# A MODEL OF ACUTE HEPATIC FAILURE IN RABBITS

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Definite progress has been made in recent studies of the pathogenesis of viral hepatitis [2, 5-7]. However, many practical problems connected with the treatment of severe forms of viral hepatitis B (HB), especially if complicated by acute hepatic failure (AHF), still remain unsolved. An adequate experimental model is necessary to study some aspects of this problem. However, the development of such a model is associated with objective difficulties, including the existence of human carriers of HB virus and the insufficient comparability of forms of virus hepatitis found in animals, notably virus hepatitis of mice [1, 7, 10], and HB in man. Attempts to create an analog of HB by administration of carbon tetrachloride to animals have also proved unsuitable because the process it causes in the liver is one of fatty infiltration rather than necrobiosis. In 1978, a model of AHF closely resembling the disease in man was produced for the first time in rabbits with aid of the selective hepatotoxin D-galactosamine (DGA) [10].

The aim of the present investigation was to continue the study of relations between function and structure of the liver in AHF induced by DGA in rabbits, to examine not only the similarity but also the differences between HB and this model, and to assess prospects for the use of the model in studying treatment problems based on the pathogenesis of HB.

## EXPERIMENTAL METHOD

Experiments were carried out on 33 New Zealand White rabbits weighing 2.9 ± 0.1 kg, aged 4-6 months. There were four series of experiments: In series I the 9 rabbits received no treatment and were given an injection of DGA (900 mg/kg to 5 animals and 600 mg/kg to 4 animals). In the remaining three series the animals received DGA in a dose of 900 mg/kg; 8 animals of series II were treated after poisoning with "Lactsol" therapeutic solution, which was injected four times in a dose of 30-40 ml into the auricular vein once every 4 h; the 8 animals of series III, after injection of DGA, received a solution of amino acids (Aminosteril-Hepa, West Germany) in the same doses and by the same scheme as in series II; the 8 animals in series IV were treated after poisoning with DGA by a special original mixture of amino acids. The study of the effect of these amino acid mixtures was not one of the aims of this part of the investigation.

DGA hydrochloride (from Chemapol, Czechoslovakia) was injected in 5% glucose solution in a concentration adjusted to 20% at pH 6.8 (by the addition of caustic soda). The solution was injected into the auricular vein at the rate of 10 ml/min. The results of biochemical tests of the blood before and 12 and 24 h after injection of DGA are given in Tables 1 and 2. In the study of aspartate (AsAT) and alanine (AlAT) aminotransferases, besides the traditional method of end points, kinetic analysis also was used on the ABA-100 bichromatic analyzer (Abbott, USA). The concentration of the 16 amino acids were determined on the KLA-3B analyzer (Hitachi, Japan).

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TABLE 1. Serum Enzyme Parameters in the Course of Experimental Galactosamine Hepatitis in Rabbits (M ± m)

| Group of animals | Dying animals | | End point method (after Pashkina) | | γ-GTP, μmoles/min·liter | Alkaline phosphatase, μmoles/min liter |
|---|---|---|---|---|---|---|
| | AsAT, μmoles/min·liter | AlAT, μmoles/min·liter | AsAt, μg/ml | AlAt, μg/ml | | |
| control (before poisoning) | 17,9±2,2 | 52,4±4,3 | 35,6±1,5 | 46,9±1,9 | 9,1±1,1 | 28,5±2,2 |
| | | | 12 h after poisoning | | | |
| All animals | 10066,2±2009,0 | 1748,7±269,8 | 298,5±12,7 | 212,3±17,1 | 142,5±23,1 | 223,9±26,5 |
| Surviving animals | 5072,0±1113,4 | 1128,6±342,6 | 307,4±25,6 | 190,8±35,0 | 139,0±14,6 | 195,4±40,5 |
| Dying animals | 13633,6±2646,4 | 2185,1±311,5 | 282,4±16,4 | 227,7±15,6 | 145,0±39,6 | 247,7±35,0 |
| | | | 24 h after poisoning | | | |
| All animals | 14346,7±3742,2 | 3703,3±860,0 | 345,2±25,1 | 332,0±21,7 | 182,8±34,8 | 290,0±40,4 |
| Surviving animals | 6426,7±431,8 | 2080,0±250,4 | 288,0 | 282,0 | 170,7±17,8 | 278,5 |
| Dying animals | 22266,7±2666,2 | 5326,7±999,8 | 383,0±16,0 | 365,3±10,7 | 195,0±74,7 | 297,7±72,7 |

TABLE 2. Serum Biochemical Parameters in the Course of Experimental Galactosamine Hepatitis in Rabbits (M ± m)

| Group of animals | Total bilirubin, μmoles/liter | Total cholesterol, mmoles/liter | Thymol test (ε × 100) | β-lipoproteins, Burstein's test (ε × 100) | Urea, mmoles/liter | Glucose, mmoles/liter |
|---|---|---|---|---|---|---|
| control (before poisoning) | 5,0±0,5 | 1,3±0,1 | 2,3±0,2 | 13,4±2,3 | 5,4±0,3 | 7,4±0,3 |
| | | | 12 h after poisoning | | | |
| All animals | 12,0±2,7 | 1,7±0,2 | 2,0±0,2 | 13,6±2,8 | 5,6±0,6 | 6,3±0,4 |
| Surviving animals | 11,5±4,0 | 1,8±0,2 | 2,2±0,4 | 18,0 | 4,2±0,6 | 6,2±0,6 |
| Dying animals | 12,7±4,1 | 1,6 | 1,9±0,1 | 10,7±3,3 | 6,5±0,7 | 6,5±0,5 |
| | | | 24 h after poisoning | | | |
| All animals | 24,3±1,4 | 1,3±0,5 | 3,0±0,1 | | 7,2±2,7 | 4,3±0,8 |

TABLE 3. Concentration of Amino Acids and Ammonia (mM) in Blood Plasma of Different Groups of Animals

| Amino acids | Groups | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Aspartic acid | 0,042±0,010 | 0,071±0,018 | 0,081±0,028 | 0,097±0,026* |
| Threonine | 0,143±0,030 | 0,276±0,103 | 0,109±0,015 | 0,379±0,148* |
| Serine | 0,373±0,037 | 0,444±0,071 | 0,420±0,095 | 0,558±0,151* |
| Glutamic Acid | 0,211±0,010 | 0,302±0,059* | 0,309±0,055* | 0,309±0,091* |
| Proline | 0,298±0,052 | 0,317±0,030 | 0,651±0,252* | 0,422±0,095 |
| Glycine | 1,190±0,153 | 1,186±0,216 | 1,012±0,247 | 1,452±0,371 |
| Alanine | 0,576±0,088 | 0,485±0,091 | 0,860±0,246 | 0,529±0,187 |
| Cystine | 0,025±0,004 | 0,017±0,004 | 0,016±0,004 | 0,049±0,014 |
| Valine | 0,263±0,027 | 0,180±0,022 | 0,231±0,025 | 0,212±0,058 |
| Methionine | 0,035±0,004 | 0,040±0,013 | 0,062±0,014* | 0,049±0,01[illegible] |
| Isoleucine | 0,075±0,008 | 0,069±0,005 | 0,076±0,010 | 0,073±0,017 |
| Leucine | 0,131±0,019 | 0,111±0,016 | 0,142±0,018 | 0,109±0,026 |
| Tyrosine | 0,083±0,008 | 0,078±0,008 | 0,161±0,019* | 0,091±0,023 |
| Phenylalanine | 0,068±0,009 | 0,054±0,012 | 0,076±0,015 | 0,059±0,016 |
| Lysine | 0,242±0,030 | 0,260±0,071 | 0,442±0,073* | 0,177±0,045 |
| Histidine | 0,202±0,018 | 0,207±0,026 | 0,326±0,057* | 0,192±0,048 |
| Total amino acids | 3,928±0,413 | 3,783±0,538 | 5,165±1,035 | 4,405±1,342 |
| Ammonia | 0,467±0,032 | 0,510±0,103 | 0,715±0,087* | 0,601±0,131 |

Legend. Groups of animals distinguished depending on development of AHE and also according to time of observation: 1) initial stage (21 animals); 2) 12-22 h after injection of DGA — rabbits in which AHE did not develop (7 animals); 3) 12-22 h after injection of DGA — rabbits which developed encephalopathy on days immediately after blood sampling (6 animals); 4) surviving animals 6-8 days after injection of toxin (4 rabbits). ×) Differences significant ($P \leq 0.05$) between Group 1 and all other groups; +) differences significant ($P \leq 0.05$) between groups 2 and 3. [The symbols "×" and "+" are mistakenly designated as asterisks in the Russian original — Consultants Bureau.]

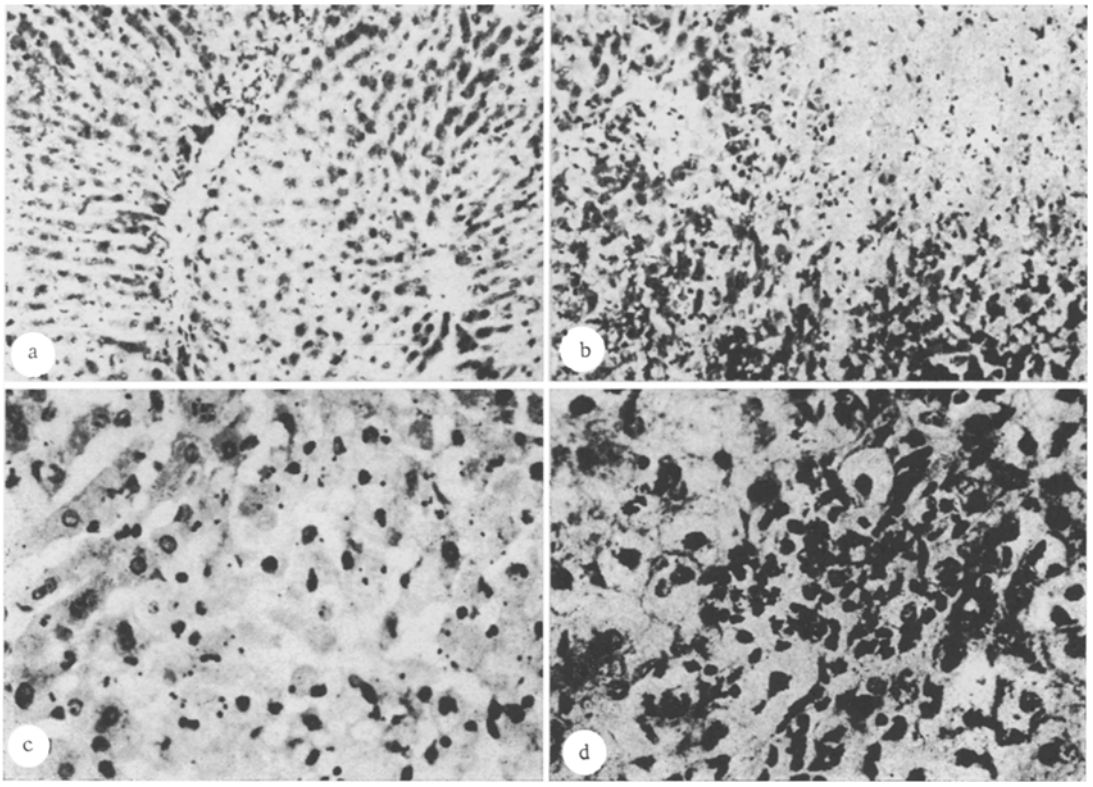

Fig. 1. Rabbits' liver: a) before injection of DGA (control); b) 23 h after injection of DGA — centrolobular necrosis, 157 ×; c) the same liver with an extensive area of necrobiosis and formation of Councilman's bodies, 397 ×; d) 6 days after injection of DGA: hydropic degeneration of hepatocytes, cellular infiltration of periportal bands penetrating into parenchyma, 400 ×. Hematoxylin and eosin.

Material for histological investigation was taken from the animals immediately after death. Rabbits which survived were killed on the 6th day. Pieces of liver, kidneys, and different parts of the brain (cortex, cerebellum, midbrain, hypothalamus) were fixed in 10% formalin solution. Secretions were stained with hematoxylin and eosin and by Van Gieson's method.

## EXPERIMENTAL RESULTS

The rabbits became apathetic, less inclined to move, and their food intake was reduced 8-14 h after injection of DGA. Cerebral disorders in the form of acute hepatic encephalopathy (AHE) developed in 11 of the 17 rabbits of the first two series, and the remaining 6 animals survived more than 6 days. When AHE developed, slowing of the frequency and an increase in amplitude of the EEG waves to 400-600 μV were observed. The results of determination of enzyme activity in the blood serum (Table 1) showed that the sharp rise in the aminotransferase level after injection of DGA was revealed most fully by the kinetic method of investigation: AsAT activity was then 550 times the normal value. The rise in the AlAT level was less marked. Accordingly, the AsAT/AlAT ratio was considerably higher than that observed in HB in man, and also than in published data [10]. These findings compel a critical re-evaluation of traditional views on the syndrome of the outflow of cytoplasmic enzymes through the injured cell membrane [3]. Changes in the blood amino acid profile (Table 3) were similar to those observed in severe forms of HB in man [9]. It can be tentatively suggested that imbalance in the blood amino acids in AHF causes changes in mediator synthesis with the accumulation of pseudomediators (octopamine, phenylethylamine, etc.), which block the monoaminergic systems of the brain and may thus contribute to the development of encephalopathy [9, 11].

The pathomorphological picture of the liver lesions in DGA-induced hepatitis in rabbits was dominated by disturbances of the trabecular structure of the hepatic lobules with the development of extensive foci of centrolobular necrosis very similar to those found in HB in man (Fig. 1a, b); under these circumstances necrosis was mainly of the coagulation type with

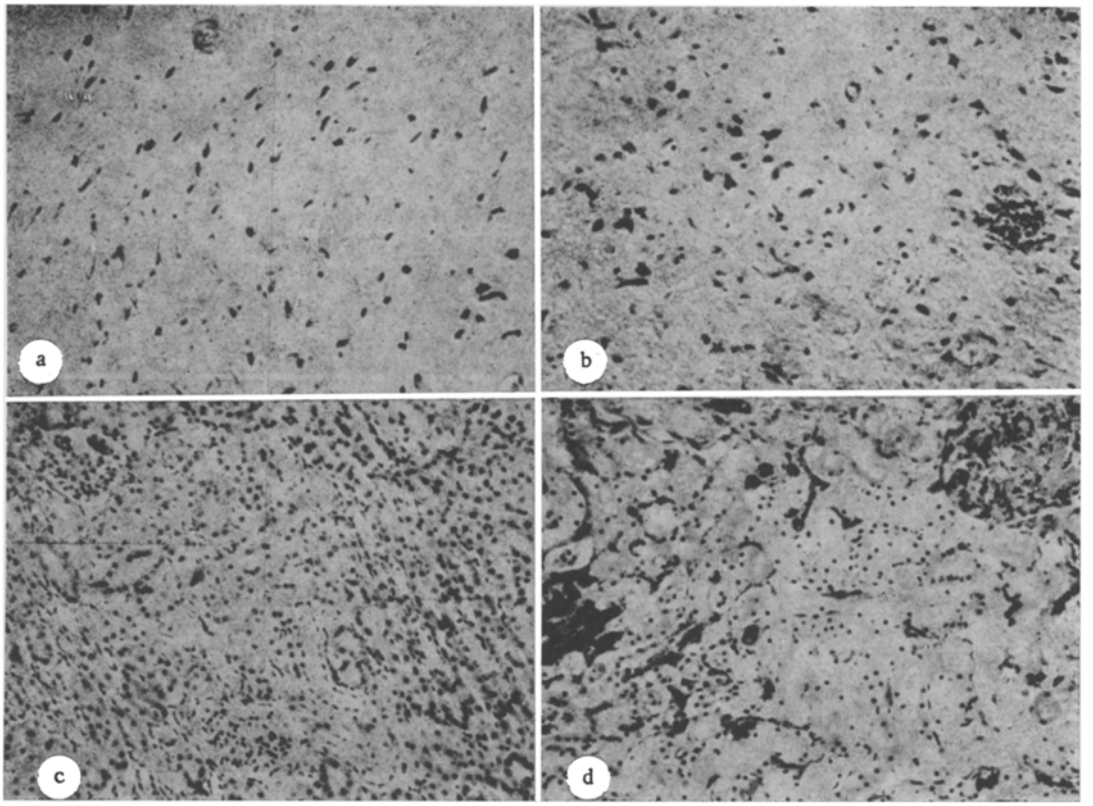


Fig. 2. Brain and kidneys 23 h after injection of DGA: a) region of hypothalamus of control animal, 157 ×; b) hypothalamus after injection of DGA: marked congestion of blood vessels, perivascular hemorrhages, edema, and acute swelling of nerve cells, 157 ×; c) area of renal cortex of control animal, 157 ×; d) kidneys after injection of DGA: marked congestion of medullary and cortical layers, distention of glomerular capillaries, edema, degeneration, and necrosis of epithelium of principal portion of nephron, 157 ×. Hematoxylin and eosin.

the formation of Councilman's bodies (Fig. 1c). In surviving animals killed 6 days after injection of DGA all the morphological features of repair processes were found in the liver with an active reaction of the connective tissue and with marked infiltration of the portal tracts (Fig. 1d). Besides the characteristic edema of AHE, multiple extensive hemorrhages with extravasation and degeneration of ganglion cells were found in the brain in HB (Fig. 2a, b), and this was associated with the more frequent development of a seizure syndrome in the experimental animals in the final stage of the disease. In the kidneys, in addition to the deposits of fibrin already described [10], we also found more severe lesions than in HB not only in the vessels but also in the tubules (Fig. 2c, d).

This experimental model of AHF thus satisfies the following basic demands required of models suitable for studying corrective pathogenetic treatment: 1) reversibility of the liver lesion; 2) reproducibility of the pathological process; 3) death of the animal from AHF; 4) adequately large size of the experimental animals; 5) minimal risk for the laboratory staff; 6) maximal similarity to virus HB in man.

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# TOXIC PROPERTIES OF SERUM OF RABBITS AND DOGS EXPOSED TO CONTROLLED HYPERTHERMIA

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Under the influence of excessive heat the blood serum acquires pathological properties connected with the appearance of burn toxins and physiologically active substances [3, 7, 8], disturbance of the lysosomal apparatus of the cells [1], and changes in the physicochemical parameters of the blood [5, 6]. The diversity of the changes in composition of the blood suggests parallel activation of various pathological and physiological mechanisms forming the response of the body as a whole.

In the investigation described below the toxic properties of the blood serum of dogs and rabbits and the degranulation reaction of mast cells, with particular reference to the system of peritoneal mast cells of rats, were studied in order to determine the relationship between the severity of the reaction of the body and the functional state of the mast cell system, one of the physiological mechanisms of immediate defense [9], in a model of heat stress.

## EXPERIMENTAL METHOD

Dogs and rabbits were exposed to hyperthermia on the 22 PG-01 apparatus, intended for creating controlled temperature conditions in man and warm-blooded animals [4]. The assigned body temperature (rectal, 42°C) was maintained automatically for 1 h in rabbits and 2 h in dogs through a system of feedback between body temperature and the heat carrier used in the apparatus. The dogs were overheated in the unanesthetized state and also under thiopental sodium anesthesia; the rabbits were unanesthetized. Serum for investigation of toxicity was collected 5-10 min after the end of the experiment and also on the 1st, 3rd, 5th, 7th, 11th, and 15th days in rabbits and dogs exposed to hyperthermia without anesthesia. The toxicity of the serum was determined by biological testing on mice with blockage of the reticuloendothelial system [2]. To estimate toxicity the serum from each experimenatl animal was injected into ten noninbred albino mice. The state of the mast-basophilic system was assessed by the degree of degranulation of the mast cells on films stained with toluidine blue. Mast cells were obtained by centrifugation of rat peritoneal washings on the 1st, 3rd, 7th, 11th, and 13th days after hyperthermia for 1 h. The cells were divided into five groups depending on the number and staining of the granules, shape of the cells, and integrity of the membrane. Round cells with clear edges, filled with many stained granules, were placed in group I. The cells of group II contained a few translucent cavities due to commencing degranulation. The cells of group III had uneven edges and paler cytoplasm because of considerable degranulation. Group IV consisted of cells with fragmented cytoplasm, including cells of "shot" type in cells of group V the membrane was absent and granules were located at the site of the former cell.

The data were subjected to statistical analysis by Student's t-test.

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