumulated in the cytoplasm of these large hepatocytes.

In the course of treatment with pyrogenal, its beneficial effect was also revealed on the blood proteins (Table 1, subgroups B and D), as shown by an increase in the albumin content and a decrease in the β-globulin content to normal. Administration of pyrogenal during the actual induction of CCl_4 hepatosis (Table 1, group 2), did not prevent the disturbance of the protein-synthesizing function of the liver produced by CCl_4. After administration of CCl_4 had stopped, and without subsequent treatment by pyrogenal, the animals of control subgroup C showed the same tendency toward normalization of the serum protein picture during resolution of the hepatosis as those of subgroups B and D. It was only in control subgroup A of group 1 that the hypoalbuminemia and increased content of α and β globulins persisted throughout the period of observation.

Increased cholinesterase activity almost to within normal limits was observed in the serum of the rats (subgroups B and D) treated with pyrogenal (6.10±0.37, and 5.20±0.6, respectively). However, it should be noted that in the rats of subgroup A and C, which were left to recover spontaneously, the cholinesterase activity also recovered to about the same level (5.75±0.56 and 6.81±0.9, respectively). The serum histidase activity in all the subgroups investigated was very stable and, despite pyrogenal administration, remained unchanged and at virtually the same level as the activity of this enzyme in the rats receiving CCl_4 only. Cholinesterase activity in liver tissue homogenate rose to within normal limits both in the experimental subgroups B and D and in the rats left to recover spontaneously (subgroups A and C). No appreciable change in histidase activity was observed in the liver homogenates in all series of the experiment compared with healthy animals. No statistically significant differences in the activity of these enzymes could be detected between groups of rats receiving CCl_4, and allowed to recover spontaneously, and groups receiving pyrogenal therapeutically or prophylactically.

These results suggest that administration of pyrogenal stimulates processes of regeneration in liver tissue and promotes a higher level of protein synthesis in the body (increased synthesis of albumins). One mechanism of its action is evidently based on the renewal and more intensive synthesis of high-energy compounds in the cells of organs and tissues [3, 4, 6].

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