and inactivated in a water bath at 56°C for 30 minutes. 0.05 cc of antigen[7] was then added and the mixture shaken for 5 minutes. It was then incubated for 30 minutes, and the results, both macro- and microscopic, read immediately thereafter.

TABLE I.
Results of the Micro Eagle Test in Syphilitic White Mice.

Duration of infection days	No. of mice	Results	
		Positive	Negative
30	28	0 0%	28 100%
60	47	2 4.3%	45 95.7%
120	38	1 2.6%	37 97.4%

As shown in Table I, the test was entirely negative in 28 mice 30 days after inoculation. Two or 4.3% of 47 mice were positive 60 days after inoculation and 1 or 2.6% of 38 mice was positive 120 days after inoculation. Of the 2 mice that were positive 60 days after inoculation one became negative when tested 120 days after inoculation; the other was still positive at the end of 120 days.

Summary and Conclusions. In a study of the micro Eagle test on the sera of 57 syphilitic white mice, we found that: (1) of 28 mice infected 30 days, none was positive; (2) of 47 mice infected 60 days 2 or 4.3% were positive; and (3) of 38 mice infected 120 days 1 or 2.6% was positive. These findings confirm those of other authors; namely, that a positive serologic test for syphilis infrequently occurs in the syphilitic mouse and is, therefore, of no value in studying the course of experimental syphilis in this animal.

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A New Method for Quantitative Measurement of Pain Sensation.

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A suitable method for quantitative measurement of analgesia (as distinguished from local anesthesia) produced by various physical and chemical agents is a crying need of modern experimental phar-

[7] Eagle, H., *J. Lab. and Clin. Med.*, 1936, **22**, 300.

macology, physiology and psychology. The method of Macht, Herman and Levy[1, 2] for determining pain threshold in normal human subjects is probably the most suitable for such drugs as the opium alkaloids, which have a wide clinical use and fully established margin of safety. Animals being indispensable for quantitative study of new and little known compounds, however, the authors have developed a "rat test" employed in the following manner.

The scrotum of a large tame male rat is a very suitable test object for the quantitative measurements of pain sensation. Richly supplied with sensory nerves, it readily responds to pain stimuli produced by a faradic current. When fine platinum electrodes connected with a standardized induction coil are applied to any given spot on the scrotum of an adult white rat and the current is increased by gradually approximating the secondary to the primary coil, a point is reached when the stimulation is sufficiently powerful to produce pain. This point, indicated by the animal's characteristic squeal, is the pain threshold for that given area, and numerous experiments performed by the authors have proven that it is surprisingly constant. This method, therefore, admits of careful study of the effect produced upon the pain threshold by various pharmacodynamic agents.

In this work the writers employed a standard Harvard inductorium operated on a single dry cell of 1.6 volts, which was carefully calibrated in the physical laboratory of a well-known university, so that the intensity of the stimulations for any given position of the secondary coil in relation to the primary could be expressed in physical units, *viz.*, volts. The inductorium was calibrated with the aid of an R.C.A. cathode ray oscillograph, and a curve was plotted to express the voltage corresponding to any given position of the secondary coil. The fine electrodes are applied to the scrotum of a large tame rat, held gently but firmly by an assistant, at a point marked for identification. The pain threshold is then established by slowly increasing the faradic current until the animal's characteristic squeal of pain is elicited. When the threshold of pain has been accurately established by repeated trials, the authors administer a drug by subcutaneous or intramuscular injection or, if the compound is insoluble, by stomach tube. The effect of the drug on the pain threshold is then measured at various intervals after its administration.

The series of drugs tested on rats in this way included various opium alkaloids, coal tar derivatives, barbiturates, bromides, etc., on

1 Macht, D. I., Herman, N. B., and Levy, C. S., *Proc. Nat. Acad. Sc.*, 1915, **1**, 582.

2 Macht, D. I., Herman, N. B., and Levy, C. S., *J. Pharm. and Exp. Therap.*, 1916, **8**, 1.

TABLE I.
Comparison of Effects of Morphine and Other Analgesics.

Rat No.	Drug administered	Dose given mg	Pain Threshold: Normal cm	Normal volts	15-30 min after injection cm	15-30 min after injection volts	1-2 hr after injection cm	1-2 hr after injection volts	4 hr after injection cm	4 hr after injection volts
1	**Morphine Sulfate**	**1**	8.0	185	7.6	250	7.8	218	—	—
2	,, ,,	**1.5**	7.6	250	6.6	530	7.1	350	7.7	232
3	,, ,,	3	7.1	350	5.4	1080	5.8	953	7.7	232
4	Codeine HCl	2	9.5	125	8.2	176	9.5	125	—	—
5	,, ,,	3	8.0	185	7.6	250	8.0	185	—	—
6	Pantopon	2	8.0	185	6.5	575	7.3	300	7.6	250
7	Dilaudid	0.25	9.0	100	7.0	368	7.0	368	—	—
							1 hr after injection		2 hr after injection	
8	Acetanilid	4	7.8	218	7.8	218	7.0	368	7.5	265
9	Antipyrin	10	7.6	250	—	—	7.2	325	7.3	300
10	Caffeine	4	8.1	180	8.1	180	8.4	170	—	—
11	Cocaine HCl	2	9.5	125	9.8	110	9.8	110	—	—
12	Phenolbarbital Na	5	7.4	280	—	—	7.8	218	7.7	232
13	Salicylate Na	10	7.2	325	6.8	445	6.8	445	—	—
14	Strychnine Nitr.	0.1	7.3	300	8.0	185	8.2	176	—	—
					45 min after administration		2 hr after administration		3 hr after administration	
15	Acetphenetidin (by stomach tube)	30	8.8	155	7.5	265	7.6	250	—	—

TABLE II.
Comparison of Analgesic Effects of Cobra Venom and Morphine.

Rat No.	Drug administered	Dose injected	Pain Threshold: Normal		45 min after injection		2 hr after injection		4-5 hr after injection	
			cm	volts	cm	volts	cm	volts	cm	volts
1	Morphine Sulfate	1 mg	6.0	809	6.1	770	7.0	368	6.0	809
2	" "	1 "	6.4	630	6.6	530	7.4	280	6.4	630
3	" "	1 "	6.5	575	6.0	809	6.0	809	6.5	575
4	" "	1 "	8.0	185	7.2	325	7.2	325	8.0	185
5	Cobra Venom	0.5 m.u.	7.0	368	6.6	530	6.7	480	6.5	575
6	" "	0.5 "	8.3	173	7.5	265	7.2	325	7.2	325
7	" "	0.5 "	8.3	173	6.8	445	6.3	665	6.2	715
8	" "	0.5 "	7.2	325	6.5	575	6.1	770	6.0	809
9	Morphine Sulfate	3 mg	7.9	205	4.9	1260	8.0	185	7.9	205
10	" "	3 "	7.1	350	5.4	1080	7.9	205	7.2	325
11	" "	3 "	8.5	166	6.5	575	8.4	170	8.4	170
12	" "	3 "	8.0	185	6.8	445	8.3	173	8.0	185
13	Cobra Venom	1 m.u.	8.0	185	7.0	368	4.0	1932	5.0	1223
14	" "	1.5 "	8.2	176	8.4	170	7.2	325	6.4	630
15	" "	1.5 "	7.1	350	6.8	575	6.8	575	5.8	953
16	" "	2 "	8.0	185	6.0	809	5.4	1080	6.4	630

the one hand, and, on the other, drugs used as controls, such as strychnine and caffeine. It was found that some of the drugs *raised* the pain threshold, that is, produced analgesia. Other drugs *lowered* it, thus indicating hyperalgesia or hypersensitivity to pain. Table I shows the results obtained with a number of such drugs. It will be seen that morphine, codeine, pantopon and dilaudid, antipyrin and acetphenetidin produce varying degrees of analgesia. Sodium phenobarbital, 5 mg, did not relieve pain while 10 mg of sodium salicylate effected some analgesia. Strychnine and caffeine lowered the threshold of pain. It was not surprising to find that the local anesthetic cocaine, which is a delirifacient when injected parenterally, induced hyperalgesia. It will be noted that the degree of analgesia produced by morphine varied with individual rats.

Of special interest were the results obtained in a comparative study of morphine and cobra venom, an analgesic now used considerably in place of the narcotics.[3, 4] Table II shows that morphine in sufficiently large doses produces an analgesia that sets in promptly after administration but wears off in a short while. The analgesia induced in rats by cobra venom, on the contrary, supervenes but slowly and lasts much longer than that of morphine. These findings regarding the effect of morphine and cobra venom, respectively, on the pain threshold of rats agree with those derived by Macht and Bryan from studies on the threshold of pain of human subjects and also of guinea pigs.[5] The results obtained by this method in regard to the comparative analgesic properties of various well-known drugs completely concur with clinical data concerning them. This new method for quantitative measurement of pain threshold and analgesia was furthermore found to be useful in the study of new and unknown compounds believed to possess pain-relieving properties such as, for instance, the various fractions obtained in connection with a biochemical study of snake venoms. A more detailed discussion of the technic employed and of the effects of numerous drugs as studied by this method will appear in the *Journal of the American Pharmaceutical Association.*

Summary. A new method for quantitative determination of pain threshold in rats is described. The results obtained with various analgesic drugs, including morphine and cobra venom, are cited as illustrations of its practicability.

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3 Macht, D. I., *Ann. Int. Med.*, 1938, **11**, 1824.

4 Macht, D. I., *M. Press*, 1939, **201**, 254.

5 Macht, D. I., and Bryan, H. F., *Compt. rend. Soc. de biol.*, 1935, **119**, 306.