In conclusion the authors are grateful to Senior Scientific Assistant E. G. Shcherbakova (Central Postgraduate Medical Institute, Moscow) for providing the preparation of prodigiosan, and to Candidate of Medical Sciences N. N. Mayanskaya for help with the biochemical investigation.

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ALPHA-FETOPROTEIN SYNTHESIS IN DIFFERENT LINES OF ADULT MICE DURING REGENERATION OF THE LIVER

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The concentration of alpha-fetoprotein (AFP) in the sera of adult mice of 12 different lines and F_1 hybrids of lines SWR and B10D2 was determined by radioimmunodiffusion in agar during regeneration of the liver after poisoning with CCl_4. In the mice of 6 of 10 lines differences in the AFP concentration between females and males were statistically significant. Interlinear differences also were found: The mean AFP concentrations in the sera of inbred C57BL/6 and B10D2 mice were significantly lower than the corresponding values for mice of most other lines. The F_1 hybrids were intermediate as regards their AFP concentration between the two parental lines. Small but statistically significant differences were found between groups of male F_1 hybrids in direct and reciprocal crosses. It is suggested that induction of AFP synthesis during regeneration of the liver in adult mice is under polygenic control.

KEY WORDS: alpha-fetoprotein; regeneration of the liver; interlinear differences; genetic control.

Alpha-Fetoprotein (AFP), the principal protein of embryonic serum is present in adult animals and man in very low (nanogram) concentrations [2, 10, 13]. During regeneration of the liver after partial hepatectomy or poisoning by various hepatotoxins, there is a sharp but transient increase in the AFP level in the animals' blood [3, 4, 6, 12, 15].

In a few cases differences in the intensity of AFP synthesis have been found in mice of different lines during regeneration of the liver [1, 12]. A detailed analysis of such differences could provide an approach to the study of the genetic control of induction of AFP synthesis in the adult.

In the investigation described below AFP was determined quantitatively during regeneration of the liver in the sera of mice of different genotypes.

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TABLE 1. AFP Concentrations in Sera of Mice of 12 Inbred Lines, Aged 13-14 Weeks, 3 Days after CCl_4 Poisoning

Index	Lines of mice									
	BALB/c		SWR		CC57Br		CC57W		C3H/Sn	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
Number of mice	23	36	36	33	24	54	38	37	23	33
M ± m, μg/ml	547±61	199±20	213±17	143±10	209±20	139±12	170±23	71±11	135±21	115±22
$P_{♀-♂}$	<0.001		<0.001		<0.001		<0.01		<0,1	
$P_{♂-♀B10D_2}$	<0.001		<0.001		<0.001		<0.001		<0,001	
$P_{♂-♀C57BL/6}$	<0.001		<0.001		<0.001		<0.001		<0.001	

Index	Lines of mice									
	AKR		A_f		129/SV—SLCP	C57BL He	C57BL/10Sn		B10D2		C57BL 6	
	♀	♂	♀	♂	♂	♂	♀	♂	♀	♂	♀	♂
	11	19	24	38	19	13	22	25	30	52	21	23
	119±33	142±13	39±7	107±18	114±24	61±11	42±7	22±9	28±4	15±3	7±2	6±1
$P_{♂-♀}$	=0.1		$P_{♂-♀}$ <0.001		—	—	<0.1		<0.01		<0.1	
P ♀—♀B10D2	<0.01		$P_{♀-♀B10D2}$	<0.1	<0.01	<0.01	<0.1		<0.01		$P_{♂B10D2-♀}$	<0.05
P♀—♀C57BL/6	<0.001		$P_{♀-♀C57BL/6}$	<0.001	<0.001	<0,001	<0,1		<0.05		<0,1	

TABLE 2. AFP Concentrations in Sera of F_1 Hybrids Aged 13-14 Weeks 3 Days after CCl_4 Poisoning

Index	Hybrids			
	F_1 (♀SWR× ×♂B10D2)		F_1(♀B10D2× ×♂SWR)	
	♀	♂	♀	♂
Number of mice	10	31	9	17
M ± m, μg/ml	135±35	86±6	111±25	67±6
P ♀—♂	>0.1		>0.1	
P♂—♂	<0,05			

EXPERIMENTAL METHOD

Virgin females and males of 12 inbred lines of mice aged 13-14 weeks, obtained from the nursery of the N. F. Gamaleya Institute of Epidemiology and Microbiology, and first generation (F_1) hybrids bred by the writers were used (Tables 1 and 2).

Regeneration of the liver was induced in mice by poisoning with CCl_4 vapor [4]. The mice were kept in groups of 8-10 animals for 15 min in a glass exsiccator with a capacity of 3.5 liters air (0.015 ml CCl_4 per liter of air).

Changes in the level of synthesis of the AFP in the blood after CCl_4 poisoning were studied in male mice of nine inbred lines. From 3 to 6 measurements of the AFP concentrations were made on each individual animal. Blood samples were taken from the retro-orbital sinus of the mice 2, 3, 4, 6, 7, 8, and 11 days after CCl_4 poisoning. To compare the AFP concentration in the sera, mice were decapitated 3 days after inhalation of CCl_4.

All the mouse sera were examined by the precipitation test in agar with a standard test system against mouse AFP [7]. Quantitative data were obtained by radial immunodiffusion in agar [9]. As the standard for the calibration curve, mouse AFP twice purified by electrophoresis in polyacrylamide gel [5] was used. The protein concentration in the AFP preparation, dialyzed against distilled water, was determined with a spectrophotometer, assuming that for mouse AFP E(1%/1 cm) 278 nm = 4.15 [14]. The quantitative limit of sensitivity of the radial immunodiffusion method in these experiments was 6 μg/ml and the qualitative limit about 3 μg/ml. The purified preparation of mouse AFP and antiserum against it were generously provided by A. K. Yazova.

EXPERIMENTAL RESULTS

The data on the AFP concentration in the blood of male mice of nine inbred lines at different times after CCl_4 poisoning are given in Fig. 1. The patterns of the dynamics of AFP synthesis in the mice of all lines were

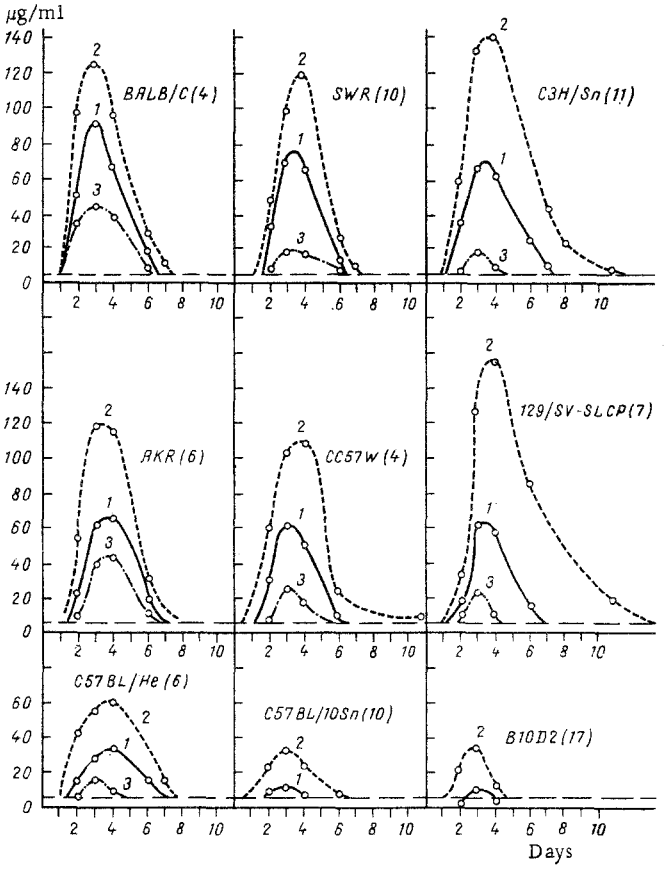

Fig. 1. AFP concentrations in sera of male mice of nine inbred lines at different times after CCl_4 poisoning.

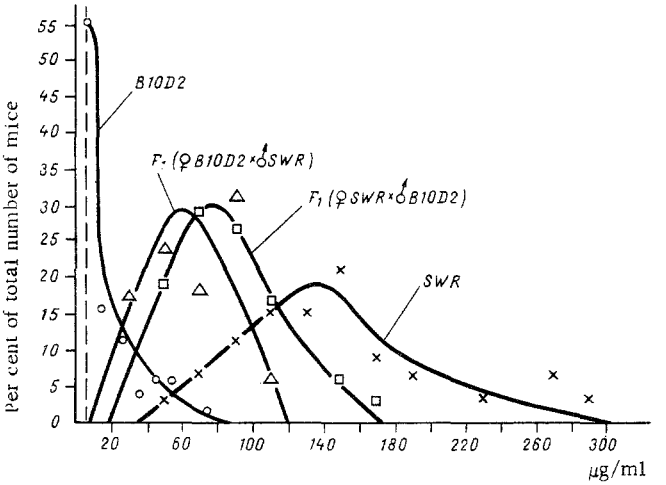

Fig. 2. Curves of distribution of male mice by serum AFP concentrations 3 days after CCl_4 poisoning.

in good agreement with data obtained by other workers [4, 6, 8, 12, 15]. An increase in the AFP level was observed after 2 h, the maximum was reached after 3-4 days, by the 6th-7th days the AFP level had started to fall, and by the 11th day after poisoning as a rule no AFP could be found in the blood of the mice. In three lines of mice – C57BL/He, C57BL/10Sn and, in particular, in B10D2 – the mean AFP levels were significantly lower than in the rest, at all times of examination.

Since the dynamics of AFP in the different lines of mice was similar, they could be compared with respect to their AFP concentration during regeneration of the liver. The sera were investigated 3 days after poisoning, for it is at that time that the highest AFP concentrations were observed in the blood of most lines of mice and, in particular, in line B10D2 (Fig. 1), which was later used to obtain F_1 hybrids. The data for AFP concentrations in the sera of mice of 12 inbred lines and F_1 hybrids are given in Tables 1 and 2.

Considerable individual variations in AFP concentrations were found in the sera of each inbred line of mice (Figs. 1 and 2). However, despite this fact, in mice of 6 of 10 lines statistically significant differences were found for the AFP concentration in females and males (Table 1). In five lines of mice (BALB/c, SWR, CC57BR, B10D2, CC57W) the mean serum AFP concentrations were higher in females than in males ($P < 0.01$). In mice of line A, however, the mean AFP concentrations in the sera were higher in males ($P < 0.001$).

Comparison of the different lines by their serum AFP concentrations showed that, despite considerable physiological differences within each line, two inbred lines could be distinguished (C57BL/6 and B10D2) in whose sera the mean AFP concentrations were significantly lower than the corresponding values for the mice of most other lines – 8 lines for B10D2 and 10 lines for C57BL/6 mice (Table 1).

Differences in the intensity of AFP synthesis in different lines of mice are evidently unconnected with differences in their sensitivity to the dose of CCl_4 used. It was shown previously that AFP synthesis is proportional to the dose of CCl_4 up to a certain limit, but with a further increase in dose the animals died [4]. A dose of CCl_4 was used in the present experiments at which the AFP concentrations were close to their maximal values, but the wastage of mice was comparatively small. Interlinear differences in AFP synthesis also were observed in another series of experiments in which CCl_4 was given in frequent repeated doses, when the dose effect was cancelled out.

Statistical analysis of the data for F_1 hybrids showed that they occupy an intermediate position for AFP synthesis between the two parental lines (Table 2, Fig. 2). Differences in AFP synthesis between females and males characteristic of the mice of the parental lines were evidently preserved in the F_1 hybrids. The low levels of significance can most probably be explained by the fact that the number of sera of female F_1 hybrids analyzed was too small (Table 2).

Small but statistically significant differences ($P < 0.05$) were found between groups of male F_1 hybrids obtained by direct and reciprocal crosses (Table 2, Fig. 2). It can tentatively be suggested that one of the genes participating in the regulation of AFP synthesis during regeneration of the liver is linked with the sex chromosome, but the role of this gene in the activation of AFP synthesis is a minor one. However, because of the insufficient number of sera from the F_1 hybrids, no final conclusions can be drawn.

It has recently been shown that the background AFP level in adult mice is controlled by one autosomal gene. However, the action of this gene is not manifested during the regulation of AFP synthesis in the early postnatal period [11]. The role of this gene in the induction of AFP synthesis during regeneration of the liver is not clear.

The results point to genetic control over the induction of AFP synthesis during regeneration of the liver in adult mice after CCl_4 poisoning. Control over AFP synthesis during regeneration of the liver is evidently polygenic in character. However, to solve this problem it will be necessary to investigate F_1 and F_2 hybrids contrasting with each other with respect to the synthesis of this protein.

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EFFECT OF ANTIHEPATOCYTOTOXIC SERUM ON DNA SYNTHESIS IN THE RAT

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Incorporation of thymidine-^{3}H into parenchymatous and reticulo-endothelial cells of the liver was studied autoradiographically in adult female rats treated with small doses (0.06 μg/100 g body weight per injection) of antihepatocytotoxic serum (AHTS), the γ-globulin isolated from it (γAHTS), and the γ-globulin fraction of normal rabbit serum (γNRS) to intact animals and to rats with liver damage caused by carbon tetrachloride (CCl_4). Following injection of γAHTS and, to a lesser degree, of AHTS into intact animals the index of labeled nuclei of both the parenchymatous and the reticulo-endothelial cells was increased. When given after preliminary CCl_4 administration, γAHTS stimulated reparative regeneration. The action of γAHTS took place in phases: A period of increase in the index of labeled nuclei was followed by a period of decrease, and this again was followed by a fresh period of stimulation of proliferative processes.

KEY WORDS: liver; antihepatocytotoxic serum; carbon tetrachloride; index of labeled nuclei.

A definite role in the activity of organs under normal and pathological conditions is ascribed to antitissue autoantibodies. The possibility of their participation in the growth of organs has been discussed [5]. The problem of their harmful and protective action is in process of solution [7, 10, 13, 15]. The use of heterogeneic antibodies may help to solve this problem.

Small doses of antihepatocytotoxic serum (AHTS) have been shown to have a normalizing action on the functions of the liver and its metabolism, when disturbed by carbon tetrachloride (CCl_4) or by exogenous bile acids [1-3, 8].

The protective action of antibodies is largely ascribed to neutralization of the antigens formed during tissue destruction [6, 7]. However, a stimulating action of antibodies on the organ may also be expected. There are two possible pathways for this to occur: Either antibodies stimulate metabolic processes only in the cytoplasm of the cell and enhance its functional capacity, or, under the influence of antibodies, DNA replication and cell proliferation take place. It has been shown that AHTS increases the mitotic index in the liver of intact rats [4, 14].

The object of this investigation was to study incorporation of thymidine-^{3}H into liver cells (parenchymatous and reticulo-endothelial) of rats following injection of small doses of AHTS, of the γ-globulin fraction isolated from it (γAHTS), and the γ-globulin fraction of normal rabbit serum (γNRS) into intact animals and to animals with liver damage caused by CCl_4.

EXPERIMENTAL METHOD

Experiments were carried out on female Wistar rats weighing 170-200 g. The AHTS for rats was obtained by immunizing rabbits with a saline extract of rat liver. The titer of the serum in the complement fixation test was 1:320. γ-Globulin was isolated from the AHTS and NRS by Kendall's method [12].

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