in the albumin and pre-albumin area. Data presented show factor specificity and freedom from other clotting factors in the case of proconvertin, Stuart, and Hageman preparations.

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Received May 18, 1959. P.S.E.B.M., 1959, v101.

Effect of Antispasmodic and Ganglionic Blocking Agents on Mortality Following Electroshock Convulsions in Mice.* (25083)

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Sporadic reports have appeared which indicate that certain agents produce a high incidence of post seizure mortality following electroshock convulsions in mice. Jenney(1) has shown that reserpine produces such an effect, while Berger(2) demonstrated a similar increase in post seizure mortality with benactyzine. Toman *et al.*(3) stated that an increase in post seizure mortality occurs with a variety of apparently unrelated agents and is nonspecific. Vernier and Meckelnburg (personal communication) observed that atropine, scopolamine, and other antispasmodics also increased post seizure mortality, and we found that ganglionic blocking agents were capable of eliciting the same response. Since no studies relating to the mechanism and site of action by which such drugs act have been reported, it was of interest to study the phenomena in more detail. The present studies were undertaken to determine if post seizure lethal effect of antispasmodic and ganglionic blocking agents was related to their peripheral anticholinergic activity and to determine if such agents altered the electroconvulsive threshold.

Method. Female mice of CF#1 strain were chosen to study ability of antispasmodic and ganglionic blocking drugs to increase mortality following supramaximal electroshock convulsions. The electroshock method was a modification of that described by Swinyard *et al.*(4). A supramaximal alternating current stimulus of 120 volts was passed for 0.3 second through saline-wick corneal electrodes from a Medcraft apparatus, type B_2, series 210. Care was taken to keep the wick electrodes saturated with physiological saline at all times. Five to 7 graded doses of the agents were administered intraperitoneally, in groups of 10 mice each, to establish response relating dose and mortality following maximal seizure. Time allowed for absorption of drugs before electroconvulsive shock was determined from average time required for maximal pupil dilatation (see below). The amount of drug resulting in mortality for 50% of mice tested was expressed as post seizure lethal dose$_{50}$ (P.S. LD_{50}) and was derived according to Litchfield-Wilcoxon procedure(5). To determine if antispasmodic and ganglionic blocking drugs altered the electroconvulsive threshold, mice were pretreated intraperitoneally with either 3.84 mg/kg atropine sulfate or 3.2 mg/kg mecamylamine. Control animals received saline, and 30 minutes after treatment groups

* We are indebted to J. L. Ciminera for statistical analysis applied to certain data.

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of 10 mice each were tested with stimuli of various intensities so that the proportion of mice exhibiting maximal seizure increased from 0-100%. Square wave pulses of 2 msec. duration and frequency of 59/second were delivered through saline-wick corneal electrodes for 0.3 second from an Offner type 736-A electroshock apparatus. The current strength (in ma.) to produce maximal seizures in 50% of mice (CS_{50}) was estimated from the stimulus response relationship by the Litchfield-Wilcoxon method. As a measure of peripheral activity of the antispasmodic and ganglionic blocking agents, their relative ability to dilate the mouse pupil was determined. The method for measuring pupil diameters was similar to that described by Pulewka(6) for antispasmodics and modified by Stone *et al.*(7) for ganglionic blocking drugs. Pupil diameters of mice pretreated with antispasmodics were measured 5 and 10 minutes after treatment and at 10-minute intervals thereafter until pupil diameters returned to normal. Ganglionic blocking drugs were studied in similar manner except that pupil diameter measurements were taken at 5, 10, 20, 40, and 80 minutes after administration of drugs. Five mice/dose were employed at each of several dose levels to encompass the entire dose-response relationship. Maximal observed dilatation for each animal was averaged, regardless of time interval at which it was found, and equiactive doses were estimated graphically from dose effect line relating log dose and maximal pupil dilatation. The amount corresponding to the dose that effected a dilatation to 10 micrometer units (1 unit = 0.082 mm) was chosen and designated the ED_{10}. Acute intraperitoneal toxicity was determined in female Carworth (CF#1) mice. LD_{50} values were calculated according to the method of Weil(8) and based on doses with 10 mice/level. The following drugs were employed: atropine sulfate, atropine methyl nitrate, benactyzine hydrochloride (Suavitil, Merck & Co.), chlorisondamine chloride (Ecolid, Ciba), hexamethonium diiodide, mecamylamine hydrochloride (Inversine, Merck & Co.), mepiperphenidol iodide (Darstine, Merck & Co.), the tertiary amine analogue of mepiperphenidol (1678), methantheline bromide (Banthine, Searle), propantheline bromide (Probanthine, Searle), pentolinium bitartrate (Ansolysen, Wyeth), scopolamine hydrobromide and scopolamine methiodide. All doses were in terms of the base or ion.

Results. The strain of mice (CF#1) employed normally was not susceptible to any great extent to lethal effect of electroshock convulsions, as has been previously demonstrated(9). Death following electroshock convulsions, produced in such mice by prior administration of various antispasmodic or ganglionic blocking agents (see below), occurred immediately after termination of maximal seizure. The apnea present during seizure was indefinitely prolonged, and death presumably resulted from respiratory failure. That respiratory failure was probably involved was revealed by the finding that the heart was still beating in apparently effective manner when convulsion ceased. The course of events described for these drug-related post seizure deaths was identical to that which results in those strains of mice normally susceptible to post seizure lethal effect of maximal electroshock convulsions(9) and to those that occur occasionally in the strain employed.

The several antispasmodic agents examined for ability to increase post seizure deaths are listed in Table I. Not only were atropine, scopolamine, and mepiperphenidol active in this respect, but also their corresponding methyl quaternary ammonium homologues were several times more active than the respective tertiary amine derivative. In addition, quaternary ammonium compounds, propantheline and methantheline, were also effective.

With several agents there appeared to be a real relationship between peripheral anticholinergic activity as measured by pupillary dilatation (ED_{10}) and ability to increase post seizure mortality (P.S. LD_{50}). Thus, the ED_{10} and P.S. LD_{50} values for all agents other than atropine, scopolamine, and mepiperphenidol were similar in magnitude. Larger doses of atropine and scopolamine were needed to increase post seizure mortality than was required to dilate pupils, but with mepiperphenidol the reverse was true. In spite of these exceptions, there was a significantly positive correlation between ED_{10} and P.S. LD_{50} value.

TABLE I. Relative Activity of Various Antispasmodic and Ganglionic Blocking Agents in Producing Death following Maximal Electroshock Convulsions in Mice.

Compound	Pupil dilatation		Post seizure mortality		Toxicity	
	Peak time,* min.	ED_{10},† mg/kg I.P.	P.S. LD_{50},‡ mg/kg I.P.	Slope§	LD_{50}, mg/kg I.P.	Slope§
Antispasmodic agents						
Scopolamine methiodide	30	0.014	0.033	10.0	104	1.16
" hydrobromide	30	0.026	0.62	4.5	317	1.12
Atropine methyl nitrate	30	0.05	0.07	3.0	49	1.16
" sulfate	30	0.18	0.70	4.7	187	1.16
Propantheline	10	0.22	0.23	8.1	54	1.10
Methantheline	10	0.38	0.48	6.3	61	1.12
Mepiperphenidol	10	0.95	0.14	8.7	101	1.17
Benactyzine	5	1.8	1.3	2.9	92	1.22
1678‖	10	2.6	3.0	8.0	171	1.12
Ganglionic blocking agents						
Chlorisondamine	30	0.05	0.064	1.9	38	1.39
Pentolinium	10	0.33	0.21	3.7	36	1.11
Mecamylamine	30	1.3	1.4	2.7	39	1.15
Hexamethonium	10	10.2	2.0	2.7	42	1.22

* Time after administration at which maximal dilatation of pupil was observed. This time was allowed for absorption prior to determining the P.S. LD_{50}.

† The dose required to produce pupil diameter of 10 μmeter units (each unit = 0.082 mm).

‡ Post seizure LD_{50} and is the dose estimated to induce 50% mortality following maximal electroshock seizures.

§ The fold change in dosage necessary to give unit stand. dev. change in response. See Litchfield and Wilcoxon(5).

‖ The tertiary amine counterpart of mepiperphenidol.

Correlation coefficient was calculated to be +0.68 (P, <0.05 with 7 degrees of freedom).

For each antispasmodic the slope of dose-response line relating per cent post seizure mortality and dose was extremely flat as compared to that ordinarily obtained in determining an LD_{50}. The slopes, in terms of fold change in dose required to effect a unit standard deviation change in response(5) for P.S. LD_{50} values, varied from 3 to 10 (Table I). Similar values obtained from LD_{50} data were considerably smaller (Table I). The characteristically flat slope obtained with P.S. LD_{50} determinations suggests that several mechanisms are involved with respect to post seizure lethal effect.

An interesting biphasic action occurred with benactyzine (Table II). Low doses, that is, those in median range with respect to dilating the pupil (see ED_{10}, Table I), produced an increase of post seizure deaths. As the dose was raised, the post seizure mortality effect disappeared and was correlated with appearance of anticonvulsant effect as indicated by absence of hind leg extensor component. Ability of benactyzine to increase post seizure mortality has been reported, although the self-limiting action was not mentioned(2).

Pretreatment of mice with ganglionic blocking drugs also resulted in immediate death following maximal seizure from electroshock-induced convulsions. As with antispasmodic drugs described, there appeared to be a close relation between ability of drugs such as chlorisondamine, mecamylamine, and pentolinium to dilate the pupil and to increase mortality following electroshock (Table I). Hexame-

TABLE II. Anticonvulsant and Post Seizure Lethal Effect of Benactyzine.

Dose,* mg/kg I.P.	Max seizure,† No. protected per 10 tested	Mortality, No. dying per 10 tested
.25	0	2
.5	0	0
1.	0	3
2.	0	7
4.	0	9
8.	0	4
16.	3	1
32.	9	0

* Administered 5 min. before electroshock.

† All mice were shocked as described in text. Protection from maximal seizure was assessed by absence of hind limb extensor component.

TABLE III. Effect of Atropine and Mecamylamine on Electroconvulsive Threshold in Mice.

Stimulus intensity, ma	Atropine sulfate (3.84 mg/kg I.P.)				Mecamylamine (3.2 mg/kg I.P.)			
	Maximal seizure, No. w.s.*/10 tested		Mortality, No. dying/10 tested		Maximal seizure, No. w.s.*/10 tested		Mortality, No. dying/10 tested	
	Control group	Drug group	Control group	Drug group	Control group	Drug group	Control group	Drug group
4.2	0	1	0	0	0	0	0	0
5.0	3	3	0	2	1	0	0	0
6.0	7	6	1	4	5	5	1	3
7.2	9	9	0	8	7	6	1	5
8.4	10	9	0	8	8	9	0	7
10.0					10	10	1	8
CS_{50}, ma†	5.5	5.7			6.3	6.5		

* w.s. = with maximal seizure. All mice were shocked as described in text.
† Stimulus intensity required to produce maximal seizure in 50% of mice; calculated after Litchfield and Wilcoxon(5).

thonium, on the other hand, was relatively more effective in producing post seizure deaths than in dilating the pupil.

In comparison with antispasmodics, the slope of dose-response lines for ganglionic blocking drugs varied only from about 2-4; nevertheless, these values were still higher than the slope functions obtained in various LD_{50} determinations (Table I). Ganglionic blocking agents, like the several antispasmodics, caused death in electroshocked mice in doses much below their lethal levels when used alone (Table I).

The influence of atropine sulfate and mecamylamine on electroconvulsive threshold was determined in mice pretreated with a dose of drug which had resulted in 90% mortality following seizures. The convulsive threshold was not altered by either atropine or mecamylamine as reflected in the almost identical CS_{50} values obtained for control and treated groups (Table III). On the other hand, there was a significant increase in post seizure mortality among treated animals that displayed maximal seizures.

Discussion. Our study reveals that antispasmodic substances and ganglionic blocking agents are highly effective in producing death following electroshock convulsions in mice. The immediate cause of death appeared to be due to respiratory failure insofar as shocked mice did not resume breathing following termination of maximal seizure. The mechanism involved in this failure was not revealed, but evidently it is not due to increase in sensitivity to the shocking stimulus. At least, no change in electroconvulsive threshold was demonstrated. The data also indicate that agents employed produced an increase in seizure mortality by a site of action outside the central nervous system. This concept is supported by close correspondence between doses required to elicit post seizure death with those required to produce typical anticholinergic action of drugs studied. Moreover, it is to be noted that some of the more potent agents were onium derivatives of nitrogen, a type of compound not well distributed within the central nervous system.

The biphasic action observed with benactyzine was of interest, since it suggested that anticonvulsant substances would protect against joint lethal action of antispasmodics or ganglionic blocking agents and electroshock convulsion. This was also shown by finding that effective anticonvulsant doses of diphenylhydantoin would prevent post seizure deaths to either atropine or mecamylamine (Torchiana and Stone, unpublished). The self antagonizing action of benactyzine and that of diphenylhydantoin indicates that death occurred only if a maximal seizure was produced. A similar relationship was observed in those strains of mice normally susceptible to lethal action of maximal seizure (9).

Effectiveness of antispasmodics and ganglionic blocking agents in increasing mortality after maximal electroshock seizures probably varies with species. Atropine has been studied in rats(10) and guinea pigs(11) with respect to its effect on electroconvulsive threshold

with no mention of death occurring in either species. Atropine is also commonly employed in man as pretreatment to electroconvulsive therapy. Certain studies(12-15) provide good evidence that when used in doses sufficient to produce peripheral anticholinergic effects, the combination of atropine and electroshock did not result in fatal outcome.

Ability of antispasmodics and ganglionic blocking agents to increase post seizure mortality resembles a similar action of reserpine, epinephrine, chlorpromazine, mescaline, azacyclonol, pipradrol and dibenamine(1,3). Little information bearing on their effectiveness in this respect is available. Such data would be helpful in determining whether post seizure lethal action of this diverse group of substances is nonspecific or whether a single property or effect underlies their action.

Summary. Ability of several antispasmodic and ganglionic blocking substances to produce immediate death following maximal electroshock convulsions in mice has been demonstrated. With most agents studied (atropine, atropine methyl nitrate, benactyzine, mepiperphenidol, methantheline, propantheline, scopolamine, scopolamine methiodide, mecamylamine, chlorisondamine, pentolinium and hexamethonium), the post seizure lethal effect was produced in doses approximating that required to produce a peripheral anticholinergic effect in the same species (pupil dilatation). Neither atropine nor mecamylamine altered the electroconvulsive threshold.

Respiratory failure appeared to be the cause of drug-induced post seizure deaths, but the mechanism of action was not revealed.

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Received May 20, 1959. P.S.E.B.M., 1959, v101.

Solubility of Hemoglobin as Red Cell Marker in Irradiated Mouse Chimeras. (25084)

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Transplantation and growth of donor-type progenitors of mouse erythrocytes in lethally irradiated mice may be demonstrated by serological markers(1) when donor and recipient red cells are of different *H-2* genotypes, and by agar(2) and starch gel electrophoresis(3) when their hemoglobins give different patterns on electrophoresis. This report describes differences in solubility of various mouse hemoglobins that can be used to identify erythrocytes of genetically different types probably not distinguishable from one another serologically or electrophoretically.

Materials and methods. Blood from de-

* Operated by Union Carbide Corp. for U. S. Atomic Energy Comm.