low residue diet and standardized pre- and post-operative care appear to be mainly responsible for the high survival rate.

1. Ingle, D. J., and Griffith, J. Q., *The Rat in Laboratory Investigation,* 2nd ed., J. B. Lippincott Co., Philadelphia, 1949.

2. Foglia, V. G., *Rev. Soc. Argent. Biol.,* 1945, v21, 360.

3. Roe, J. H., Jr., Smith, B. W., and Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., in press.

4. Somogyi, M., *J. Biol. Chem.,* 1945, v160, 69.

5. Nelson, N., *ibid.,* 1944, v153, 375.

6. Smith, B. W., and Roe, J. H., *ibid.,* 1949, v179, 53.

Received July 12, 1954. P.S.E.B.M., 1954, v86.

Reduction of Pain-Conditioned Anxiety by Analgesic Doses of Morphine in Rats. (21262)

HARRIS E. HILL, RICHARD E. BELLEVILLE, AND ABRAHAM WIKLER.

From the National Institute of Mental Health, NIMH Addiction Research Center, Public Health Service Hospital, Lexington, Ky.

Previous investigations(1,2) have demonstrated that an analgesic dose of morphine (15 mg) reduces the behavior-distrupting effects of anticipation of pain in man. The present study was designed to test the hypothesis that morphine also reduces anxiety associated with anticipation of pain in animals. Confirmation of this hypothesis may lead to the development of an analgesic testing method in animals which differs in principle from previously reported technics(3-6).

In accordance with this objective, it was considered essential that the method meet the following criteria: a) The response must be easily quantified, b) Induced changes in the response should be based upon principles similar to those underlying the human work, and c) The response change must be sensitive to graded doses of opiates. In addition, the method should, if possible, meet a requirement proposed by Wikler(7): It should distinguish between analgesic, hypnotic, and paralytic effects of drugs. The present paper is a presentation of the methodology employed with rats and of the results obtained on a first experiment on morphine.

Methods and materials. The conditioned operant or instrumental response of bar-pressing for food rewards under food deprivation was studied in a modified Skinner Box(8). Partial or complete cessation of bar-pressing, considered to be conditioned anxiety or inhibition, was produced by pairing a tone with electric shock. The degree of restoration of bar-pressing followed subcutaneous administration of graded doses of morphine provided measures of the reduction of the inhibition. Correspondence between the drug dose and the % of restoration of the previous response rate was the pertinent datum. The subjects were 12 male albino rats approximately 4 months of age when the experiment was begun. However, the N's as shown in Table 1 for the various test days are not equal since an animal was omitted from the experiment when its bar-pressing behavior was severely impaired by the morphine injections. To produce a high and fairly constant rate of response which would survive the conditioning procedure and drug injections, the animals were maintained at approximately 70% of satiation weight; they were weighed daily and fed individual amounts of Purina laboratory chow. Water was always available in the home cage but not in the experimental apparatus. The Skinner Box was modified in several respects. It was somewhat smaller than the original design. A conditioning chamber (8 x 7 x 10 inches), the floor of which was a barred shocking grid, was mounted in a sound proof box (14 x 14 x 18 inches); the bar and other accessories were similar to those described by Skinner. Circulation of air was insured by the action of a small vacuum pump.

Observation windows were placed in one end of the apparatus, and its interior was illuminated by a small electric light bulb. Conditioned bar-pressing was developed by the Skinner method, and the animals were trained on a periodic 2-minute schedule which was later changed to a periodic reinforcement with a 2-minute mean. After the animals had acquired a rapid bar-pressing rate and produced relatively smooth curves on a cumulative recorder, the technic of Estes and Skinner(9) for developing conditioned anxiety was introduced.* Conditioned inhibition of bar-pressing was developed by sounding a 60-cycle tone for 4 minutes which was terminated by an A. C. shock delivered through the floor grid. The tone was applied through a headphone which was fastened to the top of the box. The electric shock, ranging from 40 to 60 volts for approximately 0.5 seconds duration, was administered through a variac, and was adjusted according to each animal's performance during the tone-shock conditioning. The administration of drugs was begun with adaptation periods of simple bar-pressing under 3 increasing doses of morphine. During testing procedures, control and experimental periods were 48 hours apart; control readings always being obtained first. Conditioning was omitted on the day preceding each of the recording periods. On drug administration days, each animal was weighed, injected subcutaneously, and placed in the home cage for 75 minutes to allow for approximate peak action of the drug before being placed in the Skinner Box. At intervals of 7 to 10 days morphine sulfate was injected in the following order, 8, 10, 6, 11, 4, 5, 14, 12, 11, and 13 mg/kg. Reproductions of typical morphine records are shown in Fig. 1, B and B′. Since morphine produced some decrease in the rate of response in the majority of animals, calculations for each animal were based on its performance during the 4 minutes immediately preceding the tone; cumulative bar-pressings for the 4 minutes of tone presentation were calculated as percentages of each animal's previous rate. Drug and non-drug records were treated in the same manner, each animal serving as its own control.

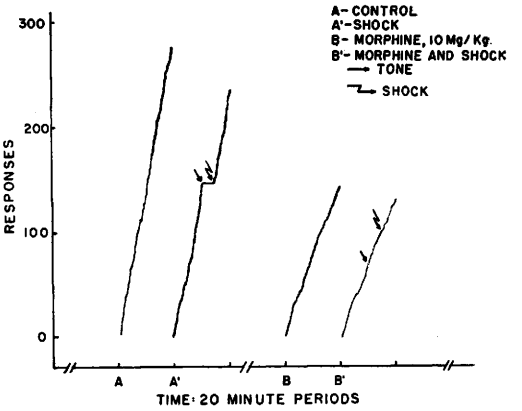


FIG. 1. 20-min. cumulative recordings. As the kymograph moved to left, responses were cumulated vertically.

Results. A 20-minute record of a well-trained animal is shown in Fig. 1, A′. It may be observed in this typical, although individual, record that responding ceased for the 4 minutes while the tone sounded and was immediately resumed after the presentation of the shock with little, if any, compensatory increase in rate. The mean control % was 13.4, the median was 17.3, and the range was 0 to 26%. Table I presents the data in rounded percentages, and Fig. 2 shows a free-hand curve fitted to the obtained points.

Correspondence between restoration of bar-pressing and the dose level of morphine is obvious. The results as presented in Fig. 2 demonstrate a range of approximately 4 to 10 mg/kg, a factor of 2½. The upper limit of effectiveness appears to be 80% restoration, or slightly greater, without complication by impairment of performance. As indicated in Fig. 1, discrepancies were found in the higher dose range. Although response rate was restored to a median of 80% by these higher doses, activity and bar-pressing were impaired

TABLE I. Mean Response Rate Percentages for Graded Doses of Morphine and the Number of Animals Tested.

MS	mg/kg 0	4	5	6	8	10	11	11	12	13	14
%	11	22	28	37	54	80	97	80	67	82	78
No.	—	6	5	9	5	6	6	6	7	4	2

* Later, Hunt and Brady(10), and Brady(11), employed a similar method for investigating the effects of electro-convulsive shock and tetraethylammonium on a conditioned "emotional" response.

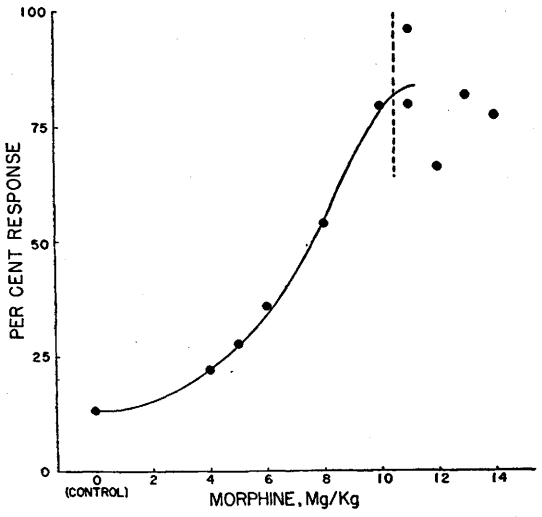


FIG. 2. Morphine dose-effect relationship. Each point on curve represents mean performance when percentage for each animal was calculated in terms of its previous rate.

at 12 mg/kg and above.

Discussion. Application of the principles derived from studies on human subjects to the behavior of the laboratory rat were found to be quite feasible. The reduction or elimination of bar-pressing during the period of the tone is considered to be the distrupting effect of anticipatory response to painful stimuli; this is analogous to the findings on man. Analogous, also, were the changes in behavior following administration of morphine; the effects of anxiety on the measured responses were either reduced or abolished. One of the chief differences between these studies was that only one dose level of morphine was employed with the human subjects whereas 13 were used with rats; in the latter the restoration of bar-pressing was proportional to the dose from 4 to 10 mg/kg.

The higher dose range, however, presents unique problems; the hypnotic and neuromuscular effects of morphine, particularly drowsiness and ataxia, are frequently marked in doses higher than 11 mg/kg. As shown in Fig. 2, a considerable degree of scatter occurred with doses of 12 mg/kg and above. This was partly due to side-effects of morphine which produced irregularities of performance including marked impairment of bar-pressing. Reliable measures of the analgesic effect of morphine, then, would extend from the smallest dose which could be differentiated from control performance, to the highest dose which produced a relatively small standard error and a statistically significant increase of bar-pressing over that produced by an arbitrarily chosen lesser amount. Thus, the maximum dose of morphine which produced analgesia without marked impairment of normal behavior might be distinguished from those doses which produced analgesia with concomitant hypnotic and unfavorable neuromuscular effects.

Further studies are being undertaken in which improved control of variables and statistical evaluation of differences will be obtained. These studies will use a greater number of animals, improved apparatus, more constant temperature and humidity control, and possibly additional measures of inhibition. Comparative measures will be obtained for the actions of a sufficiently wide range of drugs to thoroughly test the described procedure.

Although as yet the method has not been shown to be specific for detecting analgesics, it is sensitive to graded doses of morphine, and differentiates between anxiety reduction and hypnotic and paralytic drug effects. In addition, the results, which were strikingly similar to those of the previous human work, provide strong supporting evidence for the hypothesis that one of the major actions of a potent analgesic is the reduction of behavior-disrupting anticipatory response to noxious stimuli.

Summary. 1. Rats, maintained at 70% of satiation weight, were conditioned to press a bar at a rapid and constant rate in a modified Skinner Box. After about 15 days of training when this behavior had been thoroughly established, a method for producing conditioned anxiety was introduced. Shortly after each animal began the daily bar-pressing session a 60-cycle tone, which sounded for 4 minutes, was terminated by the application of a strong electrical shock. After several days of conditioning this procedure produced almost complete cessation of bar-pressing during the tone period. In testing the effect of a known analgesic the administration of graded doses of morphine (4-11 mg/kg) produced proportional restoration of the inhibited bar-pressing.

2. The reduction or elimination of inhibition by morphine was considered to be a reduction of anxiety associated with anticipation of noxious stimuli. The results parallel in all essential details previous methodological work on man, and strongly suggest that the present procedure may be useful as a technic for the screening of possible analgesic drugs.

1. Hill, H. E., Kornetsky, C. H., Flanary, H. G., and Wikler, A., *J. Clin. Invest.*, 1952, v31, 473.

2. Hill, H. E., *Arch. Neurol. and Psychiat.*, 1952, v67, 612.

3. Goetzl, F. R., Burrill, D. Y., and Ivy, A. C., *Quart. Bull. Northwestern Univ. Med. Sch.*, 1944, v17, 280.

4. Miller, L. C., *Ann. N. Y. Acad., Sci* 1948, v51, 34.

5. Wikler, A., *Neurology*, 1953, v3, 656.

6. ———, *Pharmacol. Rev.*, 1950, v100, 435.

7. ———, Fed. Proc., 1952, v67, 612.

8. Skinner, B. F., *The Behavior of Organisms.* New York, Appleton-Century, 1938.

9. Estes, W. K., and Skinner, B. F., *J. Exp. Psychol.*, 1941, v29, 390.

10. Hunt, H. F., and Brady, J. V., *J. Comp. Physiol. Psychol.*, 1951, v44, 88.

11. Brady, J. V., *ibid.*, 1953, v46, 311.

Received July 13, 1954. P.S.E.B.M., 1954, v86.

Observations on G-25671, A Phenylbutazone Analogue (4-(phenylthioethyl) -1,2-diphenyl 3,5-pyrazolidinedione).* (21263)

BERNARD B. BRODIE, T. F. YÜ, J. J. BURNS, THEODORE CHENKIN, BRUCE C. PATON, J. MURRAY STEELE, AND ALEXANDER B. GUTMAN.

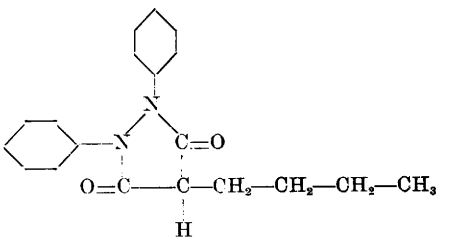
From the National Heart Institute, National Institutes of Health, Bethesda, Md., the New York University Medical Division, Goldwater Memorial Hospital; Departments of Medicine, Mount Sinai Hospital, and Columbia University College of Physicians and Surgeons, New York.

Phenylbutazone (Butazolidin) has been shown to be a potent antirheumatic agent but it may produce such side effects as edema, gastric distress, skin reactions and, on rare occasions, gastrointestinal hemorrhage and bone marrow depression. In an attempt to develop a drug retaining the antirheumatic action of phenylbutazone but devoid of its side effects, a series of phenylbutazone analogues are being screened in animals and man for anti-inflammatory activity.† It is the purpose of this paper to report preliminary observations in man on one of the compounds (G-25671) which showed marked anti-inflammatory effects in the animal screening tests.

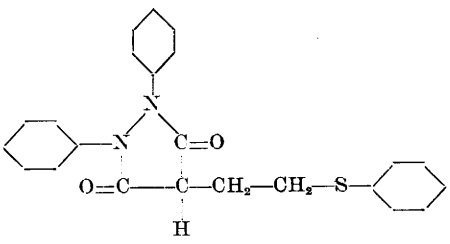
* Supported by grants from the Public Health Research Institute of City of New York, Josiah Macy Jr., Foundation, National Institute of Arthritis and Metabolic Diseases, and New York Chapter of Arthritis and Rheumatism Foundation.

† The compounds, supplied by Geigy Pharmaceuticals, were synthesized by Haefliger, F., *et al.*, and screened in animals by Domenjoz, R., and Wilhelmi, G.

For comparison, the structures of phenylbutazone and G-25671 are shown:



Phenylbutazone



G-25671

Materials and methods. Concentration of G-25671 in plasma was determined by a