we prepared the sulphates from the two alkaloids and administered the solutions of these salts by intraperitoneal injection. Table III gives the results of this test. This tabulation shows that although the differences are smaller, they are still in the same direction.

A further test was made using laboratory-recrystallized alkaloids in comparison with the original commercial products. Six different lots of the commercial material were recrystallized in the laboratory and the twelve lots of poison thus obtained were tested. The laboratory-recrystallized product had a fairly uniform crystal size, whereas the original alkaloids were decidedly variable. The commercial alkaloid and the laboratory crystals from that alkaloid did not show any difference in toxicity.

CONCLUSIONS.

1. Commercial strychnine alkaloids of C.P. quality show definite and marked differences in toxicity.
2. These differences are of such magnitude that serious variation in results are noted in their use in economic poisons.
3. These differences have not been associated with determinable changes in chemical or physical properties.
4. Recrystallization of the various alkaloids does not alter their lethal efficiency.

THE INFLUENCE OF CERTAIN SALTS ON MORPHINE TOXICITY AND NARCOSIS IN MICE AND RATS.*

BY J. M. ORT AND W. G. CHRISTIANSEN.[1]

The study of synergism and antagonism among drugs has, admittedly, many fascinating theoretical and practical possibilities. In the field of morphine pharmacology, for instance, it is conceivable that certain drugs exist which can greatly potentiate the action of morphine in all of its manifestations, thus decreasing the dosage required. But, much more important, such a potentiator may some time be found which will affect only the narcotic action of the drug or at least will not proportionally increase its vicious habit-forming effects.

The influence of atropine on the activity of morphine is, of course, well recognized. F. Schmitz, for example, studied not only atropine in this connection but also lobelia, coramine, cardiazole, hexetone and pyramidon. (On the influence of central nervous stimulants on morphine poisoning (1).) W. Peters has reported that antipyrine is a morphine synergist. (A morphine-sparing ampul preparation (2).)

Bancroft (3) and his co-workers have made a most extensive study in this field, making use of the facts and theories of the modern science of colloid chemistry to expand the theory of anesthesia and the behavior of nervous tissues originally proposed by Claude Bernard. The essence of this theory is that anesthesia or narcosis results when the colloids of the cells of the nervous tissues are either abnormally reversibly dispersed or coagulated.

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According to Bancroft's views morphine produces its effects by reversibly coagulating the cell colloids of the nervous system. These cells in the morphine addict, then, are over-coagulated. Morphinism, therefore, should yield to drugs which tend to disperse the cell colloids. The long recognized sedative action of bromides must be due to the ability of bromides to peptize the colloids of nerve tissue cells, to which property is also due whatever success has been realized in the treatment of morphinism with bromides. This theory demands that a drug which is a coagulating agent will serve to aggravate the effects of morphine and to prolong its action. Thus he found that sodium tartrate would appreciably prolong the effect of morphine in rabbits while sodium thiocyanate had the opposite effect in both rabbits and dogs.

The actual therapeutic value of sodium thiocyanate as an aid in restoring morphine addicts to health and happiness, the subject of considerable discussion and difference of opinion, need not be discussed in this paper. The importance of the fundamental principles involved, however, seemed to us to be so great as to justify still further pharmacological study. As the peptizer or disperser to test, we chose sodium thiocyanate, on which so much work has already been done by Bancroft. We selected mono-sodium phosphate as an example of a coagulator and sodium chloride as a somewhat intermediate compound. Morphine hydrochloride and acid phosphate were tested separately for comparison. Mice and rats were chosen as the test animals in order to make feasible a larger number of experiments, thus minimizing as far as possible the significance of individual biological variations.

There is, of course, a species difference in the response of various animals to morphine. Nevertheless, morphine is certainly a narcotic drug for mice and rats, and the fundamentals of its action in these animals must be considered as essentially parallel to those in man and the higher animals. Two things were investigated: toxicity and narcotic effect. The toxicities of the various combinations tested were determined in mice in terms of the M. L. D. of the morphine content of the combinations, that is, the single dose in mg./Kg. which will kill half of the animals receiving subcutaneous injections.

The narcotic activities of the various combinations tested were determined in several ways. L. Maier ("Quantitative Determination of Morphine by Bioassay" (4)) has further developed the method of Straub and Herrman in which the presence of morphine is manifested by the animal drawing up its tail over its back in an S-shape. The time between the injection and the onset of the reaction is recorded, as is also the duration of the reaction. O. W. Barlow in "The Tranquillizing Potency of Morphine, Pantopen, Codeine, Papaverine and Narcotine" (5) confined rats on their backs on boards and recorded their movements after their first excitement had worn off. Threshold or higher doses of the drugs decrease the number of these movements in a given period of time. We have found a tail pressure method to give perhaps the most accurate and consistent results of the three methods we tried. In this method a graduated cylinder containing mercury is carefully set on the rat's tail. By consecutive tests, the minimum height of mercury that will elicit a response from the animal is determined. As the morphine dosage is increased over the threshold dose, this height of mercury increases somewhat proportionally up to the point of complete anesthesia.

EXPERIMENTAL RESULTS.

All combinations tested were injected subcutaneously and prepared by adding the various ingredients to a 3% aqueous morphine hydrochloride solution in the proportion of 3 Gm. ingredient to 1 Gm. morphine hydrochloride, except that the morphine acid phosphate solution was prepared by adding 4 cc. of normal phosphoric acid to 0.2 Gm. of morphine alkaloid and then diluting to give a 3% solution.

TABLE I.—EFFECTS IN MICE.

	Mouse Toxicity.		"S" Tail Reaction.			
					Averages for Animals in Which Reactions Occurred.	
Material Injected.	No. of Animals Injected.	Approximate M. L. D. Mg./Kg.	Dose Mg./Kg. Morphine Hydrochloride Equivalent.	Incidence of Reactions.	No. of Minutes before Start of Reactions.	Duration of Reaction in Minutes.
Morphine Hydrochloride	65	300	5	3/15	37	120
			10	8/8	21	141
Morphine hydrochloride + mono-sodium phosphate	56	300	5	4/15	40	60
			10	8/8	28	116
Morphine hydrochloride + sodium sulphocyanate	49	90	5	10/16	40	65
			10	8/8	22	151
Morphine hydrochloride + sodium chloride	57	300	5	7/14	40	100
			10	8/8	38	101
Morphine acid phosphate	57	300	6	0/8	..	..

TABLE II.—NARCOTIC ACTIVITIES IN RATS.

	Tranquilizing Effect.		Response to Tail Pressure.			
			Relative Average Number of Movements of the Group after Injection as Compared to the Group Average of 100 before Injection.		Relative Pressure on Tail Required to Elicit Response after Injection as Compared to Pressure Necessary before Which Equals 100.	
Material Injected.	No. of Rats Tested.	Morphine Hydrochloride Equivalent Mg./Kg.	End 1st hr.	End 2nd hr.	End 1st hr.	End 2nd hr.
Morphine hydrochloride	5	1.67	60.3	39.1	100.2	122.2
	10	2.50	60.3	52.8	159.0	97.0
	10	3.75	45.2	60.3	236.8	211.0
	10	5.00	29.4	43.0	260.9	210.9
	10	7.50	28.9	29.5	513.1	351.8
Morphine hydrochloride + mono-sodium phosphate	5	1.67	50.0	33.3	133.0	143.0
	5	3.75	44.7	25.0	188.4	157.6
	5	7.50	4.8	34.9	578.4	299.4
Morphine hydrochloride + sodium sulphocyanate	5	1.11	28.6	14.3	110.4	118.0
	5	1.67	43.2	10.2	207.0	177.4
	5	3.75	60.2	53.4	238.8	248.4
	5	7.50	1.1	12.9	828.0	593.2
Morphine hydrochloride + sodium chloride	5	1.67	127.4	9.1	108.4	115.4
	5	3.75	69.8	37.2	241.4	206.0
	5	7.50	30.7	58.0	285.0	190.8
Morphine acid phosphate	5	1.67	51.5	12.1	149.4	111.5
	5	3.75	48.7	30.8	208.2	166.8
	5	7.50	0.0	0.0	326.0	299.8
Controls	26	..	51.7	28.1	117.8	127.4

DISCUSSION.

Toxicity—Table I.—It was found that for all the combinations tested, except that containing sodium sulphocyanate, the curve of dosage administered–incidence of death flattened out noticeably above a dosage of 150 mg./Kg. This makes an exact determination of M. L. D. for such combinations not only difficult but, for practical purposes, somewhat meaningless. No significant difference in M. L. D. could be determined among these four combinations. No consistent correlation could be detected between the incidence of death at a given dosage and the recent dietary history of the animals, *i. e.*, whether or not the animals had been fasted for 16 to 24 hours immediately preceding the test.

The results with the mixture of sodium sulphocyanate and morphine were much more sharp and consistent. The M. L. D. for this combination was definitely between 75 and 115 mg./Kg. and approximately at 90 mg./Kg. Thus our tests have shown that sodium sulphocyanate markedly increases the toxicity of morphine in mice when the two are injected simultaneously.

"S" Tail Reaction in Mice—Table I.—We found no outstanding difference in the behavior of the combinations, except that the morphine acid phosphate combination gave no response in the dosage tried. Certainly there is no evidence that mono-sodium phosphate potentiates morphine in mice or that sodium sulphocyanate antagonizes it. The results obtained by this method are apt to be irregular and inconsistent when intermediate doses are tested.

Tranquility Effect in Rats—Table II.—While this method also gave somewhat inconsistent results, it can again be seen that sodium sulphocyanate certainly does not appear to antagonize the action of morphine. The action of phosphates is seen to be irregular. With small dosages of morphine, there was little evidence of potentiation. In the largest dose tested, the tranquilizing effect of the morphine was increased by the phosphate at the end of the first hour—but so was it with sodium sulphocyanate.

Pressure on Rats' Tails Required to Elicit Responses—Table II.—This method gave us the most consistent results of the three methods tried. Comparing the effects, concentration by concentration, it is seen that, on the whole, both marked potentiation and marked antagonism of the morphine action are lacking. However, the most pronounced evidence of potentiation in this series of tests occurred with the combination of morphine and sodium sulphocyanate and the greatest evidence of antagonism was seen in the tests on morphine and sodium chloride mixtures.

SUMMARY AND CONCLUSIONS.

We are aware, of course, that, with respect to the relation between added ingredient and morphine, and to the dosage of morphine given, we have by no means studied exhaustively the possibilities of synergism and antagonism between morphine and sodium phosphate, chloride or thiocyanate. Our experiments were also confined to mice and rats. However, under the conditions of these experiments, we found on the whole no evidence of any marked increase or decrease of the action of morphine itself, either in the case of an added peptizer—sodium sulphocyanate—or added coagulators—mono-sodium phosphate and sodium chloride.

If anything, sodium sulphocyanate showed some tendency to potentiate the effect of morphine. It undoubtedly considerably increased its toxicity, which might be expected of any substance which could potentiate morphine in all of its manifestations. Our results are not those which could be predicted from Bancroft's theory on the mechanism of the pharmacological actions of morphine, sodium sulphocyanate and mono-sodium phosphate.

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REFERENCES.

(1) *Arch. exptl. Path. Pharmakol.*, 118, 258–374 (1926).
(2) *Münch. med. Wochschr.*, 79, 224 (1932).
(3) *J. Phys. Chem.*, 35, 214–268; 1184–1211, 1606–1623, 3036–3057, 3189–3206, 3452–3479 (1931); 36, 1521–1548, 2011–2082, 3162–3174 (1932).
(4) *Arch. exptl. Path. Pharmakol.*, 161, 163–172 (1931).
(5) *J. A. M. A.*, 99, 986–988 (1932).

THE TOXICITY OF BARBITAL DERIVATIVES.*,[1]

BY IVOR JONES[2] AND E. V. LYNN.

Notwithstanding the large number of derivatives of barbituric acid which have been introduced into medicine, we have as yet only meager information as to comparative toxicity and efficiency. The textbooks make certain standard statements about the older ones which are not based upon existing evidence. Whatever reports can be found in the literature have originated largely in the laboratories of manufacturers, and the few unbiased records give very little data upon which the physician can base accurate judgment. The present study was designed as a start towards making such information available.

Of the marketed compounds seven of those most often used were selected for study: Barbital U. S. P. and phenobarbital U. S. P., introduced about 1904; dial, 1912; amytal, 1924; neonal, 1926; phanodorn, 1928; pentobarbital (nembutal), 1930.

The rabbit was chosen as the experimental animal because there seems no stated objection and several disadvantages have been found for other animals.

Using oral administration for rabbits, the M. L. D. in mg. per Kg. has been determined by Fitch and Tatum (1) as follows: Barbital 275, amytal 575, pentobarbital 175, phenobarbital 150, neonal 160, phanodorn 450. Roemer (2) gave for barbital 400. By subcutaneous injection in rabbits, the only results reported have been from the laboratories of Eli Lilly & Co.: Barbital 290, amytal 110, neonal 100. Intraperitoneally, Fitch and Tatum (1) gave: Barbital 225, amytal 90, pentobarbital 65, phenobarbital 150, neonal 115, phanodorn 130. Intravenously, Herwick and Knoefel (3) reported for barbital 250, for amytal 50. Intramuscularly, Louvier (4) gave for phenobarbital 150. As far as could be determined no one else has given any results on rabbits.

* Scientific Section, A. PH. A., Washington meeting, 1934.
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