after injection of the oligopeptide ability of the rats to remember was again maintained at a high level, when AChE activity regained its initial value (Figs. 2 and 3), indicates that changes in the acetylcholine system must play an essential role only in the initial phase of memory fixation. It is more likely that the increase in AChE activity under the influence of oligopeptides after 30 and 60 min creates favorable conditions for effective functioning of the consolidation apparatus [3].

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REDUCTION IN THE TISSUE ASCORBIC ACID LEVEL IN GUINEA PIGS BY THALIDOMIDE

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KEY WORDS: thalidomide; microsomal enzymes; ascorbic acid.

Many investigations have been devoted to a study of the mechanisms of the teratogenic action of thalidomide (T) [2, 16]. Nevertheless the problem is still unsolved after more than 20 years. It has been postulated that fetal malformations are caused by T itself [9, 14]. Yet at the same time it has been shown that T can undergo both aqueous hydrolysis and biotransformation in vivo in mammals [8, 12]. It has accordingly been suggested that degradation products of T have a teratogenic action, that they are antagonists of glutamine, glutamate, or folic acid [2, 16], that they are toxic arenoxides [10], and that they inhibit protocollagen proline hydroxylase and thereby inhibit collagen formation and disturb embryonic limb development [12]. The species-specificity of the action of T (mice and rats are insensitive to this teratogen, whereas monkeys, man and, to a lesser degree, rabbits are sensitive) has been linked with species differences in its metabolism [15]. The possibility cannot be ruled out that the target of T is the connecrive tissue of the limb anlagen [18], an essential element of whose composition is collagen. The rate of collagen synthesis is known to depend largely on the ascorbic acid concentration in the tissue [4].

It was accordingly decided to study the effect of T on the ascorbic acid concentration in the organs of guinea pigs which, like primates, cannot synthesize this vitamin [4]. Since induction of enzymes of the liver microsomal fraction can sharply increase the rate of ascorbic acid metabolism [7], the action of T on microsomal hydroxylase activity from the liver of several species of mammals also was studied in experiments in vivo and in vitro. On the basis of the results a mechanism of the teratogenic action of T, capable of experimental verification, was put forward.

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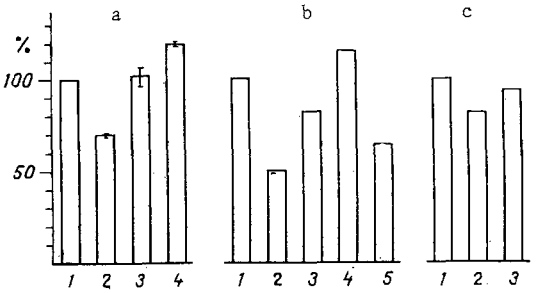

Fig. 1

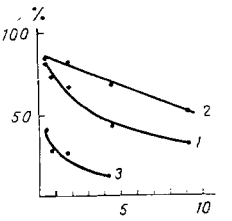

Fig. 2

Fig. 1. Action of DMSO and T on activity of aniline p-hydroxylase of ME from rabbit (a), pig (b), and guinea pig (c) liver. 1) Normal, 2) DMSO (0.5%); 3) T (100 μg/ml) and DMSO (0.5%); 4) T (powder); 5) phenobarbital (100 μg/ml) and DMSO (0.5%).

Fig. 2. Action of DMSO on aniline p-hydroxylase activity of liver ME from guinea pigs (1), from guinea pigs receiving T intraperitoneally for 4 days in a dose of 75 mg/kg daily (2), and from pigs (3).

EXPERIMENTAL METHOD

Experiments were carried out on chinchilla rabbits and guinea pigs. The microsomal fraction was isolated from pig liver as described previously [5]. The rate of p-hydroxylation of aniline was measured by the method in [3], but the reaction was carried out for 10 min. The ascorbic acid concentration was measured in several organs of guinea pigs, after homogenizing the tissue in 5% metaphosphoric acid, and then titrating extracts with 2,6-dichlorophenol-indophenol [4]. In the experiments with administration of T a suspension of it in physiological saline was made up immediately before use.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of T was studied on the velocity of p-hydroxylation of aniline, catalyzed by microsomal enzymes (ME) including cytochrome P-450 [3]. Reactions catalyzed by cytochrome P-450 are known to proceed mainly with the participation of NADPH. If T takes part in oxidative reactions with appreciable velocity, its addition to the reaction medium ought to lead to additional consumption of NADPH, and consequently, to a recordable decrease in the velocity of the main reaction – the p-hydroxylation of aniline. The results of an experiment carried out on ME from rabbit liver are shown in Fig. 1a. T (final concentration 100 μg/ml) was added to the reaction mixture either dissolved in dimethyl sulfoxide (DMSO) or in powder form. In the latter case the T concentration was close to the concentration of its saturated solution (about 100 μg/ml). DMSO itself (0.5%) inhibited the p-hydroxylase reaction by 1.4 times. T not only did not reduce the recorded rate of hydroxylation of aniline but, on the contrary, appreciably protected ME against the destructive action of DMSO. Moreover, when added to the medium in powder form, T increased the reaction velocity compared with the control (without DMSO) by 1.21 times. The stabilizing ability of T was exhibited more strongly still on pig ME (Fig. 1b). Guinea pig ME were relatively resistant to the action of DMSO, but nevertheless in the presence of T the reaction velocity in medium with DMSO was higher by 14.6% than in the absence of T.

The effect of T on hydroxylase activity of liver microsomes in vivo was studied in experiments on guinea pigs. T was injected intraperitoneally into female guinea pigs in a dose of 75 mg/kg daily for 4 days. The animals were autopsied 4 h after the last injection. The aniline p-hydroxylase activity in liver microsomes of the experimental animals was found to be twice as high as in the control. Incidentally, ME isolated from the liver of pigs receiving T were appreciably more resistant to DMSO than ME from control guinea pigs (Fig. 2). Injection of T thus caused a marked increase in ME activity, probably largely due to the ability of T to stabilize microsomes. It can be tentatively suggested that this action of T in rabbits and pigs will be even stronger (compare Figs. 1 and 2). According to our preliminary data, ME from human liver are similar in stability to pig ME. There are thus grounds for considering that T in primates will increase liver ME activity.

In the experiments of series II the effect of T on the ascorbic acid concentration in the liver, kidneys, spleen, and adrenals of guinea pigs was studied. For comparison, phenobarbital, a known ME inducer, was used. The results are shown in Fig. 3. A number of essential factors must be noted. Intraperitoneal injection of T

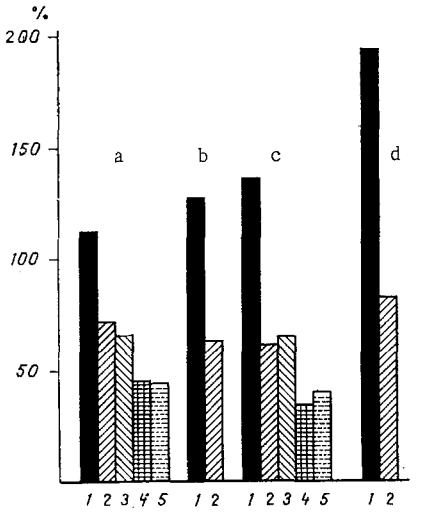

Fig. 3. Action of T and phenobarbital on ME activity and ascorbic acid concentration in several organs of guinea pigs. a) T injected by the intragastric route in a dose of 120 mg/kg for 4 days; c) T injected intraperitoneally in a dose of 75 mg/kg for 3 days; d) phenobarbital injected by the intragastric route in a dose of 50 mg/kg for 4 days. 1) Specific activity of ME p-hydroxylase, 2) ascorbic acid concentration in liver, 3) the same, in kidneys, 4) the same, in spleen, 5) the same, in adrenals.

(75 mg/kg daily for 3 days) induced microsomal hydroxylases appreciably more strongly than administration by the intragastric route (120 mg/kg daily for 3-4 days). Administration of T caused a fall in the ascorbic acid concentration in the guinea pigs' organs by 1.4-3 times even when the increase in ME activity was relatively small. The amount by which the ascorbic concentration decreased correlated with the degree of increase in ME activity. The effect of T on ascorbic acid was found to be specific. To induce a fall in the ascorbic acid level of the same extent as that produced by T with phenobarbital, a much greater increase in ME activity was required than in the experiments with T. The ascorbic acid concentration fell most sharply in those organs in which under normal conditions its concentration was maximal. In their response to ME inducers, guinea pigs, which cannot synthesize ascorbic acid, differed sharply from rats, which are capable of endogenous synthesis of this vitamin. In rats the principal reactions of ascorbic acid synthesis are effected by ME [6]. Probably, therefore, inducers of ME in rats lead to a sharp increase in the rate of ascorbic acid synthesis [7], much higher than the increase in the rate of its breakdown. As a result, induction of ME in rats is accompanied by a sharp rise in the concentration of the vitamin in the organs and blood. In guinea pigs inducers of ME, notably T, can cause an increase only in the rate of breakdown of ascorbic acid, with the result that its concentration in the tissues probably falls (Fig. 3).

The results are evidence that T can induce a deficit of ascorbic acid in female guinea pigs. On this basis we can postulate a mechanism of the teratogenic action of T. Since guinea pig fetuses, like fully grown females, cannot synthesize ascorbic acid [13], T creates a deficit of this vitamin in tissues of the embryos also. The ascorbic acid deficit leads to marked inhibitions of collagen synthesis [4], which may damage the anlagen of the limbs, in which collagen synthesis normally takes place at a rapid rate. Ganglia of the nervous system which, under normal conditions, like the adrenals are structures very rich in ascorbic acid, also need this vitamin. It has been shown that T actually damages the nervous ganglia of embryos [11, 17]. It can be tentatively suggested that disturbance of the functioning of these ganglia is also the result of a T-induced ascorbic acid deficit. It has been shown [1] that the sensitivity of rat embryos to T increased when the mothers were fed on a diet without folic acid and riboflavin. In rats with a deficiency of these vitamins, ability to synthesize ascorbic acid is sharply reduced [4]. Probably under these conditions T can create an ascorbic acid deficit in rats also.

There are thus grounds for considering that the teratogenic action of T is due to its specific induction of ME, which, in species unable to synthesize ascorbic acid (and accordingly sensitive to T), leads to a sharp fall in the concentration of this vitamin in the organs and blood of pregnant animals, and also in the fetal tissues.

That is why an ascorbic acid deficit in the fetus, first, inhibits collagen synthesis in the developing limbs and damages mesenchymal cells in them, and second, damages the limb ganglia. The combined action of these two factors probably leads to disturbance of morphogenesis of the limbs.

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QUANTITATIVE DETERMINATION OF THE INTENSITY OF FLUORESCENCE OF 5-HT ORGANELLES IN THE STUDY OF PLATELET FUNCTION

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The role of platelets in the pathogenesis of thrombohemorrhagic complications of meningococcal infection has been insufficiently studied. The role of the granular apparatus of the platelets in disturbances of their function has been particular little studied, especially in endotoxinemia accompanying the generalized form of meningococcal infection.

The work of da Prada et al. (1965-1978), who used luminescence microscopy [5], showed that it is possible to study the state of the granular apparatus of platelets with the aid of a fluorescent marker (mepacrine, acridine orange – AO, etc.), which is selectively taken up by the serotonin-containing granules (5-HT organelles, dense bodies, β-granules). The quantity of marker taken up reflects the functional state of these granules, which play an essential role in hemostasis, for they release serotonin, ADP, Ca^{++}, and platelet factor 3 in response to activation.

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