number of mitoses in the thymus occurring before the decrease in the total cell number may confirm this assumption.

The marked changes occurring in the thymus cannot be attributed solely to the effect of IFN delivered into the bloodstream. Interferon may find its way into regional peripheral nodes, where it activates some cells. A portion of these cells then enters the bloodstream and other organs. It cannot be excluded that most of the observed changes are induced by substances secreted by IFN-activated cells.

Our results indicate that IFN administration may lead to the involution of the thymus, allergization of the organism, and an increase in vascular permeability.

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Evaluation of Microsomal and Mitochondrial Oxidation in Rat Liver in Tetracycline-Induced Hepatosis

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A satistically significant decrease in the content of cytochromes P-450 and b_5 and in the activity of aniline hydroxylase and p-nitroanisole demethylase occurs in rat liver microsomes during the development of experimental acute fatty hepatosis develoing within a 24-h period after intraperitoneal administration of 125 mg/kg tetracycline hydrochloride. Under these experimental conditions tetracycline hydrochloride elicits only an insignificant disintegrating effect on oxidative phosphorylation in liver mitochondria.

Key Words: *tetracycline-induced fatty hepatosis; microsomal and mitochondrial oxidation; oxidative phosphorylation*

The toxic effect of tetracycline (TC) at the molecular and submolecular levels is due to its high membrane tropism and chelating properties. Binding of TC by mitochondria and chelation of Mg^{2+}, which plays an important role in oxidative phosphorylation, are thought to be implicated in TC toxicity [8,11]. Depending on dose and duration of the administration period, TC elicits either a disintegrating or an inhibitory effect on cell respiration [6,15].

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TABLE 1. Liver Content of Triacylglycerides and Cytochrome P-450 in Rat Liver Homogenate 24 h after Intraperitoneal Administration of TC in Different Concentrations ($M \pm m$)

TC dose, mg/kg	Triacylglycerides, mg triolein/g dry weight		Cytochrome P-450, nmol/g dry weight	
–	8.84±0.37	(100)	96.27±17.79	(100)
50	12.30±1.60	(139)	72.75±4.28	(76)
125	48.25±4.25**	(546)	63.79±5.09	(67)
250	139.70±0.50**	(1579)	51.05±4.85*	(54)

Note. Here and in Tables 2-4 one asterisk indicates $p<0.05$ and two asterisks $p<0.01$, and three asterisks $p<0.01$. The percentage of changes is given in parentheses.

At the same time, information regarding the influence of TC on microsomal oxidation is scant and contradictory. Histological studies of the liver of animals treated with relatively high doses of TC (160-330 mg/kg) revealed damage to the endoplasmic reticulum [12]. We have found that the content of cytochrome P-450 decreases by 25% in homogenate of liver obtained from animals treated with 500 mg/kg TC (intragastral administration) during a 5-day period [5]. However, there is evidence that high doses of TC do not change the cytochrome P-450 and b_5 contents in the microsomes or the rates of M-demethylation of ethylmorphine and of p-hydroxylation of aniline. At the same, numerous studies of fatty hepatoses induced by other chemical agents indicate that microsomal oxidation is damaged in this pathology [1,2].

Our objective was to assess the microsomal and mitochondrial oxidation in the liver during the development of tetracycline-induced hepatosis.

MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 180-220 g fed the standard vivarium diet. Fatty hepatosis was induced by intraperitoneal administration of 4 ml/100 g TC (Serva). The antibiotic was dissolved in normal saline (pH 8.9) and injected in a single dose of 50, 125, or 250 mg/kg. Control animals received equal volumes of normal saline. Biochemical studies were performed 24 h after the administration of TC.

The liver was perfused with normal saline under ether anesthesia. Pieces of the liver weighing 100 mg were then homogenized in normal saline (1:10). The remainder of the liver was used for isolation of microsomes and mitochondria. Microsomes were isolated by ultracentrifugation at 105,000 *g* in 1.15% KCl. Microsomes were suspended in medium containing 1.15% KCl, 20 mM Tris-HCl, and 1 mM EDTA (pH 7.4) to a final concentration of 10 mg protein/ml. Mitochondria were isolated as described elsewhere [13]. The following media were used for the preparation of mitochondrial fractions: the isolation medium contained sucrose 0.25 M, EDTA 1.00 mM, Tris-HCl 10 mM (pH 7.4) and the incubation medium contained sucrose 0.22 M, EDTA 0.20 mM, Tris-HCl 10 mM, KCl 10 mM, and KH_2PO_4 10 mM (pH 7.4).

The severity of fatty hepatosis was assessed by the content of triacylglycerides in the liver homogenate with the use of standard Lachema kits. Microsomal oxidation in the liver homogenate was estimated from the cytochrome P-450 content [14].

The contents of cytochromes P-450 and b_5 [14] and the activity of aniline hydroxylase [3] and p-nitroanisole demethylase [16] were determined in liver microsomes.

Oxidative phosphorylation was estimated in the mitochondria. The oxygen absorption rate was measured by the polarographic method [4] with the use

TABLE 2. Some Biochemical Parameters of the Microsomal Oxidation System in Rat Liver 24 h after Intraperitoneal Administration of 125 mg/kg TC ($M \pm m$)

Parameter	Control		Experiment	
Cytochrome P-450 content, nmol/mg protein	0.65±0.04	(100)	0.34±0.04**	(52)
Cytochrome b_5 content, nmol/mg protein	0.28±0.02	(100)	0.20±0.02*	(71)
Activity of aniline hydroxylase, nmol p-aminophenol/g protein/min	160.50±12.07	(100)	102.00±19.52*	(64)
Activity of p-nitroanisole demethylase, nmol p-nitrophenol/g protein/min	430.00±41.62	(100)	269.75±48.24*	(63)

Note. The control and experimental series each consisted of 6 animals.

TABLE 3. Effect of a Single Administration of Various TC Doses (Intraperitoneally, 24 h) on Oxidative Phosphorylation in Rat Liver Mitochondria ($M \pm m$)

TC dose, mg/kg	V_2	V_3	V_4	V_{DNPH}	ADP/O	RC_L	RC_{Ch}	V_{ph}	t_{ph}
Control	4.8±0.10	31.3±1.40	3.7±0.19	27.7±1.46	2.9±0.07	6.5±0.22	8.7±0.42	188.0±7.89	1.2±0.06
125	5.3±0.29	29.5±1.90	4.1±0.48	28.5±1.44	2.9±0.07	5.7±0.47	7.8±0.89	170.3±11.0	1.5±0.10
	(110)	(94)	(111)	(103)	(100)	(88)	(89)	(91)	(115)
Control	7.0±0.58	35.0±1.71	5.3±0.41	33.9±3.11	3.0±0.07	5.2±0.35	6.9±0.41	211.8±14.85	1.1±0.07
250	9.0±1.09	38.1±1.73	7.3±0.55	45.1±1.82	3.1±0.08	4.4±0.32	5.3±0.28	225.7±9.31	1.0±0.37
	(129)	(109)	(138)*	(133)**	(103)	(85)	(77)**	(107)	(90)
330	15.8±1.94	22.2±1.52	12.0±2.56	18.6±1.50	3.00±0.19	1.4±0.08	1.9±0.32	130.11±3.05	1.7±0.02
	(226)**	(63)**	(226)*	(55)**	(100)	(27)**	(28)**	(61)**	(155)**

Note. Substrate: glutamate (4 mM) with malate (1 mM). Here and in Table 4 the percentage of changes compared with the control is given in parentheses. V_2, V_3, V_4, and V_{DNPH}, nmol O_2/mg protein/min; V_{ph}, nmol ADP/mg protein/min, t_{ph}, min/mg protein.

of Clarke's electrode in a sealed 1.5-ml cell. Mitochondria were added to a final concentration of 2-3 mg protein/ml medium. Succinate (5 mM) and glutamate (4 mM) with malate (1 mM) were employed as substrates. Mitochondrial respiration was assessed under the following metabolic conditions [9]: relative to the state of rest (V_2), in the active state upon phosphorylation of 200 μM adenosine diphosphate (ADP) (V_3), in the adjusted state (V_4), and in the disintegrated state in the presence of 50 μM 2,4-dinitrophenol (V_{DNPH}). The phosphorylation rate (V_{ph}) and time (t_{ph}), the ADP/O value, and the Lardie ($RC_L = V_3/V_4$) and Chance (RC_{Ch}) respiratory control coefficients were calculated. Rates V_2, V_3, V_4, and V_{DNPH} were expressed in nmol O_2/mg protein/min, V_{ph} in nmol ADP/mg protein/min, and t_{ph} in min/mg protein. The protein content was determined by the microbiuret method [10].

RESULTS

Statistically significant changes in the accumulation of triacylglycerides in rat liver were detected only after the administration of 125 mg/kg TC (Table 1). As the antibiotic dose increased, the liver content of cytochrome P-450 decreased (Table 1), the decrease (46%) being statistically significant after administration of 250 mg/kg TC. The cytochrome P-450 content in the liver homogenate was calculated per gram dry weight, i.e., the total cytochrome P-450 content in the liver was determined. The results did not resolve the question as to whether the P-450 reduction results from a lowering of the absolute content of the enzyme or from a decrease in number of membrane structures in the endoplasmic reticulum and how these changes influence the functional activity of the microsomal system.

In order to answer these questions it was necessary to assess the functional state of isolated microsomes. Twenty-four hours after intraperitoneal administration of 125 mg/kg TC the content of cytochromes P-450 and b_5 in rat liver microsomes decreased 48 and 29%, respectively (Table 2). The activity of aniline hydroxylase and p-nitroanisole demethylase decreased similarly (37%). All the

TABLE 4. Effect of a Single Intraperitoneal Administration of Various TC Doses (after 24 h) on Oxidative Phosphorylation in Rat Liver Mitochondria ($M \pm m$)

TC dose, mg/kg	V_2	V_3	V_4	V_{DNPH}	ADP/O	RC_L	RC_{Ch}	V_{ph}	t_{ph}
Control	11.4±0.37	44.7±1.88	9.3±0.43	44.9±1.87	2.3±0.05	3.9±0.09	4.9±0.18	217.1±10.62	1.3±0.07
125	11.7±0.53	44.0±1.53	10.0±0.89	49.2±0.80	2.2±0.05	3.8±0.18	4.6±0.35	191.6±11.12	1.5±0.08
	(103)	(98)	(108)	(109)	(97)	(97)	(94)	(88)	(115)
Control	14.7±0.86	46.1±2.68	11.1±0.93	37.4±2.93	2.0±0.03	3.0±0.44	4.3±0.28	183.0±14.53	1.2±0.08
250	19.6±0.80	54.3±3.13	16.7±0.86	51.3±1.38	1.9±0.08	2.8±0.10	3.2±0.14	212.4±15.32	1.0±0.11
	(133)**	(118)	(150)**	(137)**	(95)	(93)	(74)**	(116)	(83)
330	22.0±1.12	34.3±0.35	23.5±1.18	36.6±0.18	1.9±0.15	1.6±0.06	1.5±0.06	134.2±8.91	1.5±0.09
	(150)**	(74)**	(212)**	(98)	(95)	(53)**	(35)**	(73)**	(125)**

Note. Succinate (5 mM) was used as the substrate.

changes were statistically significant. Thus, under these conditions TC induces significant alterations in the activity of the microsomal oxidation system in the liver.

Fatty hepatosis developing within 24 h after intraperitoneal administration of 125 mg/kg TC was not accompanied by significant alterations in oxidative phosphorylation occurring in the liver mitochondria. A slight decrease in V_{ph} and, correspondingly, an increase in t_{ph} were observed upon oxidation of succinate by mitochondria. When administered in this dose, TC elicited a weak disintegrating activity (Tables 3 and 4). Upon oxidation of an NAD-dependent substrate V_2 and V_4 increased insignificantly and, correspondingly, RC_L and RC_{Ch} decreased. This may indicate an increase in the ionic conductivity of the mitochondrial membranes and a weakening of the energetic regulation of hepatic respiration in animals with fatty hepatosis.

After the TC dose was increased (250 and 330 mg/kg) (Tables 3 and 4), the disintegrating effect on oxidative phosphorylation upon the oxidation of both the NAD-dependent substrates and succinate was potentiated, being maximal at the dose of 330 mg/kg. The phosphorylation rate dropped 40% and the respiratory controls decreased more than 70%. Under these conditions, 2,4-dinitrophenol no longer abolished the inhibition of the rate of phosphorylative respiration.

Thus, the development of hepatosis induced by intraperitoneal administration of 125 mg/kg TC led to the following: lipid metabolism disorders manifested in an increase of the liver triacylglyceride content, a decrease in the cytochrome content and in the activity of the microsomal oxidative enzymes, and a slight disintegrating effect on oxidative phosphorylation.

Our results suggest that it is the disorders in microsomal oxidation rather than in the mitochondrial chain which create the conditions for intense lipogenesis after TC administration.

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