A COMPARATIVE STUDY OF THE EFFECTS OF FIVE CHOLINE COMPOUNDS USED IN THERAPEUTICS: ACETYLCHOLINE CHLORIDE, ACETYL BETA-METHYLCHOLINE CHLORIDE, CARBAMINOYL CHOLINE, ETHYL ETHER BETA-METHYLCHOLINE CHLORIDE, CARBAMINOYL BETA-METHYLCHOLINE CHLORIDE

H. MOLITOR

Merck Institute of Therapeutic Research, Rahway, New Jersey

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Although the powerful parasympathetic action of acetyl choline was discovered more than thirty years ago (Hunt and Taveau (1), Dale (2)) and a large number of choline derivatives had been prepared by Hunt and his co-workers between 1906 and 1930, no therapeutic use of this class of drugs was made until 1927, when Villaret and his co-workers (3) began to use acetylcholine in a variety of pathological conditions. It soon became apparent that acetylcholine, because of its rapid hydrolysis in body fluids into choline, was not the most advantageous representative of parasympathetic stimulating drugs, and a number of more stable choline compounds, some of which had already been prepared by Hunt, were more intensively investigated. Acetylcholine chloride urea (Glaubach and Pick (4)), acetylmethylcholine (Villaret (5)), Pacyl, a compound said to be a bromcholine ester, acetyl-beta-methylcholine (Simonart (6)) and carbaminoyl choline (Kreitmair (7), Nöll (8), Velten (9), Simonart (10)) were studied, the last two being the most promising. Acetyl-β-methylcholine chloride and the ethyl ether of β-methylcholine chloride, both synthetized by Major and Cline (11), were clinically investigated by Starr and his co-workers, (12, 13, 14) and subsequently by a number of other clinicians. Of the many choline compounds mentioned above only four are

commercially obtainable: acetylcholine, acetyl-β-methylcholine (Mecholyl), carbaminoyl choline (Doryl) and Pacyl. The ethyl ether of β-methylcholine and carbaminoyl-β-methylcholine have been studied pharmacologically by Simonart (15, 16) and clinically by Comroe and Starr (17), Kovacz, Saylor and Wright (18) and Rutenbeck (19).

While many valuable data concerning these drugs can be found in the literature, a critical comparison of their pharmacological potency has not yet been published. Such an investigation seemed desirable in view of the increasing interest in their therapeutic application and is presented in the following pages.

The various choline compounds differ pharmacologically principally in their stability in body fluids and in the degree to which they possess a so-called nicotinic action, in addition to their parasympathetic, "muscarinic" effect. The resistance of a choline compound to hydrolysis by the blood esterases determines its clinical field of usefulness, especially its pharmacologic activity after peroral application; the presence or absence of nicotinic action is, according to our present knowledge, of minor practical importance, since it is exhibited only after paralysis of the parasympathetic system with atropine and administration of a dose many times greater than that necessary to produce a maximum muscarinic effect.

In a systematic study of choline compounds, a third question deserves attention, namely, that of a possible difference in affinity for different parts of the parasympathetic system. Statements are found in the literature, that some compounds stimulate primarily glandular secretions, others the vascular system, others the gastrointestinal division of the parasympathetic. Such a selectivity would obviously have not only theoretical interest but also practical importance; however, current statements pointing in this direction are based on impressions rather than on controlled experiments.

In this paper five choline derivatives: acetylcholine chloride (A), acetyl beta-methylcholine chloride (M) carbaminoyl choline chloride (L), ethyl ether of beta-methylcholine chloride (E) and carbaminoyl beta-methylcholine chloride (U), are compared

TABLE 1

SYMBOL	NAME	FORMULA	CHEMICAL PROPERTIES		PHYSIOLOGICAL PROPERTIES	
			Stability in air	Stability in water	Stability in blood	Nicotinic action
A	Acetylcholine chloride	$(CH_3)_3NClCH_2CH_2OOCCH_3$	Hygroscopic	Unstable	−−	+
M	Acetyl β-methylcholine chloride	$(CH_3)_3NClCH_2CH(CH_3)OOCCH_3$	Hygroscopic	Unstable	−	−
L	Carbaminoyl-choline chloride	$(CH_3)_3NClCH_2CH_2OOCNH_2$	Non-hygroscopic	Stable	++	++
E	Ethyl ether β-methylcholine chloride	$(CH_3)_3NClCH_2CH(CH_3)OCH_2CH_3$	Hygroscopic	Stable	++	−
U	Carbaminoyl β-methylcholine chloride	$(CH_3)_3NClCH_2CH(CH_3)OOCNH_2$	Non-hygroscopic	Stable	++	−

under identical experimental conditions. While only the first three of these are available commercially, all of them have been used clinically. In table 1 a summary of their characteristic properties is given. In view of the large number of experiments (more than 2500 over a period of four years), it is necessary to summarize the results in tables.

TOXICITY

The toxicity of A, M, L, E and U was determined in the usual way in white mice and rats. The compounds were injected intravenously into the tail vein, subcutaneously under the abdominal skin or fed by stomach tube. Animals of the same strain were

TABLE 2

L.D. 50 of choline derivatives in milligrams per kilogram

COMPOUND	IN MICE			IN RATS		
	Administered intravenously	Administered subcutaneously	Administered perorally	Administered intravenously	Administered subcutaneously	Administered perorally
A	20	170	3,000	22	250	2,500
M	15	90	1,100	20	75	750
L	0.3	3	15	0.1	4	40
E	30	250	500	25	400	2,000
U	10	120	250	21	175	1,500

used for all experiments. The average weight of the mice was 17 grams, that of the rats 150 grams. At least 8 points were determined for the drawing of a toxicity curve and between 20 and 30 animals were used at each dose level. The observation time for recording a lethal effect was limited to twenty-four hours. While occasional animals die after this time and death also must be attributed to the drug, it was felt that such late deaths were due rather to a complication following the drug action (pneumonia, enteritis) and should not be included in a comparative toxicity study. Table 2 summarizes these experiments. Given intravenously the L. D. 50 of four of the choline compounds is about 20 mgm. per kilogram; the L.D. 50 of L however in mice is 0.3 mgm. per kilogram and in rats 0.1 mgm. per kilogram.

This difference appears to be caused by the great stability of this compound in body fluids, as can be seen from the increasing difference in the L.D. 50 between L and the other compounds when administration is made subcutaneously or perorally. The peroral L.D. 50 of L in mice is 50 times that of the intravenous; that of A, 150 times; and that of M, 73 times that of the intravenous. On the other hand, the difference is smaller with E and U, being only 16 and 25 times, respectively. The fact that the intravenous toxicity of the latter two compounds is so much less than that of L, is explained by their lower pharmacological potency (about one-hundredth of that of L). Further evidence that the great difference in toxicity between A, M and L, all of which are about equally effective as parasympathetic stimulants,

TABLE 3

Influence of physostigmin on the toxicity (L.D. 100) of cholines in mice

MODE OF ADMINISTRATION	DOSE IN MG. PER KG.						
	A	A + 3 per cent physostigmin	M	M + 3 per cent physostigmin	L	L + 3 per cent physostigmin	Physostigmin
Intravenous	25	10	25	5	0.5	0.5	0.4
Subcutaneous	225	20	140	15	4	4	2.0
Peroral	3,300	50	1,600	50	30	20	15

is partly caused by the instability of the two former, is obtained in experiments in which small amounts of physostigmin (3 per cent) were added to A and M. A and M, given subcutaneously or perorally, become much more toxic when protected from decomposition by blood esterase; no such change is produced in the toxicity of L (table 3).

CIRCULATORY ACTION

The various choline derivatives were tested in the usual way on the carotid blood pressure of etherized cats and rabbits under urethane anesthesia. No significant difference in the relative potency or type of action of the five compounds was found between the two animal species, or the different methods of anesthesia, although, as a rule, cats showed a greater sensitivity.

Two procedures were followed in comparing the five choline derivatives: one consisted in the administration of one drug into the same animal by *various* ways, namely, intravenously, subcutaneously, perorally and, in a special group of experiments, by iontophoresis, as a salve, nasal spray or per rectum; in each case the intensity and duration of action were compared. In another series of experiments the minimum effective doses of several compounds were administered by *one* of the previously mentioned methods to the same animal and the results compared. Obviously, a combination of both methods would have been the best. Whenever possible this was tried; however, the action of some of the cholines given by mouth, subcutaneously or by iontophoresis, lasts so long that the general condition of the animal does not permit the consecutive application of more than 3 or 4 doses in these cases. In order to obtain complete data for each compound, it was therefore necessary to compare results obtained in different animals. In view of the variations in individual sensitivity to parasympathetic stimulation, the blood pressure lowering action of a standard dose (0.001 mgm.) of intravenously injected M was determined in each animal before the other tests were begun. It was thus possible to eliminate animals with unusually strong or weak response to parasympathetic stimulation and to base the comparison on animals of comparable sensitivity. The solutions of all the choline derivatives investigated showed practically no change in strength during several weeks.

Intravenous injections were made into the jugular vein, subcutaneous injections under the abdominal skin. Peroral administration was made by introducing a stomach tube through the intact stomach under visual control into the duodenum and fastening it here by means of soft cotton threads. Although the application of cholines made in this way is not strictly peroral in the clinical sense, the introduction of the stomach tube into the duodenum instead of the stomach was chosen because of the varying quantities of ingesta present in rabbits' stomachs. Moreover, several experiments on cats, where the tube was placed into an empty stomach, showed results similar to those with intro-duodenal

application. In view of the great difference in results between parenteral and peroral application, especially with the unstable choline compounds, we did not use the technic of intra-duodenal injection, as recommended by Villaret, Besançon and Cachera (20) and later employed by Simonart. The possibility of a small quantity of injection fluid being resorbed through the lesion caused by the injection cannula cannot be avoided with certainty. Simonart reports that M is effective from the duodenum in a dose as small as 1 mgm. per kilogram; although we were able to reproduce occasionally this result by using intra-duodenal injec-

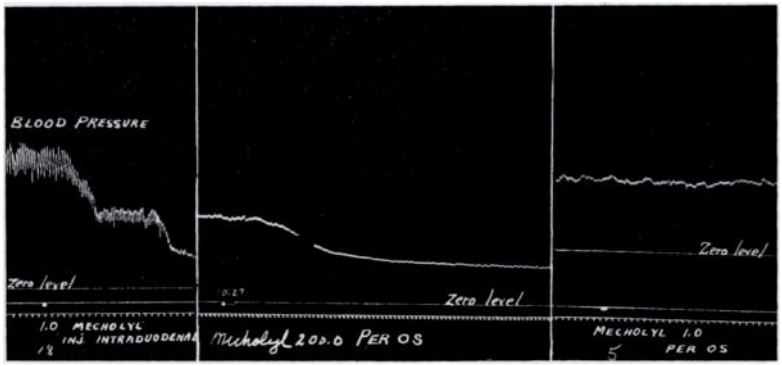

FIG. 1. EXPERIMENT 106 (NOVEMBER 6, 1935), 186 (NOVEMBER 4, 1935), 194 (NOVEMBER 5, 1935)

Carotid blood pressure of rabbits, anesthetized with urethane (0.8 gram per kilogram). Time intervals, 10 seconds. (*a*) 1 mgm. M injected intraduodenally; (*b*) 200 mgm. M introduced by stomach tube; (*c*) 1 mgm. M introduced by stomach tube.

tion (fig. 1), doses as high as 200 mgm. were necessary when given by stomach tube to produce regularly the same effect in rabbits.

Results similar to those after peroral application were obtained by giving cholines rectally, either in form of a clysma or a suppository. Application of the potent and more stable compounds (L, E and M) to the mucous membranes of the nose and pharynx both of which have been used clinically (in cases of Ozena), was made in form of ointments (10 per cent) or sprays (20 per cent). As expected, an effect on the blood pressure was

obtained; however, the action, depending upon area and condition of resorbing membranes, varied so much that the inclusion of these experiments in a quantitative summary is impossible.

The application of choline ointments to the broken skin gave similar results. While even a vigorous rubbing of ointments containing M or L in concentrations up to 40 per cent into the *unbroken* skin is followed only by a rash on the site of application, the application of ointments containing between 1 and 10 per

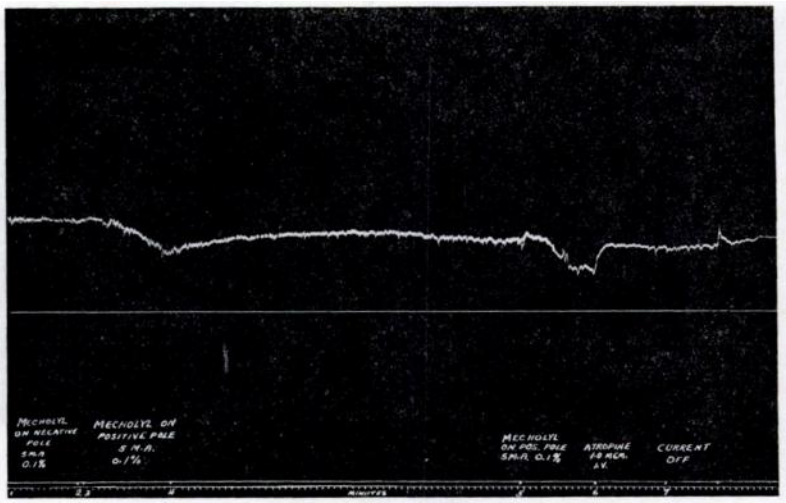

FIG. 2. EXPERIMENT 59 (MAY 11, 1934). RABBIT, 2.3 KGM.; URETHANE ANESTHESIA
Carotid blood pressure. Time intervals, 1 minute. (*1*) M solution 0.1 per cent on negative pole; 5 m.a.; (*2*) current off; (*3*) M solution 0.1 per cent on positive pole; 5 m.a.; (*4*) current off; (*5*) M solution 0.1 per cent on positive pole; 5 m.a.; (*6*) 1 mgm. atropine sulfate intravenously; (*7*) current off.

cent of M or L to the *denuded* skin results in a marked and prolonged fall of blood pressure. Indeed, the action of L ointments in high concentrations is sometimes so persistent that it may cause death. However, the effects following local application of choline salves to ulcers or wounds cannot be kept sufficiently under control as to make this method desirable. Absorption, and hence systemic action, depend not only upon the area of denuded surface, but also upon the condition of the wound. While a choline salve can be adapted to various conditions by a change of its con-

centration, a correct estimation of all factors influencing the result is difficult.

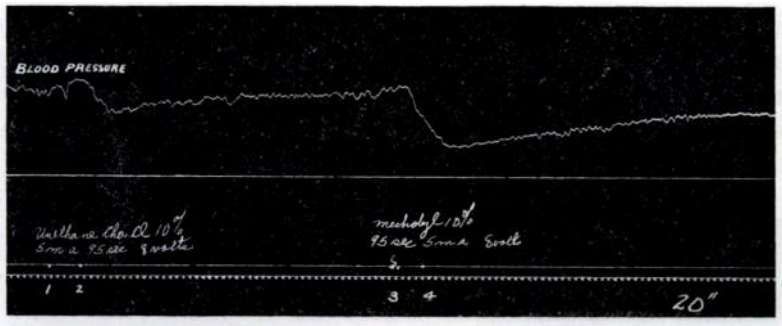

FIG. 3. EXPERIMENT 1100 (MAY 11, 1936). RABBIT, 2.5 KGM.; URETHANE ANESTHESIA

Influence of type of choline. Carotid blood pressure. Time intervals, 20 seconds. (*1*) U solution, 10 per cent on positive pole; 5 m.a., 8 volts; (*2*) current off; (*3*) M solution, 10 per cent on positive pole; 5 m.a., 8 volts; (*4*) current off.

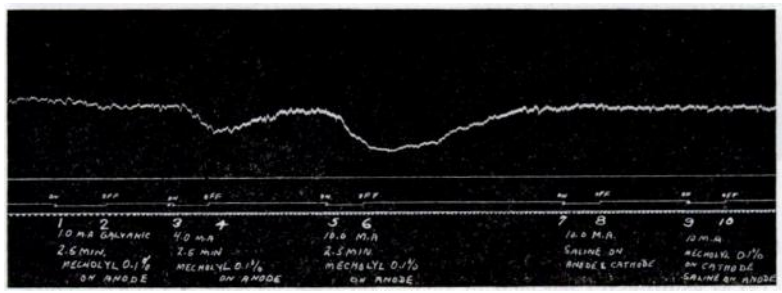

FIG 4. EXPERIMENT 1093 (JULY 13, 1936). RABBIT, 3.0 KGM.; URETHANE ANESTHESIA

Influence of strength of current. Carotid blood pressure. Time intervals, 20 seconds. (*1*) mecholyl solution on positive pole 0.1 per cent; 1.0 m.a.; (*2*) current off; (*3*) mecholyl solution on positive pole 0.1 per cent; 4.0 m.a.; (*4*) current off; (*5*) mecholyl solution on positive pole 0.1 per cent; 10 m.a.; (*6*) current off; (*7*) saline on positive and negative pole; 10 m.a.; (*8*) current off; (*9*) mecholyl solution on negative pole 0.1 per cent; 10 m.a.; (*10*) current off.

A far more dependable method of introducing choline derivatives through the unbroken skin is afforded by iontophoresis. This was first tried clinically by Kovacz (21) and was confirmed in animal experiments, performed upon request of this author in our laboratory (fig. 2). Systematic studies of choline iontophoresis

have been published by Rutenbeck (22) and Kotkis and Melchionna (23).

Aqueous or alcoholic solutions of choline compounds can be driven into the body from the positive pole of a galvanic machine. The intensity of action depends upon the nature of the choline compound (fig. 3); the strength of the electromotive force (depending upon size of the electrode, strength of current and individual resistance of the skin) (fig. 4); the solvent (fig. 5);

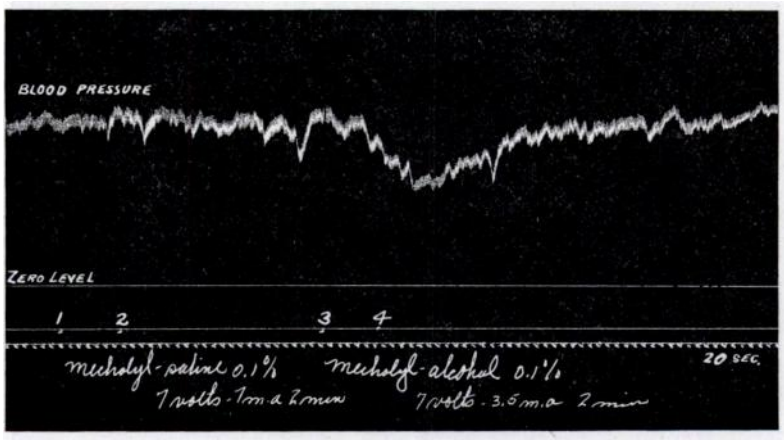

FIG. 5. EXPERIMENT 1102 (MAY 13, 1936). RABBIT, 3.4 KGM.; URETHANE ANESTHESIA

Carotid blood pressure. Time intervals, 20 seconds. Influence of solvent. (*1*) M solution 0.1 per cent in saline on positive pole; 7 m.a., 7 volts; (*2*) current off; (*3*) M solution 0.1 per cent in alcohol on positive pole; 3.5 m.a., 7 volts; (*4*) current off.

and, only to a minor degree, upon the concentration of the solutions (fig. 6). Since the strength of the current passing through the skin is indicated on the milliammeter, the patient's individual sensitivity to parasympathetic stimulation is the only variable not under control.

It is not within the scope of this paper to present the results of our studies on iontophoresis. However, in a comparative investigation on choline derivatives, their adaptability to iontophoresis should be included, since this method of administration has become widely used. That the effects produced by choline ionto-

phoresis are typical choline effects, and not due to an action of the current alone, can be seen from figure 2, where no action on the blood pressure is seen when the polarity is reversed, and where the M action is immediately terminated by the injection of atropine. Figure 5, which demonstrates the influence of the solvent on the intensity of choline action, points in the same direction. The same current, which, when passed through a solution of M in

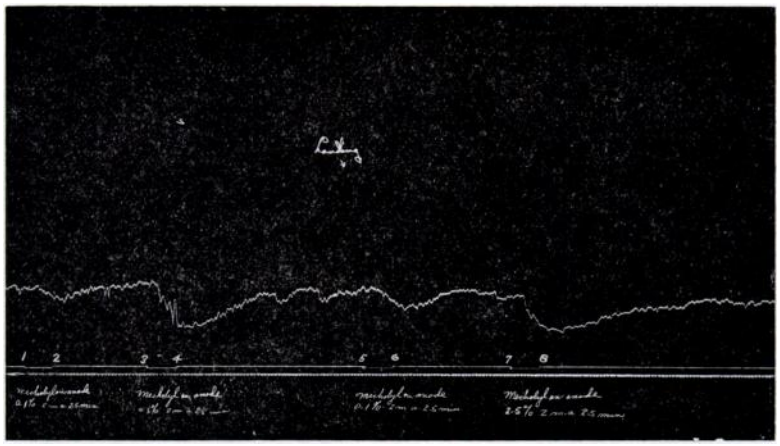

FIG. 6. INFLUENCE OF CONCENTRATION

Rabbit 3 kgm., urethane anesthesia. Carotid blood pressure. Time intervals, 20 seconds. (*1*) M solution on positive pole 0.1 per cent; 2 ma.; (*2*) current off; (*3*) mecholyl solution on positive pole 0.5 per cent; 2 m.a.; (*4*) current off; (*5*) mecholyl solution on positive pole 0.1 per cent; 2 m.a.; (*6*) current off; (*7*) mecholyl solution on positive pole 2.5 per cent; 2 m.a.; (*8*) current off.

saline, causes but little effect, induces a marked fall of blood pressure when passing through a solution of M in a non-electrolyte.

The greater choline action at the site of the active electrode, which is the principal reason for the use of iontophoresis, is shown in figure 7. It is well known that the galvanic current itself produces hyperemia and increased skin temperature. In order to investigate whether a specific choline action was added to this general effect of the galvanic current, the current was first applied to a rabbit's ear by means of an electrode moistened with saline; after some time a solution of M was dropped on the

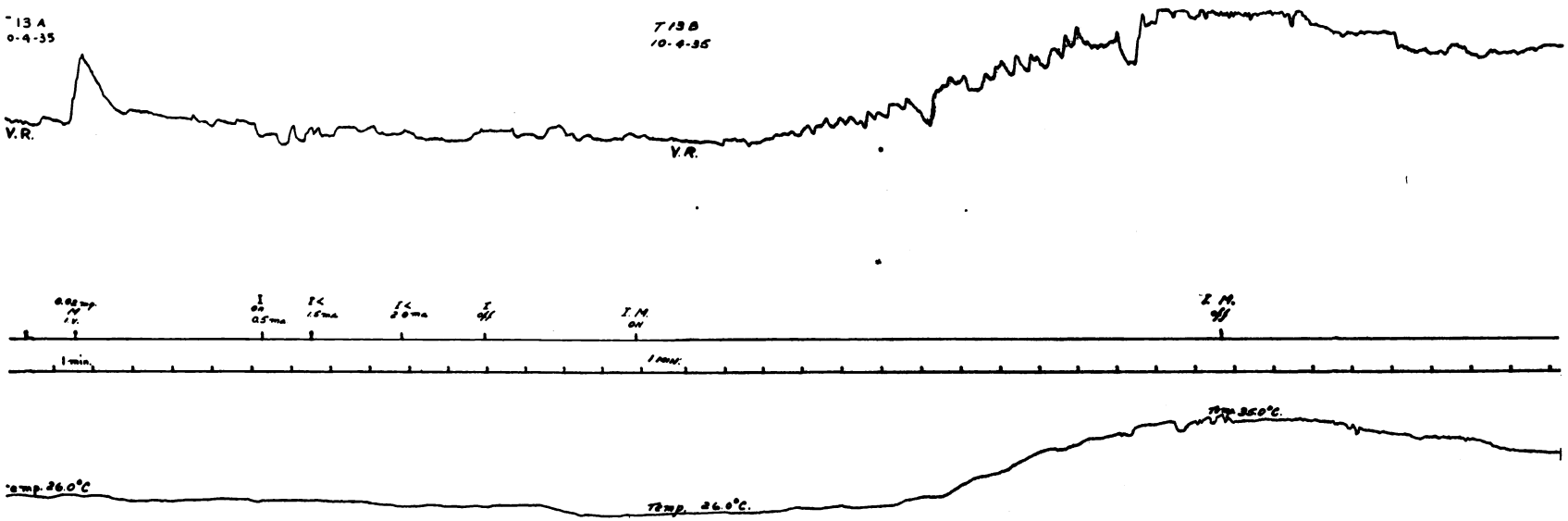

FIG. 7. EXPERIMENT 1113 (OCTOBER 4, 1935). RABBIT, 2.5 KGM.; NO ANESTHESIA

Upper curve: Light transmission through ear. Lower curve: Skin temperature. Time intervals, 1 minute. (*1*) 0.02 mgm. M intravenously; (*2*) saline solution on positive pole; 0.5 m.a.; (*3*) saline solution on positive pole; 1.5 m.a.; (*4*) saline solution on positive pole; 2 m.a.; (*5*) current off; (*6*) M solution on positive pole; 2 m.a.; (*7*) current off.

electrode, the strength of the current remaining unchanged. Continuous records of the skin temperature and peripheral circulation were taken from a skin area in the immediate vicinity of the active electrode by the method of Molitor and Kniazuk (24). A marked rise in skin temperature and vasodilatation occurred after the choline compound had been added to the electrode,

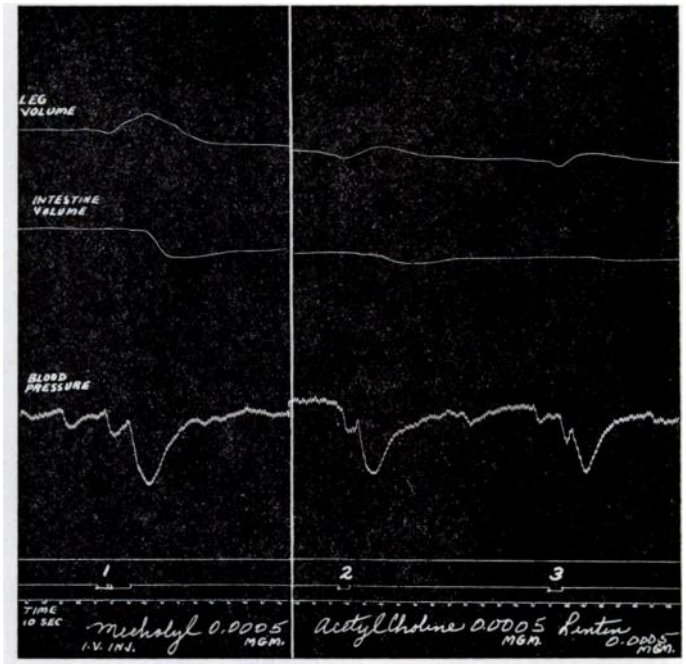

FIG. 8. EXPERIMENT 1192 (APRIL 14, 1936). RABBIT, 3.2 KGM.; URETHANE ANESTHSIA

Upper curve: leg volume. Middle curve: intestinal volume. Lower curve: blood pressure. Time intervals, 10 seconds. (*1*) M 0.0005 mgm. intravenously; (*2*) A 0.0005 mgm. intravenously; (*3*) L 0.0005 mgm. intravenously.

while no such effects were observed during the application of the galvanic current alone.

Many animals which had been subjected for several hours to effective choline iontophoresis died apparently from asphyxia. Atropine, given at this late date did not stop the symptoms and artificial respiration was equally ineffective, athough breathing

of a pure oxygen-carbon-dioxide mixture seemed to prolong life somewhat. A post-mortem examination showed edema of the lungs, apparently the cause of death, since the heart was frequently found still beating The danger of developing pulmonary complications due to prolonged choline action is lessened when

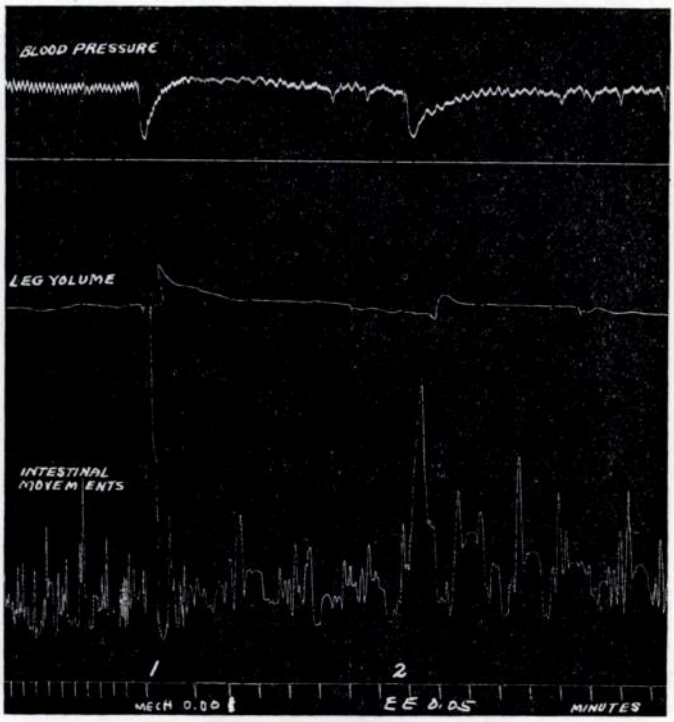

FIG 9. EXPERIMENT 1198 (MAY 5, 1936). RABBIT, 3 KGM.; URETHANE ANESTHESIA

Upper curve: carotid blood pressure. Middle curve: leg volume. Lower curve: intestinal movements. (*1*) 0.001 mgm. M intravenously, (*2*) 0.05 mgm. E intravenously.

iontophoresis is performed with the unstable choline compounds A and M. The reason for this is obvious: the systemic action of these compounds disappears almost immediately after discontinuation of the current because of their rapid destruction in the circulating blood, while the stable compounds continue to circu-

late in an active form for a long period. The danger increases with the potency of the compounds used, L involving greater risks than U or E (Rutenbeck (19)).

In a group of experiments plethysmographic records of the leg and intestinal volume (fig. 8) and records of the intestinal movements in situ (fig. 9), using Straub's technic (25), were taken simultaneously with the blood pressure readings. It was found

TABLE 4

Effect of choline derivatives on the carotid blood pressure, leg volume and intestinal motility of rabbits

COMPOUND	(DOSES IN MG. PER KG.), EFFECT ON BLOOD PRESSURE				EFFECT ON LEG VOLUME	EFFECT ON INTESTINAL MOTILITY
	Minimum effective dose intravenously	Equally effective doses			Smallest intravenous effective dose	Smallest intravenous effective dose
		Intravenous	Subcutaneous	Peroral		
A	0.0002	0.002	1.0	1,000	0.0003	0.0003
M	0.0002	0.002	0.2	50	0.0003	0.0003
L	0.0002	0.002	0.1	2	0.0003	0.0003
E	0.02	0.2	3.3	100	0.02	0.02
U	0.02	0.2	3.3	100	0.02	0.02

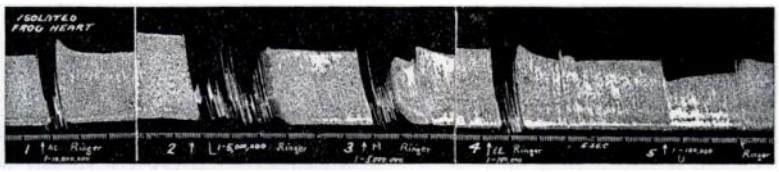

FIG. 10. EXPERIMENT (JUNE 19, 1936). RANA PIPIENS, ISOLATED HEART ON STRAUB CANNULA

Time intervals, 5 seconds. (*1*) A 1:10 million; (*2*) L 1:5 million; (*3*) M 1:5 million; (*4*) E 1:100,000; (*5*) U 1:100,000.

that a dose which was just sufficient to produce a distinct fall of blood pressure caused also an increase of leg volume and of intestinal movements. While this dose varied greatly with the different choline compounds, there were no significant differences among the five compounds with respect to the relative strengths of their cardiac, vascular and intestinal actions. Table 4 summarizes the results of more than 400 experiments.

FROG HEART ACTION

On the isolated heart of Rana pipiens (with Straub cannula) the following concentrations were found to be approximately equally effective in causing decrease of amplitude and diastolic standstill: 1:10 million of A; 1:5 million of M and L; 1:100,000 of U and E (fig. 10). These experiments were performed during spring and summer.

MIOTIC ACTION

Choline compounds with muscarinic action generally act as miotics. However, statements in the literature regarding this effect are somewhat contradictory. The only compounds which have been used clinically as miotics are A, (Comland and Cahane (26)) and L, (Velhagen (27)), the first injected subconjunctivally, the second applied externally in a 0.1 per cent solution. Hunt (28) has described a strong miotic effect of acetyl-beta-methylcholine in topical application (0.1 to 1 per cent solution) while Simonart (29) and Schmidt could not observe such an effect.

One of the reasons for these differences of opinion lies in the fact that some authors, like Comland and Cahane, or Simonart observed the miotic effect after subconjunctival, respectively subcutaneous injection, while others used external application. It is not surprising that a choline derivative as stable as E, given in a lethal dose (10 mgm.), as in Simonart's experiment, should produce a more intense parasympathetic stimulation including miosis than the unstable esters, all actions of which are of rather short duration. However, when, as in Schmidt's and Hunt's experiments the drugs are given in the same manner, another reason must account for the different results. The following observation may provide the explanation. It was noted that a different result was obtained when the solution was dropped into an eye, kept open with a lid holder, from that obtained, when after the administration the eyelid was gently massaged (fig. 11). A series of experiments, in which the two procedures were compared in the eyes of the same animal, showed striking differences. This agrees with Starr's observation, that massage on the site of application greatly increases the effect of subcutaneously

injected M. Changes in the pupillar diameter were measured in rabbits by placing over the eye a strip of celluloid in which holes with increasing diameter had been cut. The diameter of the pupil was recorded at the beginning of the experiment and at regular intervals thereafter. A similar method was used in cats, estimating maximum length and width of the pupil. The animals were kept throughout the experiment in a semi-dark room.

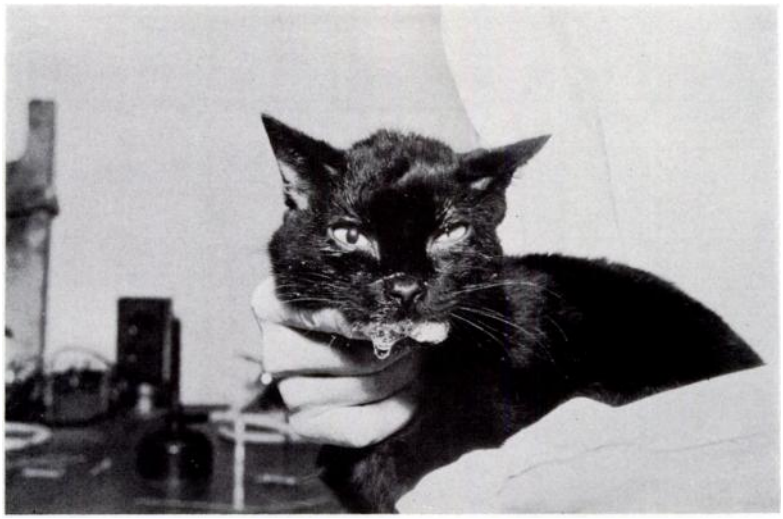

FIG. 11. EXPERIMENT (APRIL 28, 1936)
Right eye: M solution, 5 per cent, instilled without rubbing. Left eye: M solution, 5 per cent, instilled with subsequent slight massage.

Observations were made and recorded independently by two individuals. A summary of the results is given in table 5.

INTESTINAL ACTION

The intestinal activity of A, M, L, E and U was tested on isolated rabbits' intestines and on non-anesthetized dogs. In the latter salivation, vomiting and defecation were taken as criteria of the parasympathetic stimulation and the smallest dose of a choline derivative was determined which would regularly produce these symptoms. The smallest concentrations of choline derivatives markedly increasing the amplitude and frequency of the

movements of isolated rabbits' intestines were about 0.001 mg. % for A, M, and L and 1.0. mg. % for U and E (fig. 12).

In the non-anesthetized animal the action was observed on seven female dogs, ranging in weight between 5.5 and 16 kgm., to which the five compounds were administered by subcutaneous injection or by mouth. The animals were kept on a uniform diet (meat, dog pellets, milk) and the drugs were given 2 to 3 hours after a meal. Under such conditions the time interval between administration of an emetic drug and the first attack of

TABLE 5

Miotic action of choline compounds in rabbits

PERCENTAGE CONCENTRATION	NAME OF COMPOUND											
	A		M		L		E		U		Ph	
	Contact	Massage	Contact	Massage	Contact	Massage	Contact	Massage	Contact	Massage	Contact	Massage
0.001											±	++
0.005												
0.01			−	−	−	±	−	−			+++	+++
0.05					−	++						
0.1	−	−	−	+	−	+++	−	±	−	−	+++	+++
0.5	−	−	−	+±	±	+++	−	++	−	±		
1.0	−	±	−	++±	+	+++	−	+++	±	++	+++	+++
5.0	−	+	±	+++	+	+++	−	+++	+	++		

Explanation of signs: −, no change in pupillar diameter; ±, 10 per cent constriction; +, 20 per cent constriction; +±, 30 per cent constriction; ++, 40 per cent constriction; ++±, 50 per cent constriction; +++, 60 per cent constriction.

vomiting is fairly constant in the same animal (Molitor (30)); the same uniformity was found for defecation. The symptoms appeared within 5 to 10 minutes after subcutaneous injection and 10 to 20 minutes after feeding; each compound was given repeatedly to the same animal; the results agreed within reasonable limits. A summary of the findings is given in table 6.

DISCUSSION

The experiments reported in this paper were undertaken to furnish data for the selection of various choline derivatives for

specific clinical purposes. Five compounds: acetylcholine chloride, acetyl beta-methylcholine chloride, carbaminoyl choline chloride, ethyl ether beta-methylcholine chloride, carbaminoyl beta-methylcholine chloride, were tested under strictly com-

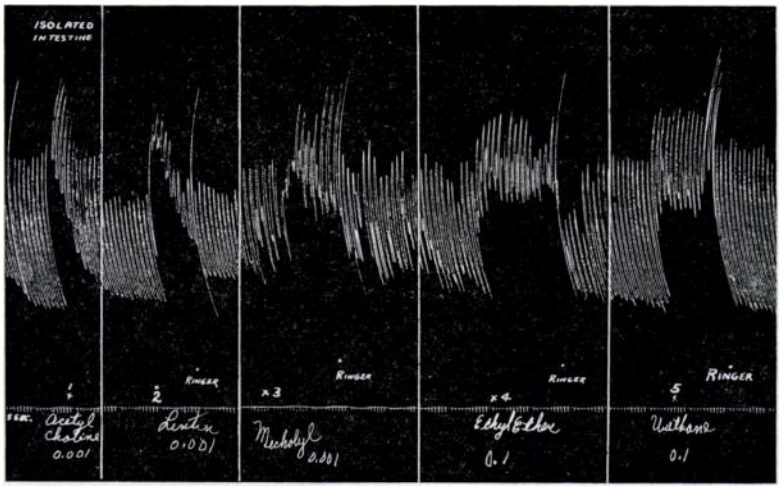

FIG. 12. EXPERIMENT 183 (OCTOBER 16, 1935). ISOLATED RABBIT'S INTESTINES (*1*) A 1:100 million, (*2*) L 1:100 million, (*3*) M 1:100 million, (*4*) E 1:1 million, (*5*) U 1:1 million.

TABLE 6

Cathartic action of choline derivatives in dogs

COMPOUND	MINIMUM EFFECTIVE DOSE IN MG. PER KG.		RATIO BETWEEN SUBCUTANEOUS AND PERORAL DOSE	COMPARATIVE STRENGTH IN RELATION TO A	
	Subcutaneous	Peroral		Subcutaneous	Peroral
A	0.8	40	1:50	1	1
M	0.05	25	1:500	16	2
L	0.01	0.25	1:25	80	160
E	0.25	5	1:20	3	8
U	0.25	5	1:20	3	8

parable experimental conditions for toxicity, circulatory action, miotic action, effect on gastrointestinal tract, effect on isolated intestines and isolated frog heart. The results of these experiments are summarized in tables 7 and 8. In table 7, the action of each compound is compared with that of acetylcholine. All

values given in this table are expressed in terms of relative potency of acetylcholine, the action of which is designated in each of the horizontal columns as 100. However, since a figure of 100 for A represents in every group of experiments different actual dose levels, it is obvious, that the columns of this table must not be

TABLE 7

Comparison of potency of choline derivatives

Relative doses or concentrations required to produce equal intensities of effect (acetylcholine = 100)

COMPOUND					ACTION TESTED	MODE OF ADMINISTRATION
A	M	L	E	U		
100	75	1.5	150	50	L.D. 50	Intravenous injection
100	52	1.7	145	64	L.D. 50	Subcutaneous injection
100	37	0.5	17	8.5	L.D. 50	Peroral
100	100	100	10,000	10,000	Blood pressure	Intravenous injection
100	20	10	330	330	Blood pressure	Subcutaneous injection
100	5	0.2	10	10	Blood pressure	Peroral
100	100	100	6,600	6,600	Intestinal action. Rabbit's intestines in situ	Intravenous injection
100	6.2	1.2	31	31	Defecation in dogs	Subcutaneous injection
100	62	0.6	12	12	Defecation in dogs	Peroral
100	100	100	10,000	10,000	Rabbit's isolated intestines	
100	200	200	2,000	2,000	Frog heart	
100	10	1	10	50	Miotic action	

Note: Only figures in the same horizontal column are comparable.

read vertically. A comparison of actual doses is given in table 8, where L.D. 50 in mice, blood pressure lowering action in rabbits and effective concentrations on the frog heart and isolated intestine are given. An important reservation must be applied to the conclusions drawn from this table, so far as the determination of the therapeutic range is concerned. The lethal doses are given

as L.D. 50 and have been determined on mice, while the blood pressure experiments were performed on rabbits under urethane anesthesia, which is known to have quantitative influence on the results. It is therefore only the general trend of the results which is significant while the actual figures, expressed in the therapeutic index, have no practical importance.

The data in tables 7 and 8 indicate clearly, that two factors govern the pharmacological efficacy of choline compounds: their stability in body fluids and their individual pharmacological potency. A, M and L have about the same potency in tests on

TABLE 8

Comparison of toxicity and pharmacologic potency of choline derivatives

COMPOUND	TOXICITY IN MICE—L.D. 50			POTENCY					RATIO OF LETHAL DOSE TO EFFECTIVE DOSE		
				In vivo (rabbits)—blood pressure lowering dose			In vitro—effective concentration				
	Intravenous	Subcutaneous	Peroral	Intravenous	Subcutaneous	Peroral	In frog heart	In rabbits' intestines	Intravenous	Subcutaneous	Peroral
	mgm. per kgm.	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per kgm.*	*mgm. per cent*	*mgm. per cent*			
A	20	170	3,000	0.002	1	1,000	0.01	0.001	1:10,000	1:170	1:3
M	15	90	1,100	0.002	0.2	50	0.02	0.001	1:7500	1:450	1:22
L	0.3	3	15	0.002	0.1	2	0.02	0.001	1:150	1:150	1:7.5
E	30	250	500	0.2	3.3	100	1.0	0.1	1:150	1:75	1:5
U	10	120	250	0.2	3.3	100	1.0	0.1	1:50	1:36	1:2.5

isolated organs or when injected intravenously. E and U under similar conditions are about 100 times less active. These values change when the intravenous administration is replaced by a subcutaneous or peroral. The effect is then a resultant of the stability on one hand and of the individual potency of the compound on the other. Stable, but less potent derivatives like E or U, become more effective under such circumstances while the more potent, but unstable, like A and M, are greatly reduced in strength. The maximum effect is obtained with a compound of the potency of A or M and of the stability of E or U, as is seen in

the experiments with L. However, while a compound of this type possesses under all experimental conditions the greatest pharmacological activity, its advantages are considerably diminished by its far greater toxicity, resulting from the combined effects of stability and potency. This difference is greatest after intravenous and subcutaneous injection and becomes less marked after peroral administration. It cannot be explained by the greater stability in body fluids only, but must in part be due to the chemical or physical properties of the drug itself. We are led to this conclusion from the experiments with A and M solutions, protected against the action of blood esterase by the addition of physostigmine. The ratio of intravenous to peroral L.D. 50 in mice is, under these conditions, markedly reduced: for A from 1/140 to 1/4, for M from 1/70 to 1/14; but even after stabilization with physostigmine the intravenous L.D. 50 of A is 5 mgm. per kilogram, of M, 3 mgm. per kilogram, as compared with 0.3 mgm. per kilogram of L, although the minimum effective doses on the blood pressure are for all three compounds about the same (0.0002 mgm. per kilogram), without and with physostigmine added.

A selective affinity of the various choline derivatives for different parts of the parasympathetic system cannot be deduced from our experiments, which, however, did not include a comparison of glandular actions (sweating, salivation). The figures which lead to this conclusion are summarized in tables 5 and 8.

It is somewhat unexpected that such a well defined and characteristic property as the presence or absence of nicotinic action has no influence upon the outcome of experiments under normal conditions. A similar observation has recently been made by Wedd (31), who found no difference in constriction of coronary vessels between A, L and M, although the former two possess to a very marked degree a nicotinic action, while it is completely absent in the latter (Simonart). No evidence has yet been presented which would indicate that the nicotinic component of a choline derivative influences its therapeutic action. However, since our knowledge of choline therapy is still limited, the conclusion of Simonart appears justified, that a choline compound free

from nicotinic action should be preferable for clinical application, provided that it is equally effective and otherwise suitable.

The selection of a choline derivative will vary with the therapeutic purpose. For a strong but transient parasympathetic stimulation, unstable compounds of equal effectiveness but smaller toxicity than the stable ones seem preferable; they will also offer advantages in iontophoretic application, since the rapid destruction of these compounds in body fluids causes an almost immediate disappearance of systemic effects after interruption of the galvanic current. When a prolonged systemic effect is desired, peroral administration will be advisable; in this case the stable choline compounds should better serve the purpose, especially since the difference in toxicity so pronounced in intravenous or subcutaneous injection is less marked in peroral application.

SUMMARY

1. The pharmacologic and toxicologic properties of acetylcholine chloride (A), acetyl beta-methylcholine chloride (M), carbaminoyl choline chloride (L), ethyl ether beta-methylcholine chloride (E), and carbaminoyl beta-methylcholine chloride (U) have been compared with the following methods of testing: toxicity in mice and rats after intravenous, subcutaneous and peroral administration; circulatory and intestinal action in rabbits and dogs; miotic action in rabbits and cats; action on isolated intestines and isolated frog heart.

2. A, M and L were found to possess the same pharmacological activity when given intravenously. The potency of E and U was found to be between 100 and 150 times less.

3. In subcutaneous and peroral administration the potency depends largely upon the stability of the choline derivatives in body fluids. Stable compounds like L are, under these circumstances, much more effective than the otherwise equally potent A and M.

4. The procedure followed in testing the miotic action of drugs greatly influences the results. Slight massage of the eyelids may increase the effect up to 100 times.

5. Within the scope of these experiments no selective affinity of the various choline derivatives for certain parts of the parasympathetic nervous system was found.

6. The presence or absence of nicotinic action in a choline derivative does not influence the results under normal experimental conditions, i.e., without previous atropinization.

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