

RESTRAINT ULCERS IN THE RAT

I. Influence on Ulcer Frequency of Fasting and of Environmental Temperature Associated with Immobilization of Varying Durations

L. Buchel and D. Gallaire

Translation of "Ulceres de contrainte chez le rat. I. Influence, sur la frequence des ulceres. Du jeune et de la temperature de l'environnement associes a des immobilisations de durees variables, Archives des Sciences Physiologiques, 1967, xxi, pp. 527-536.

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16. Abstract			
This article reports results of the production of experimental ulcers in rats. The conclusion indicates two experimental conditions which regularly provokes the appearance of gastric ulcers in a high percentage of rats. a) Two-and-a-half hour restraint, proceeded by a 24-hour fast; b) one-and-a-half hour restraint with lowering of the environmental temperature while fasting.			
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## RESTRAINT ULCERS IN THE RAT

### I. Influence on Ulcer Frequency of Fasting and of Environmental Temperature Associated With Immobilization of Varying Durations

L. Buchel and D. Gallaire\*

The etiology of gastric ulcers has preoccupied physicians /527\*\* for several years in addition to physiologists, pharmacologists and psychologists.

Production of experimental ulcers has allowed the development of studies designed to bring some light upon the pathogenesis of this disease; in addition, it has facilitated research on substances possessing protective properties capable of being used in therapeutics. As ulcerogenic agents, different chemical substances have been employed as well as mechanical procedures. These latter methods involve either a direct action within the gastrointestinal sphere [1, 32], or a single or repeated stress applied to the animal: cold, traumatic shock, muscular exercise, cutting the spinal cord, forced immobilization [29, 30, 31] electrical stimulation [28, 14].

Although the psychosomatic origin of gastric ulcers is still /528 discussed, the procedure of forced immobilization of the animals, involving a "psychotomimetic" factor seems to be of particular interest for the production of experimental ulcers. This procedure, which gives rise to gastric ulcers which are termed restraint ulcers, has been applied to several animal species: mouse, rat, guinea pig, hamster, rabbit, monkey [7], and cat [23]. The better results have been obtained with the rat and the mouse. However, the fragility of the gastric mucosa of the mouse makes

\*Pharmacology Institute Laboratory of Professor Jeanne LEVY, 15 rue de l'Ecole de Medicine, Paris (6th).

\*\* Numbers in margins indicate foreign pagination.

measurement of the lesions difficult, and so the rat has been considered to be the animal of choice.

In 1956, ROSSI, BONFILS et al [26] refined a technique for the regular production of gastric restraint ulcers in the rat. This technique, employed by numerous researchers [2;3, 4, 5, 6, 7, 13, 15, 19; 20, 23] consists of subjecting the rats to an immobilization varying from 7 to 25 hours, during which the number of afflicted animals progresses from 58 to 100 percent. However, a long period of restraint is inconvenient for the study of protective substances whose elimination may be more or less rapid. In this work, we set the experimental conditions so as to permit reduction to the minimum of the duration of restraint while including two supplementary stresses, i.e. fasting and cold.

## RESULTS

We used rats of the Wistar breed.

We will describe in succession the immobilization procedure utilized and the influence on ulcer frequency of age, sex, the source of animals, the duration of restraint, fasting before the restraint and of environmental temperature.

### 1. Immobilization

Different procedures for immobilization have been employed: flexible wire mesh enveloping the animal's body [3, 4, 5, 6, 26], plaster bandages [34], maintenance of the animal on its back by fixation of its paws [24], confinement in a plexiglass box [22].

Other physical stresses have been associated with the restraint: maintenance of the animals in a cold enclosure causing a hypothermia [9, 11]; immersing the animals in a water bath at 25°C [35]; fasting prior to the restraint [7]. Besides, a prolonged fast by itself can cause the appearance of gastric ulcers in the

rat [12, 17, 25].

On the other hand, restraint has also been applied to conditioned rats [27].

In our experiments, we use the immobilization procedure /529 recommended by ROSSI, BONFILS et al [3, 4, 5, 6, 26]. After ether anesthesia, just enough to transitorially reduce the rats' mobility, they are immobilized in a flexible wire gauze whose mesh is five millimeters square. The wire gauze is pierced with four orifices; corresponding to the location of the animals' paws whose dimensions (2 x 2 cm for the anterior paws, 2 x 2.5 for the posterior paws) are such that their rigid contour does not interfere with the circulation of blood. Each paw is introduced into the orifice up to the root of the limb and attached under the mesh to its contralateral homologue by means of adhesive tape. The wire gauze is finally pulled up over the animal's back and molded to the shape of its body. In this way a cylinder is produced whose diameter is adjusted to the size of the rat and whose length is fixed so that the head is completely covered but is not immobilized. Lateral movements of small amplitude remain possible; the tail is mobile and rests only by its base on the mesh. Each rat, equipped with its metallis corselet is suspended horizontally on a support, so that its paws cannot touch the ground; groups of 10 or 20 rats are placed on the same support in such a manner that they are not face to face; they are maintained at a constant ambient temperature. An appropriate duration of restraint, under these conditions, very regularly produces the appearance of gastric ulcers.

## 2. Examination of the lesions

At the end of the period of restraint, the animals are immediately sacrificed by decapitation; the sampled stomachs

are opened by an incision running the length of the greater curvature and examined after a light rinsing with distilled water.

Macroscopic examination. The juxta-esophageal portion or the rumen as well as the duodenum (\*) present no visible macroscopic lesions. All the lesions are located on the glandular portion of the preduodenal stomach, and essentially on the summit of the longitudinal gastric rugae, which cover the anterior and posterior surfaces of the stomach; they are often located symmetrically in relation to the lesser curvature.

We generally find ulcerations with clean edges, punched-out, whose base is covered with a layer of blackish blood, burrowing into the thickness of the stomach wall, without ever causing a perforation, surrounded by an edematous, whitish rim. Their shape depends on their size: they are either punctiform, round or oval, the size of a pin-head, or else they are composed/530 craters capable of reaching 5 to 10 mm in length and 2 in width. An ulceration is however not always so obvious; it can exist in the wall as only a bloody infiltrate, blackish resembling a scab, whose removal allows the ulceration to appear. This observation has already been made by BONFILS et al [3, 4]. In the exposition of the results, we include this type of lesions with the ulcerations. Sometimes, the mucosa is dark red, as if congested, and does not present ulcerations. These last two observations allow one to suppose that a vasomotor phenomenon exists which intervenes in the genesis of the restraint ulcers. But, as BONFILS et al [3, 4] and BRODIE et al [8] have already noted, there is nothing, beyond the capillary alterations observed microscopically, to confirm this pathogenesis.

(\*) One of us demonstrated further in experiments in vitro, that the isolated duodenum of rats having gastric ulcers shows a normal reactivity to epinephrine and to acetyl- -methylcholine [16].

The number of ulcers is variable. It is a function of the severity of the disease and is on an equal footing with the appearance of hemorrhages in the digestive tract. These are sometimes observed with no macroscopically visible ulcerative lesion and are coincident, in this case, with the congested appearance of the gastric mucosa.

Histological examination<sup>(\*)</sup>. The stomachs, opened the length of the greater curvature, are spread out and fixed in formalin; hematoxylin-and-eosin and van Gieson stains are used. This examination discloses the superficial character of the lesions observed.

Outside of the ulcerations, the mucosa and the sub-mucosa have a normal appearance.

Within the ulcers are seen great changes of structure particularly involving the sub-mucosa. The muscular layer is not affected. Above it the connective layer composed nearly exclusively of collagen fibers is greatly thickened. It contains dilated capillaries and a very discrete lymphocytic infiltrate, of a roughly triangular form at the muscular base, it sends forth at its summit prolongations which separate rows of mucosal cells and accompany dilated capillaries filled with red cells. These capillaries open in the bottom of the ulcer. At this level the superficial layer of the mucosa is altered, the cells have lost their mucous character, they are pressed against one another and are approaching necrosis. The base of the ulcer is covered with a brownish material which is only blood undergoing digestion.

(\*) We thank Miss M. Tissier, Technical Director of the National Laboratory of Public Health, who kindly performed the histological examinations and reported the results obtained to us.

Radiological examination. The contrast media employed are: barium sulfate, Lipiodol (\*\*), ultra-fluide, diluted or not, Vasurix 38 (\*\*\*) , pure or diluted. Better observation of the details of the gastric mucosa are obtained with Vasurix 38 diluted.

After gavage with a gastric sound with 2 ml of the contrast/531 medium, the rat is given a light ether anesthesia; the radiographs are made on the film without filters, from different views: right lateral, left lateral, decubitus, frontal and oblique.

Despite obtaining a very fine mucosal relief, it was not possible for us to objectivize the ulcerations. This is explained, on the one hand, by the small size and the superficial character of the ulcers, and on the other hand by the lack of penetration of the contrast medium into the ulcerated place which is filled up with a bloody infiltrate.

### 3. Reporting the results

The experiments were performed, most often, on groups of twenty rats. To express the results obtained, we make use of, in a parallel manner, two systems of gradations. The first, used by most authors, consists of noting the percentage of rats bearing ulcers. The second, takes into account the number of ulcers which each animal presents. This number, if it depends on the individual susceptability of the rats; as BONFILS et al [3, 4] observed, does not seem, in our experiments, to vary proportionately with the duration of the restraint. Following the example of LEVIS and BEERSAERTS [20], we adopt the following gradation [10]: 0 - normal stomach; 1 - one ulcer; 2 - 2 ulcers; 3 - 3 ulcers; 4 - 4 or more ulcers. For each group of

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(\*\*) Ethyl esters of fatty acids from iodinated dianthus oil, [see 38, p. 100]

(\*\*\*) Acetylamino-3-triiodo-2,4,6 benzoate of N-methyl glucamine, solution at 75 p. 100.



rats, we calculate the average index, the standard error of the average (standard deviation) and the probability. This mode of expression is also used by MARAZZI-UBERTI and TURBA [21] in their study on gastric ulcer provoked by histamine in the guinea pig.

4. Duration of restraint whether or not preceded by fasting

Restrictions of variable duration are applied to rats either fed normally just at the time of immobilization or deprived of food but not drink, before the application of restraint. The experiments are performed at an ambient temperature of  $22 \pm 1$  °C.

Restraint without prior fast. The frequency of ulcers obtained after restraints varying in duration from one to seven hours [10] is represented in figure 1.

No ulcerative lesion was observed after one hour of restraint.

When the duration of the restraint is increased from two and a half hours to five hours, the percentage of animals bearing ulcers remains at approximately 40%; it reaches 55% with an average index of 1.40 after a restraint of seven hours.

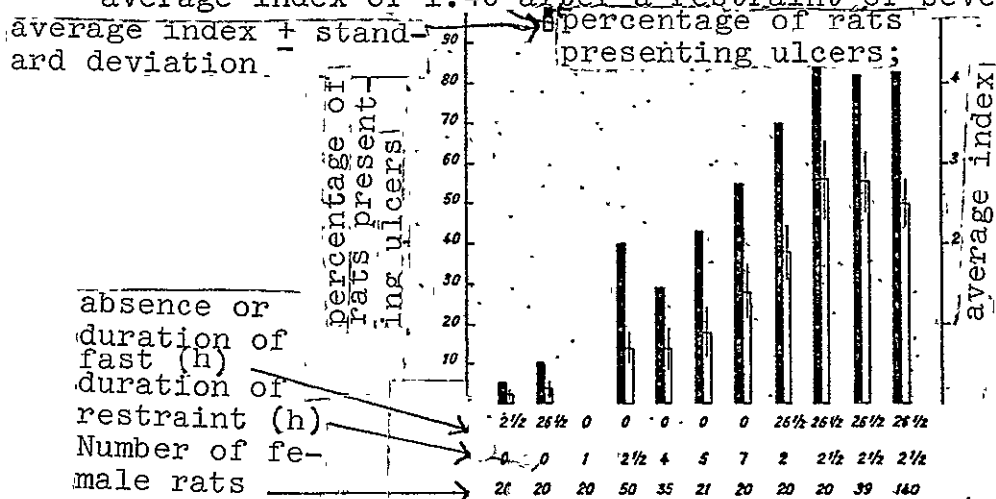


Figure 1. Influence of the production of ulcers of the duration of restraint, of hydrated fast and of their association.

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Restraint with prior hydrated fast. HANSON and BRODIE [18] subjected the rats to a prior fast of 48 hours; under these conditions, 69% of the animals presented gastric ulcerations after a four hour restraint.

In our experiments, the animals were submitted to a prior fast of twenty-four hours, during which they lived in the animal quarters in groups of 10 in cages equipped with wood chips, this fast being prolonged for the duration of the restraint. As seen in figure 1, in animals thus prepared, a restraint of two and a half hours regularly caused, in groups of 20, 39 and 140 rats ulcer formation in 82, 83 and 85% of the animals, the average index varying from 2.5 to 2.8; while the fast of twenty-six and a half hours (24 hr. of prior fast and 2 and a half hours of fast during the restraint) by itself alone only provoked ulcers in 10% of the animals.

5. Variations in results as a function of the individual state of the rats

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In the experimental conditions described above, that is to say while using a two and a half hour restraint preceded by a hydrated fast of twenty-four hours, we varied successively the age, the sex and the source of the rats. The results are shown in figure 2.

A significant deviation is noted, as much for the frequency of ulcers as for the average index, with two lots of rats belonging to the Wistar breed, but coming from two different breeders. In contrast, in each of these lots, the sex and age of the animals (50 and 90 days) changes neither the percentage of ulcerated stomachs nor the average index. Let us note that BRODIE and HANSON [7] observe in young rats a more elevated number of ulcers than in adult rats, but according to these authors the age factor intervenes very little, when the duration of restraint is less

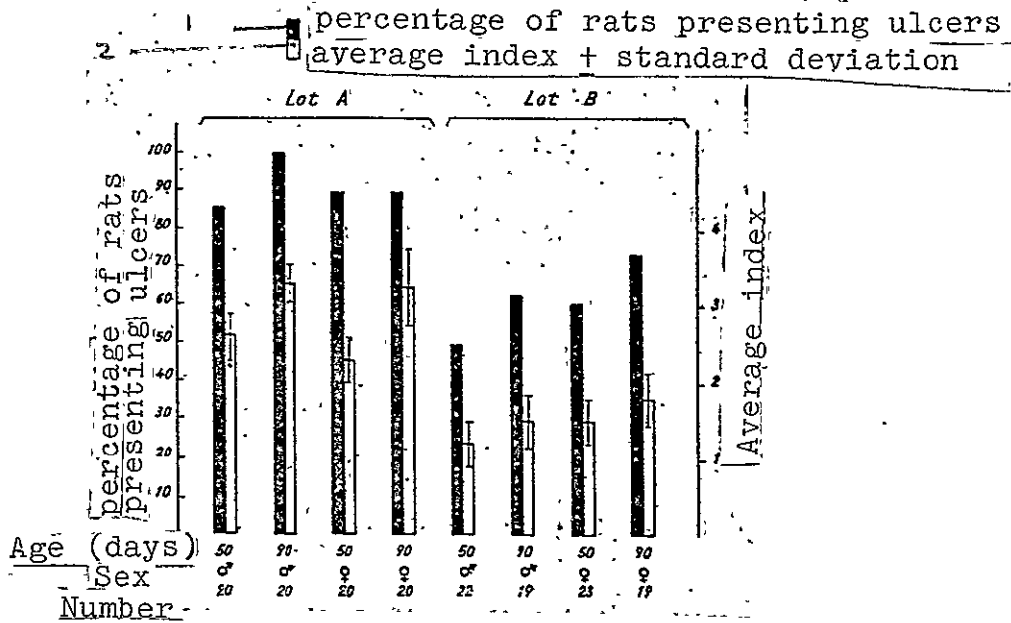


Figure 2. Influence on the production of ulcers of age, sex and source of the rats.

than twenty-four hours.

As a result of these facts and for reasons of technical convenience, we use on the one hand rats 50 days old, of a weight varying from 150 to 200 gm and, on the other, stock coming from the same breeding.

6. Variations in results as a function of the environmental temperature.

The ambient temperature at which the rats are maintained during the restraint exerts an influence on the frequency and the severity of the ulcers [11]. Control animals (groups of 20 rats) not subjected to restraint, do not develop ulcers.

We have represented in figure 3, the results obtained on rats having undergone a prior twenty-four hour fast, subjected to restraints of one and a half and two and a half hours, at different ambient temperatures, kept constant during the dura-

tion of each experiment, but varying from 14 to 32°C.

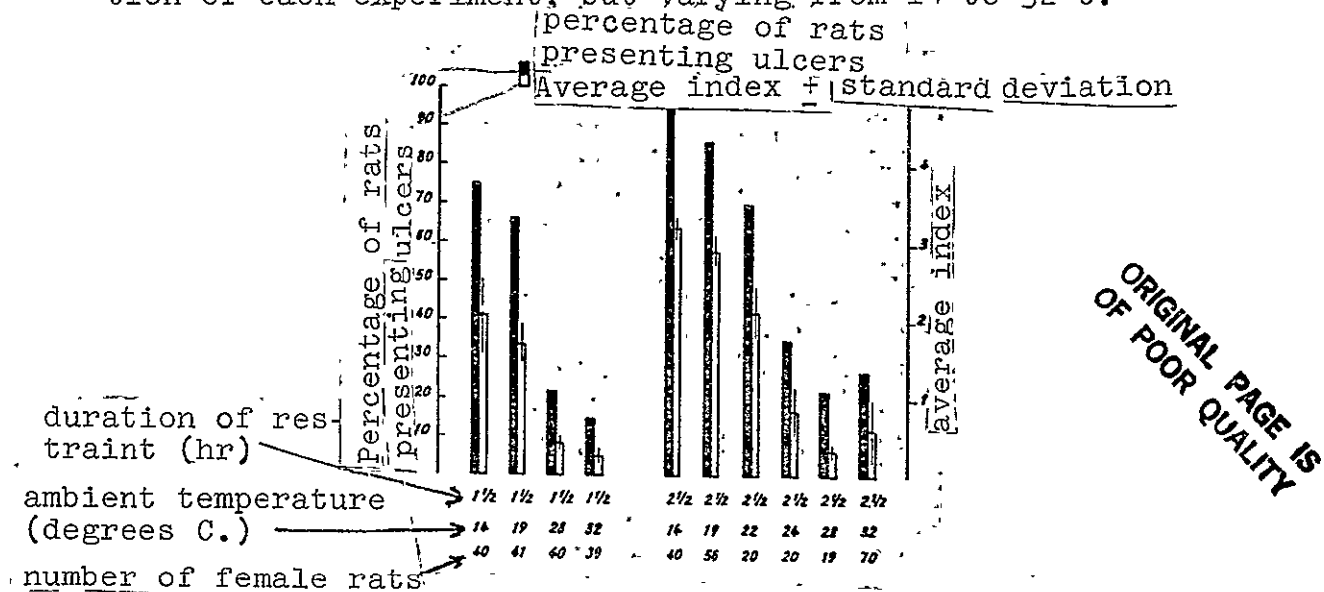


Figure 3. Influence on the production of ulcers of the duration of the restraint and of the environmental temperature in rats subjected to a prior 24 hour fast.

One and a half hour restraint exerts only a weak ulcerogenic action at ambient temperatures of 28 and 32°C., but become effective when the temperature drops; to 19°C. we obtain 66% of the animals developing ulcers; at 14°C., 75% of the animals are afflicted, with an average index of 2.05 ± 0.50.

Two and a half hour restraint gives parallel results. The percentage of animals bearing ulcers does not exceed 35% when the environmental temperature lies between 24 and 32°C. As soon as the temperature falls below 24°C., the frequency of ulcers increases and reaches 95% at 14°C., at the same time that the severity of the ulcers (average index) is augmented.

These experiments demonstrate the favorable action of cold on the frequency and severity of ulcerative lesions. In 1963, BRODIE and VALITSKI [9] also noted the aggravating role of cold when associated with a one hour restraint for the appearance of gastric hemorrhages in the rat.

## DISCUSSION

To reduce the duration of restraint to a minimum, so as to allow regular production in the rat of a high percentage of gastric ulcers, we took recourse to two supplementary stresses: fasting and cold.

A twenty-four hour fast preceding the restraint made the ulcer frequency rise from 40% (average index: 0.7) to 82-85% (average index: 2.5 to 2.8), the experiments being performed at an ambient temperature of  $22 \pm 1^{\circ}\text{C}$ .

Addition of cold to the restraint and the prior fast permitted reduction of the duration of restraint from two and a half hours at  $22^{\circ}\text{C}$ . to one and a half hours at  $14^{\circ}\text{C}$ , in order to obtain ulcers with a similar frequency (75%) and the same number (average index 2.05).

Association of restraint with fasting and cold, which causes a high percentage of gastric ulcers after immobilizations respectively of two and a half hours and of one and a half hours, allows study of protective substances after single administration, even if their elimination is rapid.

On the other hand, the experimental conditions in which the ulcer frequency is very low, notably a greater reduction in the duration of restraint, and an elevation of the environmental temperature, make possible examination of substances capable of exerting an ulcerogenic action, in the rat.

## CONCLUSIONS

1. Some experimental conditions are described in which an immobilization of short duration regularly provokes the appearance of gastric ulcers in a high percentage of rats: two and a half hour restraint, when it is preceded by a twenty-four fast;

one and a half hour restraint, when lowering of the environmental temperature is associated with fasting.

2. It is planned to utilize the techniques described for the study either of protective substances or of aggravating substances.

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