Inhibition of Drug Metabolism in the Mouse by a Hypocholesterolemic Agent, AY-9944

The enzymes localized in liver microsomes are not only considered to be the most important ones in drug metabolism, but they are also involved in some stages of cholesterol biosynthesis[1,2]. KATO et al.[2] reported that some well known inhibitors of cholesterol biosynthesis (i.e. triparanol, benzmalacene) had inhibitory action also on the metabolism of certain drugs. On the other hand one of the most representative inhibitors of microsomal drug-metabolizing enzymes, SKF 525-A (β-diethylaminoethyl-3, 3-diphenyl propylacetate), was also found to be an inhibitor of cholesterol biosynthesis[3].

Recently, a powerful inhibitor of hepatic cholesterol synthesis was described by CHAPPEL et al.[4,5]: AY-9944 [*trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride]. The purpose of the investigation reported here was to assess a possible inhibitory action of this novel hypocholesterolemic agent on drug metabolism. A battery of three pharmacologic assays to estimate inhibition of 'non-specific' enzyme system was used as suggested by LESSIN[6]: (a) prolongation of pentobarbital hypnosis, (b) intensification of chlorpromazine-induced hypothermia, and (c) reduction in the acute toxicity of Schradan (OMPA, octamethyl-pyrophosphoramide). These assays are based on the assumption that a 'non-specific' liver oxidase system is involved in all these three phenomena, thus potentiation of barbiturate hypnosis and chlorpromazine hypothermia are the result of inhibition of these enzymes, while the inhibition of the same enzymes leads to a reduction of Schradan toxicity, because they are necessary for the *in vivo* conversion of Schradan into a highly toxic anticholinesterase[7].

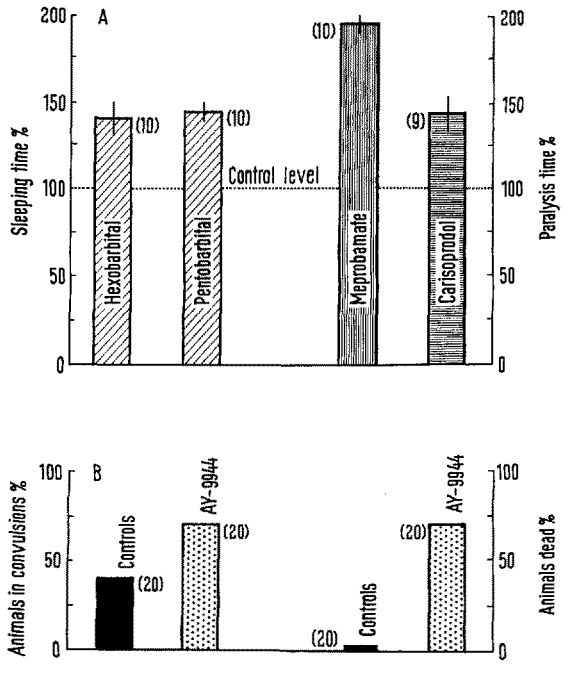
Methods. Male mice (albino, Charles River CD-1) weighing 20 to 25 g were used. All drugs were injected intraperitoneally. Room temperature was maintained at 23°C. Hypnosis induced by pentobarbital sodium and hexobarbital sodium, paralysis induced by meprobamate and carisoprodol were determined from the duration of loss of the righting reflex. The toxic effects of strychnine were assessed from convulsions and mortality.

Chlorpromazine hypothermia. Two measurements were taken during pretreatment. The animals were treated with 2.5 mg/kg of chlorpromazine hydrochloride, and the body temperature was measured 1 h later using a thermocouple apparatus. AY-9944 was given at various intervals before the chlorpromazine.

Schradan toxicity. The mean survival time of groups of ten mice was determined after a dose of 50 mg/kg of Schradan. The interval between injection and death was recorded as survival time.

Results and discussion. Figure 1 shows that 9.3 mg/kg (i.e. 20 μmole/kg) of AY-9944 potentiated hypnosis induced by the two barbiturates tested, prolonged meprobamate and carisoprodol paralysis and increased the toxicity of strychnine.

The effect of AY-9944 on body temperature of mice is summarized in the Table. The fall in rectal temperature



(A) Potentiation of hypnosis induced by hexobarbital and pentobarbital, and prolongation of paralysis induced by meprobamate and carisoprodol in male mice treated with AY-9944. 9.3 mg/kg (i.e. 20 μmole/kg) of AY-9944 was administered 20 min before the injection of hexobarbital (120 mg/kg), pentobarbital (80 mg/kg), meprobamate (180 mg/kg) or carisoprodol (140 mg/kg). Vertical bars represent ± S.E. Figures in brackets are numbers of animals. (B) The effect of 1 mg/kg of strychnine in control mice and animals treated with AY-9944. 9.3 mg/kg of AY-9944 was administered 20 min before the injection of strychnine.

Hypothermia and potentiation of chlorpromazine hypothermia induced by AY-9944

Treatment	Mean body temperature, ± S.E., 8 mice/group	
	Experiment No. 1	Experiment No. 2
Controls	37.1 ± 0.3	37.2 ± 0.2
Chlorpromazine (2.5 mg/kg)	34.0 ± 0.9	35.3 ± 0.6
	Dose of AY-9944	
	30 mg/kg	90 mg/kg
AY-9944	35.9 ± 0.6	34.4 ± 0.8
AY-9944 + chlorpromazine		
5 min[a]	31.1 ± 0.4	29.3 ± 0.6
30 min	31.4 ± 0.9	30.7 ± 0.7
1 h	32.3 ± 0.7	30.8 ± 0.9
2 h	33.2 ± 0.8	33.1 ± 0.6

[a] Time interval between the injections of AY-9944 and chlorpromazine. The rectal temperature was always measured 1 h after the administration of chlorpromazine

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caused by 2.5 mg/kg of chlorpromazine was between 1.9 and 3.1°C. These values are in agreement with the findings of others[8,9]. AY-9944 produced a maximal decrease of 1.2°C and of 2.8°C at 30 mg/kg and 90 mg/kg dose levels respectively. When both compounds were administered a fall of 6.0 to 7.9°C was recorded. The maximal decrease of temperature was found when AY-9944 was given 5 min before the injection of chlorpromazine.

When a dose of 30 mg/kg of AY-9944 was given to mice 30 min before treble the median lethal dose of Schradan (i.e. 50 mg/kg) the agent conferred on the animals some resistance to the anticholinesterase. The mean survival time of mice treated with AY-9944 was 42% longer than that of controls ($p < 0.01$).

These results suggest that in mice AY-9944 has an inhibitory action on the metabolism of certain drugs. Qualitatively similar results were reported for isoniazid, iproniazid, SKF-525-A when the above three pharmacologic assays were used to estimate inhibition of drug oxidation[6]. Hexobarbital sleeping time is regarded as a good index of the activity of microsomal enzymes[10]. The enzymes responsible for the metabolism of pentobarbital, hexobarbital, carisoprodol, strychnine and Schradan were only found in microsomes of liver[11]. It is reasonable to conclude that AY-9944 is also an inhibitor of the 'non-specific' microsomal oxidase system in the liver.

These results also show that AY-9944 produces hypothermia in mice. Various agents are known which lower body temperature and also potentiate barbiturate hypnosis[8,12,13]. It is evident that the activity of the microsomal oxidase system like that of many other enzyme systems, can be modified by changes in temperature. The questions, however, how the hypothermia and the potentiation of barbiturate hypnosis are related in the case of AY-9944, and what is the underlying mechanism responsible for the hypothermia induced by AY-9944 alone, are to be investigated.

In the treatment of poisoning with organophosphorus anticholinesterases 2-PAM (pyridine-2-aldoxime methiodide) in general is as effective therapeutically as atropine. However, it is reported that 2-PAM treatment is not successful with organophosphorus amides, such as Schradan, which form a very stable complex with the enzyme, acetylcholinesterase[14]. In poisoning with this type of compound, agents will be useful therapeutically only if they have a strong reactivation effect, but AY-9944 may be of use prophylactically because of its inhibitory action on the oxidative conversion of Schradan into a toxic anticholinesterase.

Further studies utilizing biochemical parameters on drug biotransformation are in progress. The study in detail will be published elsewhere.

Résumé. Un composé hypocholestérolémique (AY-9944) administré par voie intrapéritonéale provoque chez les souris une action hypothermique de même qu'une action inhibitrice sur le système enzymatique d'oxidase «non-spécifique» du foie.

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Genetic Tumours of Nicotiana Hybrids and the Hormone Balance

Hybrids of *Nicotiana glauca* × *Nicotiana langsdorffii* have been cultured for five successive years. They were rendered autofertile by amphidiploïdy with colchicine. KOSTOFF[1] and others observed those hybrids bearing tumours regularly on roots and twigs, the so-called 'genetic tumours'. The neoplasms start by the union of two different genoms and are bacteriologically sterile. We have noted by macroscopic account of the number of tumours on the roots, that the tumour-inciting power diminished progressively in each generation of plants and disappeared completely after five years of successive culture. Our plants cultivated outdoors progressively recovered normal appearance as they restored the normal hormonal equilibrium. No variations or mutations are noted on chromosomes.

THIMANN[2] suggested that the growth hormones liberated by the *Rhizobium bacterium* may incite the nodule development in leguminous plants. KEHR and SMITH[3] argued that the tumours in Nicotiana hybrids may have a hormonal origin through a defective functioning of the plant hormones. It is well known that a sufficient amount of nitrogen in the soil does inhibit or suppress completely the formation of nodules on leguminous plants. From our experiments over a sufficient number of years, it seems likely that the culture of Nicotiana hybrids on soils with a sufficient amount of mineral nutrients can progressively suppress the so-called 'genetic tumours' in the hybrids. This surprising fact is presented to other researchers because it may invalidate the theory of the genetic origin of those tumours and reduce the phenomenon to a hormonal disturbance.

Résumé. Les tumeurs «génétiques» des hybrides de Nicotiana sont dues à une perturbation de l'équilibre hormonal, susceptible de restitution. L'origine génétique de ces tumeurs paraît improbable.

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