of Oxford[6] for anaerobes. It is not identical with glutamine and p-amino benzoic acid because these substances have no activating effect on the growth of *Str. hemolyticus.*

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Comparative Potentiating Effects of Certain Therapeutic Agents on Sodium Evipal Hypnosis.

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Barlow and Gledhill[1] demonstrated the potentiation of isoamyl-ethyl barbituric acid and ethyl (1-methyl butyl) barbiturate on morphine. More recently, Loewe[2] utilized this principle to show that active cannabis preparations, when administered orally to mice, materially prolonged the hypnotic effect of subcutaneously administered pernoston.

We have observed that the intravenous administration of evipal sodium under carefully standardized conditions produces a remarkably consistent reaction on the adult male rat. This is particularly true if the *initial* voluntary movement is taken as the criterion of recovery from hypnosis. Female rats differ materially from males in their reactions to evipal in that hypnosis is more irregular and persists 2 to 5 times as long. Immature animals react inconsistently.

Materials and Methods. Adult male rats, weighing from 325-400 g were used throughout this study; all animals were fasted for 14-18 hours prior to medication. The normal period of hypnosis from a standard dose of evipal sodium administered by the saphenous vein as a 4.0% aqueous solution at an injection rate of 0.1 cc per 15 seconds was determined. This period was estimated within 10 seconds by counting time from the establishment of hypnosis (the completion of the intravenous injection) until the *first* voluntary movement occurred following the injection. A period of 5-7 days was allowed to elapse before the animals were again medicated. The compound to be tested in conjunction with the hypnotic was adminis-

[6] Oxford, A. E., Lampen, J. O., and Peterson, W. H., *Biochem. J.*, 1940, **34**, 1588.

[1] Barlow, O. W., and Gledhill, J. D., *J. Pharm. and Exp. Therap.*, 1933, **49**, 36.

[2] Loewe, S., *J. Am. Pharm. Assn.*, 1940, **29**, 572.

tered orally or subcutaneously to fasted animals 30 minutes prior to the injection of the hypnotic by vein. In other experiments, the oral medication preceded the intravenous administration of the hypnotic by 60 minutes. The animals were again allowed to rest for a period of 5-7 days, when the hypnotic effect of the intravenously administered standardized dose of evipal sodium was again determined. Under such conditions, a single animal could be used for not less than 5 or 6 separate tests.

The method described above is amenable to graphic recording. The procedure[3] necessitates the restraint of the rat on its back by means of specially adapted animal boards and the recording of muscular movements on a smoked paper by means of a recording lever attached by thread to a small serrafine clamp, which in turn is attached to the anterior abdominal wall. During the period in which the animal is under the influence of evipal sodium, only respiratory movements are recorded. The end point is defined as that moment when voluntary movements reappear.

Results. The results are summarized in Table I.

Na Bromide: No potentiation was observed in spite of the large dose administered. This was felt to be due to the fact that insuf-

TABLE I.
Potentiation of Hypnotic Effect of Na Evipal by Various Therapeutic Agents.

No. of animals	Dose of Evipal Na (intravenous) mg per kg	Additional medication			Mean duration of anesthesia (min.)	P.E.$_M$*
		Drug	Route	Dose, mg/kg		
183	40	—	—	—	11.3	± 0.26
30	40	Na bromide	p.o.	350	11.4	± 0.36
33	40	" "	p.o.	350†	15.9	± 0.56
27	40	Amidopyrine	p.o.	250	19.1	± 0.57
21	40	Acetylsalicylic acid	p.o.	175	14.2	± 0.55
19	40	Morphine SO_4	s.c.	1.0	17.6	± 0.49
24	40	" "	s.c.	2.0	18.6	± 0.52
21	40	" "	s.c.	4.0	29.1	± 0.72
17	40	" "	s.c.	6.0‡	30.4	± 0.94
48	40	" "	s.c.	42.0§	37.2	± 1.12
26	40	Demerol (D-140)	p.o.	50	22.4	± 0.72
18	40	" "	p.o.	20	18.3	± 0.45
52	60	—	—	—	30.4	± 0.69
29	60	Demerol (D-140)	p.o.	50	87.7	± 3.88
29	60	Amidopyrine	p.o.	250	84.6	± 2.39

*$P.E._M = \frac{\sigma \times 0.6745}{\sqrt{n-1}}$

†1 daily, 5 days.
‡2 deaths.
§35 deaths.

[3] Barlow, O. W., *J. A. M. A.*, 1932, **99**, 986.

ficient time was allowed for saturation of the tissues with bromide. Accordingly, a second group of animals was employed, which received daily doses of sodium bromide (350 mg per kg per day, p.o.) for a period of 5 days before the second dose of evipal sodium was administered. These animals showed no gross depression or impairment of spontaneous movements during the course of bromide medication. However, the potentiating effect of such continued therapy on evipal hypnosis is clearly demonstrable.

Acetylsalicylic Acid: A slight degree of potentiation resulted from the administration of this dose. No significant depression was observed in these animals prior to the administration of the evipal sodium.

Amidopyrine: Marked potentiation of the hypnotic effect was observed. This dose did not produce any significant depression.

Morphine: The initial dose of morphine as 10% of the lethal dose, based on a lethal dose of 420 mg per kg as established by Hunt,[4] caused 35 deaths in a group of 48 animals. We subsequently observed that this figure for the generally accepted M.L.D. of morphine was not applicable to our rat colony, a point which is borne out by the fact that doses as small as 1.0 mg per kg produced a definite synergistic effect with evipal by vein. This synergism became more pronounced as the dose level increased.

Demerol (D-140): This is a new type of potent analgesic originally described by Eisleb and Schaumann[5] and more recently by ourselves.[6] It is 1-methyl 4-phenylpiperidine 4-carbonic acid ethyl ester. A detailed account of the pharmacological properties of this material is now in preparation.[7] Single doses of this order have a marked analgesic effect, but only a slight sedative action. We have been able to demonstrate this sedation objectively by Barlow's[3] tranquilizing procedure. Under the conditions of this test, this drug has a definite potentiating effect on the hypnotic action of sodium evipal, which is superior to that of amidopyrine and approaches that of morphine.

Summary. The hypnotic effect of sodium evipal, administered intravenously, may be markedly potentiated by acetylsalicylic acid, amidopyrine, demerol (D-140) and morphine. The synergistic effectiveness is in the order named.

4 Hunt, R., *U. S. Hygiene Lab. Bull.* 1910, p. 83.

5 Eisleb, O., and Schaumann, O., *Deutsch. med. Wochschr.*, 1939, **65**, 967.

6 Climenko, D. R., and Barlow, O. W., in preparation.

7 Barlow, O. W., and Climenko, D. R., in preparation.