Effect of Some Surface Active Agents on the Action of Barbiturates. (17741)

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Interest in the effect of surface active agents on drugs stems mainly from their inhibitory action on enzyme systems(1-5), although penetration can not be entirely disregarded as a factor. Eiseman(6) by *in vitro* experiments has shown that by combining a known tuberculostatic agent with a surface active agent the tuberculostatic action was increased. The study described below was undertaken to investigate the effects of these agents on barbiturates. The doses of surface active agents used were such that no observable effect was produced. Pentobarbital and thiopental were used as examples of those barbiturates which are destroyed in the liver(7,8). Barbital was used as an example of a barbiturate not destroyed in the body(7). The onset of hypnosis was considered to be the loss of righting reflex and the duration the time between loss of righting reflex and the regaining of the reflex.

Experiments with Pentobarbital Sodium. Eleven surface active agents were selected from the group which had been tested for toxicity(9), and one-half the LD_{50} was given with a hypnotic dose of sodium pentobarbital

TABLE I.

Copyright and Chemical Names of the Agents Studied with One-half the Intravenous LD-50 for Mice. The amounts stated represent the amounts of active material contained in the commercial preparations.

Agents	Type	½ LD-50, mg/kg	Chemical names
Emulsept	C	10	Lauric and myristic acid esters of colamino-formylmethyl-pyridinium chloride
Igepal CA	N	35	Polymerized ethylene oxide condensate
Aerosol O.S.	A	60	Isopropylnaphthalene sodium sulfonate
Santomerse 3	A	52	Dodecyl benzene sodium sulfonate
Areskap 100	A	90	Monobutylphenyl-phenol sodium monosulfonate
Aresket 300	A	125	Monobutyl diphenyl sodium monosulfonate
CDMEN Br.*	C	25	Cetyl dimethyl ethyl ammonium bromide
Aerosol O.T.	A	30	Dioctyl ester of sodium sulfosuccinic acid
Emcol 888	C	4	Polyalkyl naphthalene pyridinium chloride, average M.W. 382
Santomerse D	A	60	Decyl benzene sodium sulfonate
Aresklene 400	A	100	Dibutylphenyl-phenol sodium disulfonate

* Our abbreviation.

1. Glassman, H. N., *Bact. Rev.*, 1948, v12, 105.

2. Kirsner, J. B., and Wolff, R. A., *Gastroenterology*, 1944, v2, 270.

3. Kirsner, J. B., and Spitzer, E. H., *Gastroenterology*, 1944, v2, 348.

4. Block, C. L., Portis, S. A., and Necheles, H., *Gastroenterology*, 1944, v3, 45.

5. Knox, W. E., Auerbach, V. H., Zarudnaya, K., and Spirtes, M., *J. Bact.*, 1949, v58, 443.

6. Eiseman, B., *J. Exp. Med.*, 1948, v88, 189.

7. Dorfman, A., and Goldbaum, L. R., *J. Pharm. and Exp. Ther.*, 1947, v90, 330.

8. Shideman, F. E., Kelly, A. R., and Adams, B. J., *J. Pharm. and Exp. Ther.*, 1947, v91, 331.

9. Hopper, S. H., Hulpieu, H. R., and Cole, V. V., *J. Am. Pharm. Assn.*, 1949, v38, 428.

TABLE II.
The Effect of Surface Active Agents (½ LD-50) on the Sleeping Time of Mice Given an Intravenous Dose of 50 mg/kg of Sodium Pentobarbital. The surface active agents were mixed with the sodium pentobarbital.

Surface active agent	No. lived	Duration of hypnosis (min.) Mean	S.E.$_m$
None	20	23.3	2.08
Emulsept*	20	32.2	2.16
Igepal CA*	19	33.1	3.78
Aerosol O.S.	20	34.0	1.17
Santomerse 3	20	34.4	2.65
Areskap 100	20	35.8	4.27
Aresket 300	19	37.0	3.35
CDMEN Br.*	20	38.4	3.22
Aerosol O.T.*	20	39.4	3.82
Emcol 888*	16	41.0	4.53
Santomerse D*	17	46.1	5.23
Aresklene 400*	18	47.7	3.37

* These agents were retested giving the surface active agents 5 min. before the pentobarbital.

to groups of mice. From this group of 11, one was selected which gave a typical effect and that one was studied on rats, rabbits and dogs. The mice used were all female; the rats and rabbits used were male; the dogs were both male and female with matched pairs between the control and those receiving the surface active agent. Table I gives a list of the agents studied with the ionic type, the chemical nature and one-half the LD_{50}. Table II shows the experiments on mice which received 50 mg per kg of sodium pentobarbital alone and mixed with surface active agents. Twenty mice were used in each experiment. In all instances where a surface active agent was given the duration of hypnosis was longer with a surface active agent than without. In order to rule out the possibility of a reaction between the surface active agents and sodium pentobarbital, mice were given a surface active agent and 5 minutes later the same dose of sodium pentobarbital was given. Seven compounds were so tested. Qualitatively, the same results were obtained as before and 6 of the agents gave the same quantitative results. Santomerse D showed a significantly longer time of hypnosis when the surface active agent was given before sodium pentobarbital than when it was given at the same time. Thus, the effects observed in our first experiment were not due to the barbiturate being mixed with the surface active agent. Twenty male rats were then given 30 mg per kg of sodium pentobarbital by intraperitoneal injection. At the time of the loss of righting reflex Aresklene 400 was given intravenously to 10 of these in the dose of 50 mg per kg. The mean time of hypnosis was increased from 66.1 to 118.1 by Aresklene 400. Sodium pentobarbital 30 mg per kg was administered intravenously to male rabbits with and without Aresklene 400, 50 mg per kg. All the controls survived but 5 out of 10 rabbits which received the Aresklene died. There was no difference in sleeping time between the survivors and the controls. Four pairs of dogs matched for sex and weight received 25 mg per kg of sodium pentobarbital. One of each pair received Aresklene 400, 25 mg per kg or one-eighth the LD_{50}. Differences between numbers of pairs were small and inconsistent.

Experiments with Thiopental Sodium. Female mice were used for these experiments and thiopental sodium was given intravenously in a dose of 50 mg per kg. The surface active agents were tried in doses of one-half the LD_{50}, but it was found that this dose caused an increase in death rate. Therefore, the experiment was conducted with one-fourth the LD_{50} of surface active agents. The same 11 compounds were studied as were studied with sodium pentobarbital. Table III shows the

TABLE III.
The Effect of Surface Active Agents (¼ LD-50) on the Death Rate and Sleeping Time of Mice Given an Intravenous Dose of 50 mg/kg of Sodium Thiopental. The surface active agents were mixed with sodium thiopental.

	No. of mice	% died	Duration of hypnosis (min.) Mean	S.E.$_m$
Control	15	6.7	8.0	0.56
Santomerse 3	10	50	5.6	2.12
CDMEN Br.	10	0	7.6	0.68
Aerosol O.S.	10	60	9.5	3.53
Santomerse D	10	0	10.0	1.59
Emulsept	10	30	10.4	2.66
Emcol 888	10	40	10.5	1.34
Igepal CA	10	10	13.0	1.68
Areskap 100	10	20	16.7	2.84
Aresklene 400	20	20	19.6	2.92
Aresket 300	10	20	20.1	5.60
Aerosol O.T.	20	10	23.8	3.23

TABLE IV.
The Recovery of Thiopental Sodium Administered Intravenously to Mice With and Without Aresklene 400 (¼ LD-50).

	Controls	Aresklene 400, 50 mg/kg
	30 min. after inj.	
No. of mice	13	14
% thiopental recovered		
Mean	66.3	77.7
S.E.$_m$	1.69	2.89
	120 min. after inj.	
No. of mice	10	10
% thiopental recovered		
Mean	57.7	69.3
S.E.$_m$	2.70	3.18

10). The range given in per cent recovered to that injected varied at 30 minutes with the controls from 53.1 to 75.0, while in those receiving the surface active agent the variation was from 61.0 to 102.0. The chance is less than 1 in 100 that the difference was not significant. At two hours the range for controls was 44 to 72%, for those receiving Aresklene the range was 54 to 85%. The chance is less than 1 in 50 that this difference was not significant. It seems probable that the interference with the destruction of the thiopental accounts for the longer sleeping time.

Experiments with Barbital Sodium. Twenty mice per group, 10 males and 10 females, received 300 mg per kg of sodium barbital by tail vein. The controls received only sodium barbital and the experimental animals received in addition Aresklene 400, 100 mg per kg, mixed with the barbital sodium. The time without righting reflex was very variable in both groups, and there was probably no difference between the two groups. However, the average time for onset of hypnosis was 40.5 minutes after the administration of barbital sodium alone, while with Aresklene 400 the average time of onset of hypnosis was 14.2 minutes. In a similar experiment on 20 rats with a dose of 200 mg per kg of barbital sodium and Aresklene 400, 50 mg per kg, there was no difference either in the duration of hypnosis or in the time from injection until onset of hypnosis between the controls and those receiving Aresklene. Ten male rabbits

TABLE V.
The Effect of Surface Active Agents on the Onset of Hypnosis of Mice Receiving Intravenously 300 mg/kg of Barbital Sodium. The surface active agents were given mixed with the barbital sodium.

	No. of mice	No. of mice anesthetized	Onset of hypnosis (min.) Mean	S.E.$_m$
Control	20	19	33.9	3.23
Emulsept	10	10	37.2	3.21
Aerosol O.T.	10	10	34.8	4.10
Santomerse D	10	10	30.1	3.97
Aresket 300	10	8	30.1	6.30
Areskap 100	10	10	21.4	2.47
Santomerse 3	10	10	19.7	2.76
Emcol 888	10	7	16.3	1.19
Aerosol O.S.	10	10	12.2	1.27
CDMEN Br.	10	10	3.7	1.24
Igepal CA	10	10	3.0	0.49

data obtained from these experiments with the surface active agents listed in order of increasing duration of hypnosis. Igepal CA and the agents listed below it in the table gave a significant increase in the time of hypnosis.

As an attempt to test the mechanism of action with thiopental sodium some mice were sacrificed and analyzed at various intervals for the content of thiopental in the body (Table IV). The mice were injected into the tail vein with thiopental sodium, with and without Aresklene 400, 50 mg per kg. Each mouse was placed in a jar and at 30 minutes or 2 hours later killed and ground in this same jar with chloroform and buffer added. The analyses were carried out according to a modified method of Jailer and Goldbaum(7,

10. Jailer, J. W., and Goldbaum, L. R., *J. Lab. and Clin. Med.*, 1946, v31, 1344.

were given barbital sodium, 160 mg per kg, and half of them received Aresklene 400, 50 mg per kg, 2 minutes before the barbiturate. The onset of hypnosis averaged 14.8 minutes for the controls and the average time with Aresklene 400 was 2.4 minutes. Five matched pairs of dogs were given barbital sodium, 140 mg per kg with one of each pair receiving in addition Aresklene 400, 25 mg per kg, 1 minute before the barbiturate. The time from an injection until the dog would not stand up was an average of 12.8 minutes for the controls and 2.0 minutes for those receiving Aresklene 400. Ten other surface active agents in doses of one-half the LD_{50} were then tested for the effect on the onset of hypnosis in mice (Table V). Female mice were used for this experiment. Six of the agents showed a decrease in time of onset of hypnosis which was probably significant.

Summary. 1. The duration of hypnosis of pentobarbital sodium was increased for mice by all 11 surface active agents tested.

2. Aresklene 400 was selected for study on other animals. It increased the time of hypnosis due to pentobarbital in rats and rabbits but not in dogs.

3. Thiopental sodium showed an increase in effect with some of the surface active agents but not all.

4. Aresklene 400 increased the activity of thiopental apparently by decreasing the rate of destruction of thiopental in the body.

5. The onset of hypnosis but not the duration of hypnosis was influenced by Aresklene 400 with sodium barbital. Aresklene 400 decreased the time of onset of hypnosis when given with barbital sodium in mice, rabbits, and dogs but not in rats. Six of 10 other agents studied only in mice had the same effect on onset of hypnosis.

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Serological Tests on Soluble Antigens from Mice Infected with *Cryptococcus neoformans* and *Sporotrichum schenckii.** (17742)

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The purpose of the present paper is to show that soluble antigens of the fungus agents occur in various tissues and body fluids of mice infected with *Cryptococcus neoformans* and *Sporotrichum schenckii,* and that these antigens are readily detectable by direct serological tests analogous to those that have been used for the detection of soluble antigens in various bacterial, rickettsial and virus infections.

Methods. Thirteen strains of *Cryptococcus neoformans,* 4 strains of *Sporotrichum schenckii;* and, for control purposes, a strain of *Blastomyces dermatitidis* were used. The *Cryptococcus* included a representative strain of each of the types (A, B, C) recently reported by Evans(1), and also the Curtis strain which Benham(2) found to differ somewhat in agglutinating properties from other strains. The mice were infected by intraperitoneal injections, and the materials to be tested were obtained from animals that were obviously ill or that had recently died from the infection.

The antigens employed for serological precipitation tests were wash fluids from the peritoneal cavity and extracts made from the liver, spleen, testes and lungs. The peritoneal wash fluids were formalinized, and cleared by prolonged centrifugation. The organs were ground in mortars, suspended in 5 ml of salt solution, shaken for about 5 minutes in tightly stoppered test tubes, then heated 30 minutes

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1. Evans, E. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 644.

2. Benham, R. W., *J. Infect. Dis.,* 1935, v57, 255.