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EFFECTS OF ACUTE AND CHRONIC TERPHENYLS AND METHYLNAPHTHALENES ADMINISTRATION ON HEPATIC PENTOBARBITAL METABOLISM IN THE RAT

by

P. SCOPPA

1966

Joint Nuclear Research Center
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SUMMARY

The aromatic hydrocarbons examined and their mixtures, excepted the para isomer of terphenyl, interfere at high doses with the metabolism of pentobarbital. A single oral dose causes a biphasic response : the rate of pentobarbital metabolism is reduced in the first hours after the intake of hydrocarbon, while after longer time intervals, up to five days, it is trongly accelerated. Chronic administration of terphenyls results in a moderate increase of pentobarbital metabolism. Simultaneous treatment of the animals with ethionine abolishes the increased rate of pentobarbital metabolism produced by the hydrocarbons.

EFFECTS OF ACUTE AND CHRONIC TERPHENYLS AND METHYLNAPHTHALENES ADMINISTRATION ON HEPATIC PENTOBARBITAL METABOLISM IN THE RAT (*)

In the course of an investigation of the toxicity of aromatic hydrocarbons used as nuclear reactors coolants it seemed desirable to look for possible synergism between, or potentiation of effect by, these hydrocarbons and such commonly used drugs as sedatives, tranquillizers, analgesics, etc., which are administered over prolonged periods.

The experiments described in the present communication were suggested by those of Arcos et al. (1) with aromatic polycyclic hydrocarbons and of Hart et al. (2) with chlorinated aromatic hydrocarbons. Several compounds used by the above-mentioned authors produced an increase in the activity of microsomal drug-metabolizing enzymes which was characterized by a latency of onset and a prolonged duration.

The compounds considered are aromatic polycyclic hydrocarbons (terphenyls, methylnaphthalenes) and their mixtures (CM-2, Thermip) used in the technology of Orgel type reactors. These products were studied by measuring their effects on the metabolism of pentobarbital in the rat.

Sleeping time following injection of pentobarbital was used as an indirect measure of "in vivo" metabolism, while "in vitro" metabolism was determined chemically in liver slices. In an attempt to demonstrate enzyme induction by such aromatic hydrocarbons, ethionine, an inhibitor of protein synthesis (3), was given with the compounds examined. Inhibition of the effect of an enzyme inducer by ethionine was put forth as evidence for an induction of "de novo" enzyme synthesis by the inducer, rather than activation of existing enzymes (4).

(*) Manuscript received on November 8, 1966

METHODS

Male rats of the Sprague-Dawley strain, weighing 230-270 g, were maintained on a synthetic diet (A.L.A.L. - Allevamento Lombardo Animali da Laboratorio - Milano. Italy) and water "ad libitum".

The effects of a single dose of the different hydrocarbons on pentobarbital metabolism were examined at various time intervals after the administration of the compound (8 millimoles/Kg body weight) by stomach tube. The parameter taken into consideration was the sleeping time in response to intraperitoneal injection of sodium pentobarbital (40 mg/Kg). Sleeping time was taken as the elapsed time between injection of pentobarbital and regaining of righting reflex. The number of rats for each group and other experimental data are reported in Table I.

Chronic treatments with mixtures of hydrocarbons were performed by the administration of 0,1 millimole/Kg/24h ; the duration of the treatment varied between one and five weeks. Sleeping times following injection of pentobarbital were measured 24 hours after the end of the treatment. The number of rats for each group and other experimental conditions are given in Table II.

The effect of ethionine toward the action of OM-2 mixture (o-terphenyl 15%, m-terphenyl 80%, p-terphenyl 4%, diphenyl<1%) on pentobarbital metabolism "in vivo" and "in vitro" was examined using 80 rats divided into the following treatment groups : (a) control, (b) OM-2, 0,65 millimole/Kg, (c) D,L-ethionine, 150 mg/Kg, (d) OM-2, 0,65 millimole/Kg, plus D,L-ethionine, 150 mg/Kg. The compounds

were given in olive oil by intraperitoneal injection : the treatment was continued for a period of three days. Sleeping time in response to sodium pentobarbital (40 mg/Kg, i.p.) was measured on 10 rats for each group 24 hours after the last treatment. Upon awakening the animals were killed and the concentration of pentobarbital in plasma and liver was measured by a spectrophotofluorimetric method (5). The remaining rats, 10 for each group, were decapitated;the liver was immediately excised and rinsed in cold, isotonic solution of potassium chloride ; 500±10 mg slices were placed in 50 ml tubes containing 6 ml of Krebs-Ringer-bicarbonate-glucose to which 1 micromole of sodium pentobarbital had been added. The tubes were incubated for 1 hour at 37°C, under an atmosphere of 5% CO_2 and 95% O_2. Duplicate control tubes received the same amount of liver slices and medium with sodium pentobarbital and were kept on ice for 1 hour. At the end of the incubation period, aliquots of the incubation medium were removed and analyzed for pentobarbital by the method of Brodie (6). The amount of pentobarbital metabolized was determined as the difference between the amount remaining in control and incubated tubes.

RESULTS

The administration of a single dose (8 millimole/Kg, by stomach tube) of the hydrocarbons examined produces a biphasic response on the metabolism of pentobarbital in the rat (Table I). This effect was detected, although with varying intensity and duration, for all the hydrocarbons and mixtures examined : the only exception was the para-isomer of terphenyl which was practically inactive. Six hours after treatment the rate of pentobarbital metabolism was strongly reduced : sleeping time increased and more than doubled in the case of treatment with ortho-terphenyl. When the injection of pentobarbital was made in the days following the administration of the hydrocarbons, the results obtained were completely different. Sleeping

time was strongly reduced, and then the rate of pentobarbital metabolism increased. The higher effect was detected on the second day of treatment with terphenyls (Figure 1), and while it is not well defined, it is certainly present between the second and the third day in the treatment with methylnaphthalenes (Figure 2). The duration of this effect was about 5 days for terphenyls and 4 days for methylnaphthalenes.

Chronic administration of small doses (0,1 millimole/kg/24h) of mixtures of the hydrocarbons did not produce marked effects on the metabolism of pentobarbital "in vivo". Sleeping time was decreased by treatment with terphenyls, but was not significantly (7) affected by treatment with alkylnaphthalenes. It is interesting to note that the reduction of sleeping time did not increase as a consequence of a prolonged treatment, but tended to decrease after three weeks (Table II).

Administration of the mixture of terphenyls for 3 days, for a total dose of about 2 millimoles/Kg, increased the ability of animals to metabolize pentobarbital. This change in the metabolic handling of the drug was first suggested by shortened sleeping times of the rats receiving the hydrocarbon (Table III). In contrast to the control group which slept 87 $\pm$ 5 minutes, the group treated with OM-2 mixture slept 41 $\pm$ 5 minutes. When ethionine was given with the hydrocarbons, the decrease in sleeping time was abolished and the mean time (97 $\pm$ 6 min) was not statistically different from mean control time. When ethionine alone was given, sleeping times were lenghtened so that the mean approached 123% of control time.

To ascertain if sleeping time following the injection of pentobarbital could be considered as an indirect measure of the metabolism "in vivo", the concentrations of pentobarbital in plasma and liver were determined upon awakening of the animals. The results (Table III) show no significant differences among the four groups : this provides evidence that the treatments to which the rats had been submitted did not produce changes in the sensitivity of the nervous system to barbiturate ; the differences in sleeping time can be attributed to changes regarding the rate with which pentobarbital is metabolized.

The increase in the rate of metabolism of pentobarbital, showed by the shortened sleeping time in the animals receiving the terphenyls mixture, was confirmed by the "in vitro" studies. Liver slices from animals receiving the OM-2 mixture were capable of degrading pentobarbital more rapidly than comparable slices of control animals. These results are reported in Table III and are shown both as "unit activity" (micrograms metabolized/g liver/hour) and "hepatic capacity" (milligrams metabolized/Kg body weight/hour): hepatic capacity was calculated as the product of unit activity and the ratio of liver weight to body weight: it is thought to be a better expression of "in vivo" metabolism since the relative mass of the metabolizing tissue is taken into consideration.

A statistical comparison of either unit activity or hepatic capacity of the treated group with the control group shows the difference to be highly significant ($P < 0,01$). Slices taken from the livers of animals treated with ethionine alone showed a reduced

capability to metabolize pentobarbital. When given in combination with the terphenyls, ethionine effectively prevented the increase of metabolic activity, observed when terphenyls were given alone. The hepatic capacity of the animals so treated was not statistically different from control values.

Liver-to-body weight ratios in treated and control animals were not significantly different.

DISCUSSION

In the last few years a large number of chemically unrelated compounds has been shown to increase the activity of the microsomal enzymes which metabolize drugs and other foreign compounds (8).

The mechanism of action has not yet been fully clarified : Conney et al. (9) put forward the hypothesis that the higher drug-metabolizing activity could be due to the synthesis "de novo" of enzymatic protein. More recent investigations by Ortega (10) and Remmer et al. (8, 11, 12) show modifications at a subcellular level in the liver of the animals treated with compounds capable of producing an increase of drug-metabolizing enzymatic activities. The results obtained by electron microscopy are in full agreement with those coming from biochemical investigations : they show the typical phenomenon of enzyme induction. In particular, the primary phenomenon seems to be an increased synthesis of a special microsomal cytochrome, which has a fundamental function in the hydroxylation of those compounds able to produce an increase in drug-metabolizing activities ; it is followed by the neoformation of intracellular membranes, part of the reticulo-endothelial system, where the drug-metabolizing enzymes are located (13).

It seems reasonable to consider the increase in enzymatic activity produced by the administration of the hydrocarbons examined to be connected with the "de novo" formation of structures of the reticulo-endothelial system, and therefore to a higher amount of microsomal substance corresponding to that complex system of membranes.

As far as the increased duration of the action of pentobarbital is concerned, when this drug is given a short time after an acute treatment with high doses of hydrocarbons, the hypothesis of a competitive inhibition of drug-metabolizing enzymes by such compounds may be advanced. Experimental evidence for this point can be provided with "in vitro" experiments on the metabolism of pentobarbital in the presence of varying concentration of hydrocarbons.

CONCLUSION

The present research on the effects of aromatic hydrocarbons used as coolants in nuclear reactors led to conclusion that such compounds are capable of interfering with the metabolism of pentobarbital only if they are introduced into the organism in high doses.

Obviously the results obtained in the rat cannot be extrapolated to human beings, although the most recent research in the clinical field (8) shows that similar results should be expected in man.

Because of the non-specificity of microsomal drug-metabolizing enzymes, the effects detected on the metabolism of pentobarbital may be extended to other foreign compounds such as food additives, pesticide residues, various chemicals of therapeutic or industrial use.

FIG. 1 - EFFECTS OF A SINGLE ORAL DOSE OF TERPHENYLS ON PENTOBARBITAL SLEEPING TIME

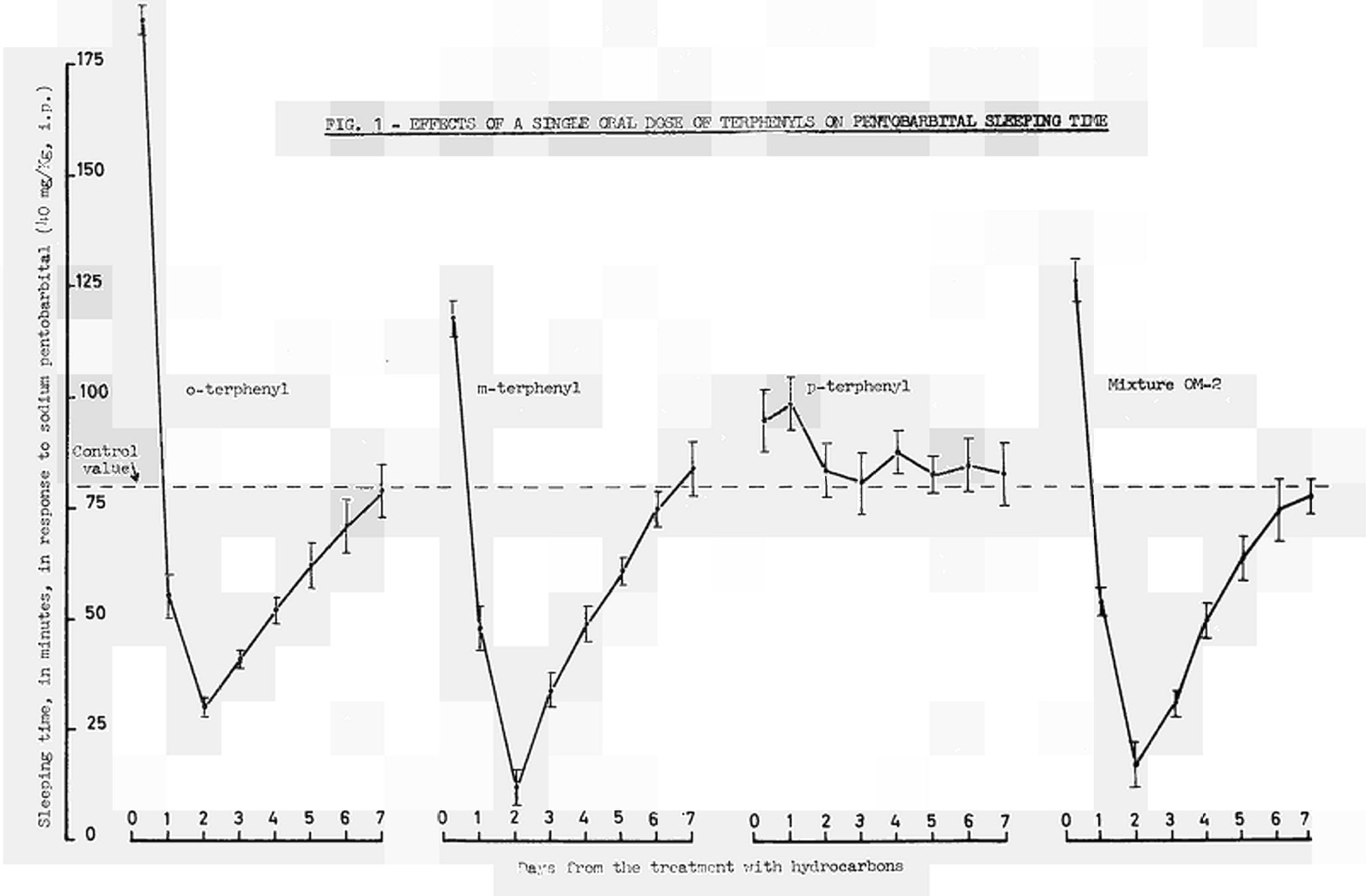

FIG. 2 - EFFECTS OF A SINGLE ORAL DOSE OF ALKYLNAPHTHALENES ON PENTOBARBITAL SLEEPING TIME

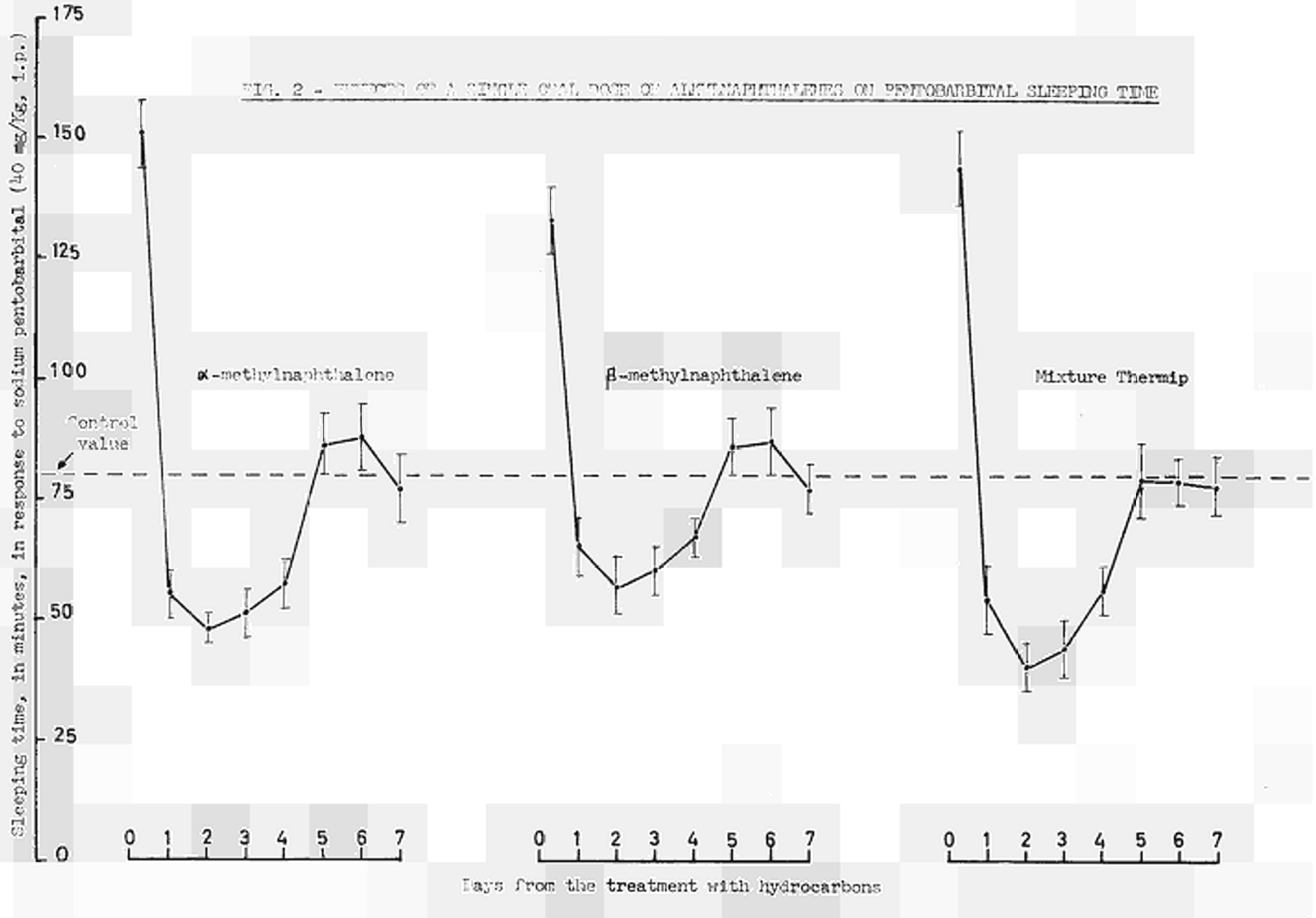

TABLE I

EFFECTS OF A SINGLE DOSE OF VARIOUS AROMATIC HYDROCARBONS (8 millimoles/Kg, by stomach tube) ON PENTOBARBITAL (40 mg/Kg, i.p.) SLEEPING TIME IN THE RAT(°)

TREATMENT(x)	TIME AFTER ADMINISTRATION OF HYDROCARBON							
	6 hours	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Control (60)	80 ± 4 (20)	-------	83 ± 4 (20)	-------	-------	79 ± 5 (20)	-------	-------
Ortho-terphenyl (80)	185 ± 4 (10)	55 ± 5 (5)	30 ± 2 (10)	41 ± 2 (5)	52 ± 3 (5)	62 ± 5 (5)	71 ± 6 (5)	79 ± 6 (5)
Meta-terphenyl (70)	118 ± 4 (10)	48 ± 5 (5)	12 ± 4 (10)	34 ± 4 (5)	49 ± 4 (5)	61 ± 3 (5)	75 ± 4 (5)	84 ± 6 (5)
Para-terphenyl (50)	95 ± 7 (10)	99 ± 6 (5)	84 ± 6 (10)	81 ± 7 (5)	88 ± 5 (5)	83 ± 4 (5)	85 ± 6 (5)	83 ± 5 (5)
OM-2 mixture (70)	127 ± 5 (10)	54 ± 3 (5)	17 ± 5 (10)	31 ± 3 (5)	50 ± 4 (5)	64 ± 5 (5)	75 ± 7 (5)	78 ± 4 (5)
α-methylnaphthalene (50)	151 ± 7 (10)	55 ± 5 (5)	48 ± 3 (10)	51 ± 5 (5)	57 ± 5 (5)	86 ± 4 (5)	88 ± 7 (5)	77 ± 5 (5)
β-methylnaphthalene (50)	133 ± 7 (10)	65 ± 6 (5)	57 ± 6 (10)	60 ± 5 (5)	67 ± 4 (5)	86 ± 6 (5)	87 ± 7 (5)	77 ± 5 (5)
Thermip mixture (50)	144 ± 8 (10)	54 ± 7 (5)	40 ± 5 (10)	44 ± 6 (5)	56 ± 5 (5)	79 ± 8 (5)	79 ± 5 (5)	78 ± 6 (5)

(°) Values in the table are time in minutes during which righting reflex was absent ± standard error ; numbers in parentheses represent number of animals per group.

(x) The hydrocarbons were given in solution in 2 ml of olive oil, or in suspension in the case of p-terphenyl.

TABLE II

EFFECTS OF CHRONIC TERPHENYLS AND ALKYLNAPHTHALENES FEEDING ON PENTOBARBITAL SLEEPING TIME IN THE RAT(°)

TREATMENT(x)	TIME ON DIET					
	Control	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Terphenyls OM-2 mixture 0,1 millimole/Kg/24 h	82 ± 4	68 ± 5	55 ± 7	50 ± 4	63 ± 5	74 ± 5
Alkylnaphthalenes Thermip mixture 0,1 millimole/Kg/24 h	80 ± 5	74 ± 3	73 ± 5	68 ± 7	75 ± 5	79 ± 4

(°) Values in the table are time in minutes during which righting reflex was absent ± standard error; animals received pentobarbital sodium (40 mg/Kg, i.p.) 24 hours after the end of treatment.

(x) Five rats for each group; the hydrocarbons were given in the food.

TABLE III

SLEEPING TIMES AND HEPATIC METABOLISM OF PENTOBARBITAL IN RATS PRE-TREATED WITH TERPHENYL MIXTURE OM-2 AND WITH TERPHENYL MIXTURE OM-2 PLUS D,L-ETHIONINE

TREATMENT(°)	No. of rats	Sleeping time (min)	PENTOBARBITAL CONCENTRATION UPON AWAKENING		HEPATIC METABOLISM		Liver/ body weight ratio (g/100 g)
			Liver (γ/g)	Plasma (γ/ml)	Unit activity (γ/g liver/h)	Hepatic capacity (mg/Kg b.w./h)	
Control	20	87 ± 5	71,6 ± 3,9	12,1 ± 1,7	156 ± 4	5,65 ± 0,24	3,62 ± 0,06
Terphenyl mixture OM-2 (0,65 mmole/Kg, i.p.)	20	41 ± 5	71,3 ± 4,5	13,6 ± 1,7	187 ± 4	6,98 ± 0,28	3,73 ± 0,07
D,L-ethionine (150 mg/Kg, i.p.)	20	107 ± 5	68,1 ± 4,8	12,8 ± 1,6	132 ± 5	4,61 ± 0,32	3,49 ± 0,11
Terphenyl mixture OM-2 (0,65 mmole/Kg, i.p.) plus D,L-ethionine (150 mg/Kg, i.p.)	20	97 ± 6	69,7 ± 3,6	12,1 ± 1,6	141 ± 4	5,25 ± 0,25	3,72 ± 0,09

(°) The treatments were continued for a period of three days. On the fourth day following initiation of treatments, 10 rats for each group received 40 mg/Kg pentobarbital sodium, i.p.; upon awakening the animals were sacrificed for the determination of liver and plasma concentrations of barbiturate. The remaining rats, 10 for each group, were used for measuring pentobarbital metabolism "in vitro".

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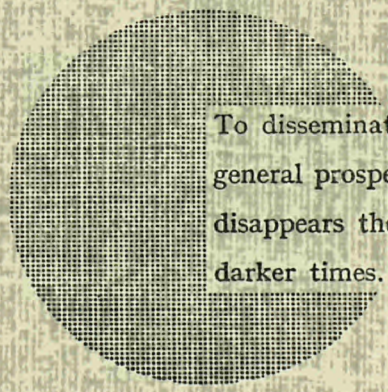

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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