Synergism Between Cold and Restraint for Rapid Production of Stress Ulcers in Rats.* (31970)

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A number of methods have been described for study of somatic expression of adverse psychic stimulation in experimental animals (1-4). Most methods are based upon the development of gastric lesions in rats subjected to some form of environmental stress. The most popular mode of stressing the rat has been simple physical restraint, which is inexpensive and easy to perform. Unfortunately, simple physical restraint leads to wide variation in incidence and types of lesions; mortality rates tend to be high and unpredictable; furthermore, 12 to 24 hours are required for the production of lesions.

In the present studies, rats were immobilized in rigid plastic devices that facilitated precise standardization of restraint volume and conditions. It was demonstrated that restraint and exposure to cold acted synergistically to produce gastric ulcers. On the basis of the results of these experiments, we propose an improved method for study of stress-induced ulcers in rats. The method offers several important advantages over those described previously. Lesions are produced in 2 hours; there is less variability in the incidence and types of lesions produced and animals die infrequently.

Materials and methods. Female CD rats (derived originally from Sprague-Dawley stock) weighing between 180 and 220 g were purchased from Charles River Laboratories, Inc., North Wilmington, Mass. For 24 hours prior to restraint, the animals were permitted to ingest nothing but tap water. They were then placed in plastic restraint boxes (Fisher Scientific Co., Catalog No. 1-280, Medium Size) (Fig. 1). The length of the restraint volume was adjusted by entering the door panel in one of 3 available positions. The door panel was then taped in place. Further immobilization of the animals was achieved by introducing glass test tubes (98 × 11 mm) through the longitudinal aperture in the bottom of the restraint boxes; the tubes were then pushed laterally. Usually 2 tubes sufficed to immobilize an animal. Immobilization was sufficient to prevent the animal from turning and wedging itself in the restraint box, thus compromising respiration. Animals were then placed in a cold room or refrigerator at 4° to 7°C. Two hours later, the animals were sacrificed, the stomachs were removed and examined for lesions.

Other groups of animals were either a) restrained as described above and maintained at room temperature, or b) refrigerated at 4° to 7°C without restraint for a period of 2 hours before sacrifice.

Results. Lesions were defined as erosions of the gastric mucosa (glandular portion) at least 1 mm in diameter that extended either to or through the submucosa; their edges were sharply demarcated and their bases were black or red (Fig. 2). Other phenomena such as diffuse erythema, which was observed frequently, and submucosal hemorrhages, which appeared uncommonly, were not scored (see below).

The results of these studies are listed in Table I. It is apparent that the combination of cold exposure and physical restraint yielded a

TABLE I. Gastric Lesions in Rats Subjected to Either Cold or Restraint or Both.

	n	All lesions* No. (%)	Multiple severe lesions† No. (%)
Restraint	12	0 (0)	0 (0)
Cold	13	2 (15)	1 (8)
Restraint and cold	119	79 (67)	53 (45)

* See text for definition of lesions.
† Three or more lesions greater than 1 mm in diameter, usually with intraluminal hemorrhage.

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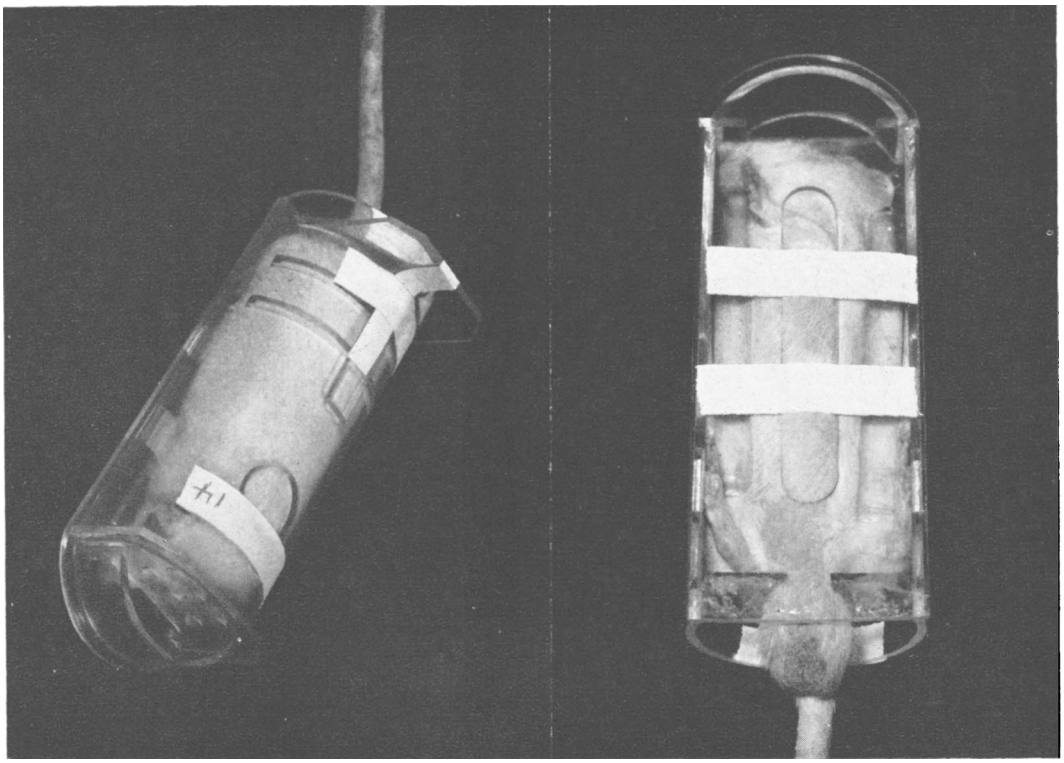

FIG. 1. Rats restrained in plastic boxes. Note 3 slots for adjusting volume. Also note test tubes occupying lateral spaces in restraint box.

much greater incidence of lesions than did either type of stress acting alone.

Discussion and conclusion. This method offers several significant advantages over those described previously. The simplicity of the technique permits an experienced operator to immobilize an animal in approximately 30 to 45 seconds. Only 2 hours are required to produce lesions, offering further saving of time. Apparently this rapid production of lesions represents a synergistic effect of the dual stresses employed. We believe that the mode of restraint also contributes to the improved results obtained. This method obviates additional environmental variables that may be difficult to control, *e.g.*, increased temperature, as with plaster of paris immobilization (5); pain, as with nailing the paws to a board(1); or ingestion of sharp metal fragments, as with wire screen immobilization(3). Furthermore, use of the plastic box permits immobilization in a relatively uniform volume. Bonfils and Lambling, on the basis of studies

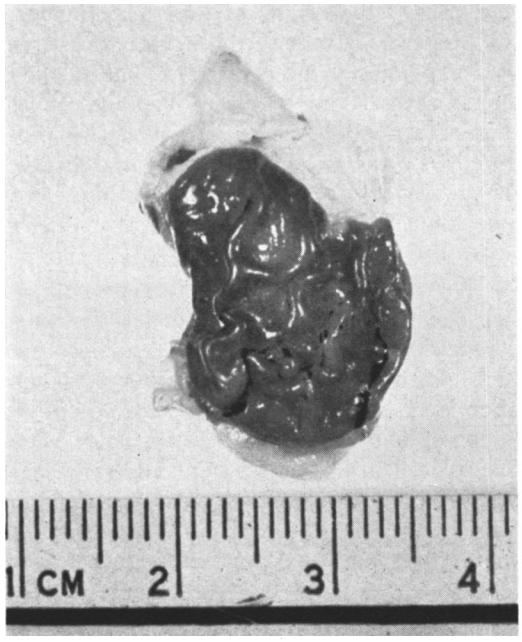

FIG. 2. Photograph of stomach of rat subjected to physical restraint and cold exposure for 2 hours. In the glandular portion of stomach there are numerous small lesions and 2 very large lesions.

in a large series of animals, described a linear inverse relationship between incidence of lesions and restraint volume(6). It was our experience that when restraint volume was decreased below a critical point, mortality rates rose sharply; furthermore, it was difficult to identify this critical point. The technique described here rarely results in death of the rat.

In comparison with simple physical restraint, this technique yields lesions that are more consistent not only in frequency of occurrence, but also in their type and location. One major problem encountered in assessing the results of prolonged simple physical restraint was that a large variety of lesions resulted, including petechiae, submucosal hemorrhages without mucosal loss, mucosal edema, mucosal erythema, and blebs and ulcer formation in the squamous portion of the stomach. The present procedure usually yields only one type of lesion, obviating the need for judgment decisions in scoring various types of lesions or in assessing their relative significance.

Brodie and Valitski reported an earlier effort to devise a method for rapid induction of stress ulcers in rats through the dual stress of cold and restraint(7). Using rats of different sex and strain, and with different experimental conditions, they found a high incidence of submucosal hemorrhages but no ulcers.

In using this technique the following precautions should insure that the results will be consistent and valid. In a series of experiments all rats employed should be of the same sex, weight, and strain and treated as similarly as possible before study. Owing to ill-defined variations, apparently related to differences in such factors as season(8), diurnal rhythms(9), and prior experience of the rats, control observations should be carried out on each day of experimentation.

Using this technique, while observing the precautions cited, we have found the design and interpretations of various studies on stress-induced ulceration are facilitated. For example, owing to the very short period required for the development of lesions, we have found this method applicable to the study of the effects of drugs with short durations of action; administration of multiple doses of such drugs, as was required in the simple physical restraint model, is no longer necessary.

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