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ERRATUM

ROBERT E. STITZEL and JEANE S. MCCARTHY: Stress and strain—Factors influencing drug metabolism. *Biochem. Pharmac.* **20,** 2085 (1971).

It is regretted that the above short communication appeared without the figures. The complete version is published below.

SHORT COMMUNICATION

Stress and strain—Factors influencing drug metabolism*

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SEVERAL investigators have begun to examine the metabolic consequences of exposure to stress. Our laboratory,[1] using cold as a stress, found a decrease in the microsomal metabolism of hexobarbital. After 2·5 hr of a hindlimb ligation stress, Rupe *et al.*[2] and Driever and Bousquet[3] reported an apparent increase in hexobarbital metabolism. The different effect on hexobarbital metabolism produced by the two stress procedures suggests that the type of stress employed may be an important factor in determining the type of metabolic response elicited. The present study examines this hypothesis by comparing the effects of several types of stress on microsomal metabolism. The possibility that different strains will respond differently to stress has also been examined.

Female Long–Evans and Holtzman rats, 150–200 g, were used. Upon arrival the animals were placed in uncrowded cages for at least 5 days and were provided with food and tap water *ad lib.* All lighting, both for control and experimental animals, was maintained on a 12-hr on–off cycle. Rats were always killed between 8:00 and 8:30 a.m. Body weights of all stressed animals were not significantly different from nonstressed control.

Animals subjected to cold stress were housed individually in stainless steel cages placed in a ventilated cold room maintained at 4°. After removal from the cold stress, the animals were immediately decapitated, their livers removed and microsomes prepared. The time-sequence of placing the animals in the cold was such that rats stressed for either 1, 2 or 4 days were all killed on the same day. Control rats which had been maintained at 25° were always sacrificed with the experimental animals.

A swim stress consisted of placing the animals in a large (75 × 60 × 28 cm) Nalgene basin filled to a depth of 18 cm with water at 28° for 45 min. The depth was such that the animals had to swim to keep their nostrils above water. After removal from the basin the animals were gently dried and kept at 28° for 1 hr and then returned to the same relatively stress-free area as the controls. All animals were killed 24 hr after their last exposure to swim stress.

Animals subjected to a restriction of movement stress were placed individually in stainless steel cylinders which permitted virtually no movement in any direction. Animals were stressed for 6-hr periods for either 1, 2 or 4 successive days. Animals were killed approximately 18 hr after their removal from the last stress session.

After decapitation livers were rapidly excised, weighed and homogenized in ice-cold 1·15% KCl. Microsomes were prepared and incubations carried out by differential centrifugation as described previously.[4]

The pathways studied, methods of assay and the amount of substrate added were: side-chain oxidation of hexobarbital,[5] 3·0 μmoles, and aromatic hydroxylation of aniline,[6] 10 μmoles. Aliquots of the microsomal suspension were assayed for protein content by the method of Lowry *et al.*[7]

Holtzman rats showed an impairment in their ability to metabolize hexobarbital after repeated exposure to a swim stress (Fig. 1). After the second and fourth stress session, hexobarbital metabolism had decreased by approximately 40 per cent. Long–Evans rats, on the other hand, responded to the

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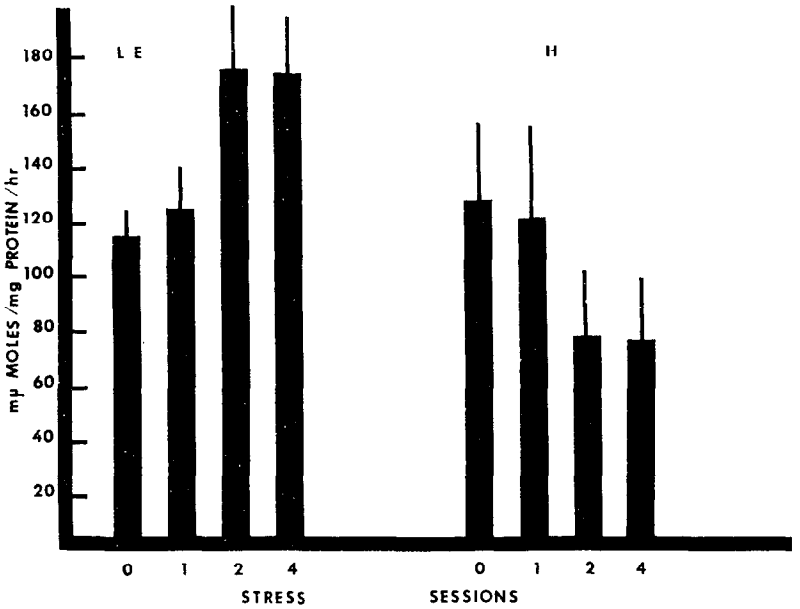

FIG. 1. Effects of swim stress on hexobarbital metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

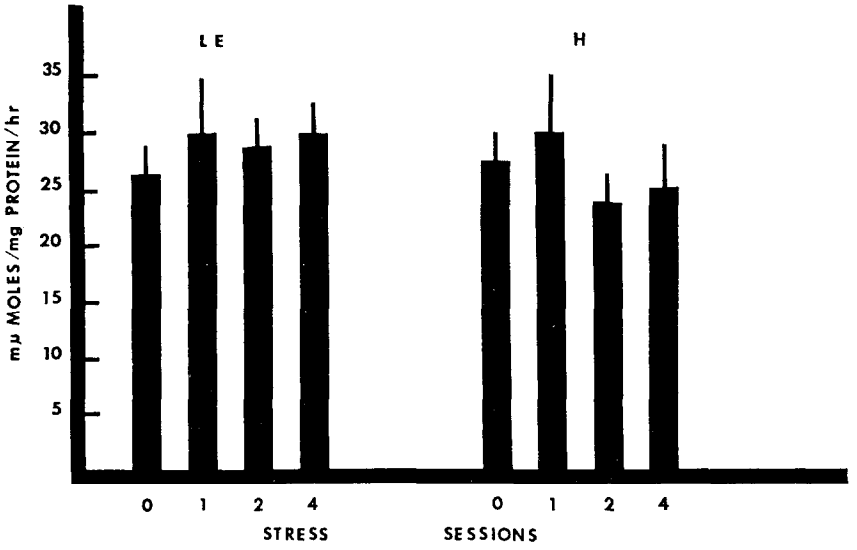

FIG. 2. Effects of swim stress on aniline metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

swim stress with increased hexobarbital metabolism (Fig. 1). The maximal effect of the stress was manifest after the second swim stress and remained elevated through the fourth stress period. The increase in rate of hexobarbital oxidation after 2 and 4 days of swim stress was approximately 52 per cent. Swim stress did not significantly alter the *p*-hydroxylation of aniline in either rat strain (Fig. 2).

Stress resulting from restriction of movement had little effect on the oxidation of hexobarbital in Long–Evans rats until the fourth day when a slight, but significant ($P < 0{\cdot}05$), increase in metabolism occurred (Fig. 3). This is in contrast to the results obtained with Holtzman rats where restrictions of movement resulted in a progressive reduction in the rate of barbiturate metabolism (Fig. 3). The extent of the decrease in hexobarbital oxidation appeared to be related to the number of days of exposure to stress. By the fourth day hexobarbital metabolism was reduced to approximately 36 per cent of control levels. The hydroxylation of aniline was unaffected by restraint stress in both rat strains (Fig. 4).

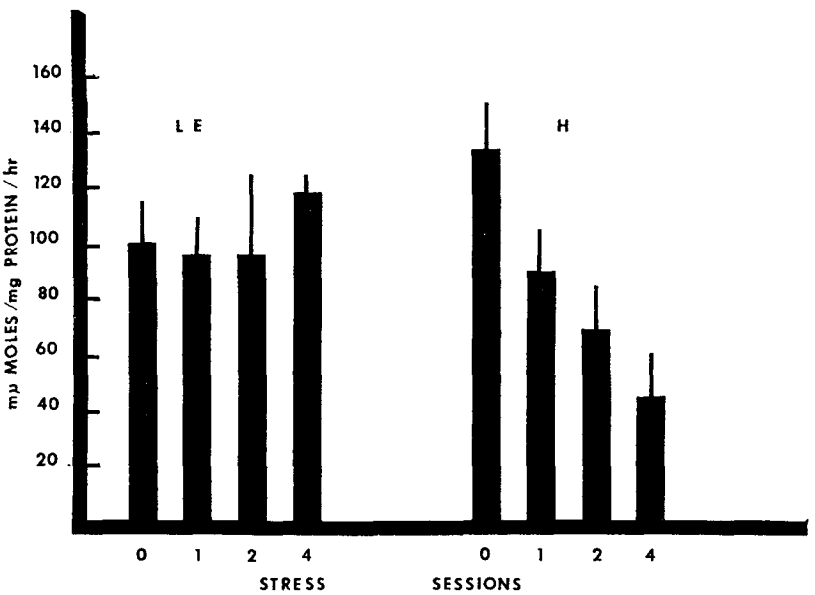

FIG. 3. Effect of restriction stress on hexobarbital metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

Exposure of animals to a cold environment for up to 4 continuous days resulted in an increase in hexobarbital metabolism in Long–Evans rats, the peak effect being reached after the fourth day of cold stress (Fig. 5). Hexobarbital metabolism was increased by about 66 per cent after 4 days of cold exposure. Holtzman rats, on the other hand, showed an initial stimulation of hexobarbital metabolism after 1 day of cold stress which was then followed by a decline in rate of barbiturate disappearance (Fig. 5).

Cold stress also elevated aniline hydroxylation in Long–Evans rats (Fig. 6). The magnitude of the stimulation increased with the duration of the cold exposure. The maximal increase observed was approximately 70 per cent. In Holtzman rats the rate of aniline hydroxylation was not significantly affected by cold stress even after 4 days of continuous exposure (Fig. 6).

Conflicting reports have appeared concerning the nature of the interaction between stress and drug metabolism. Reports of both stress-induced stimulation and depression of microsomal enzyme rates have been published. The demonstration by Rupe *et al.*[2] that rats subjected to unilateral hindlimb ligation show decreased barbiturate sleeping times suggests that stress increases barbiturate metabolism. On the other hand, Furner and Stitzel[1] exposed rats to a cold stress and found barbiturate metabolism *in vitro* to be depressed. The studies of Bousquet's group and ours differed in two important aspects: the type of stress employed and the strain of animals used. The present experiments tested these variables by measuring the rates of microsomal drug metabolism in rats of two different strains, Long–Evans and Holtzman, which had been exposed to three types of stress.

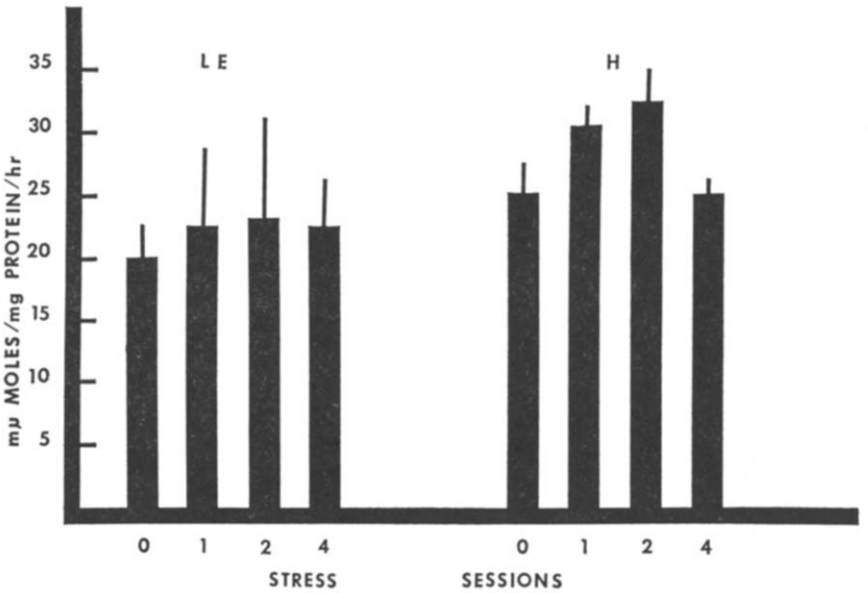

FIG. 4. Effects of restriction stress on aniline metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

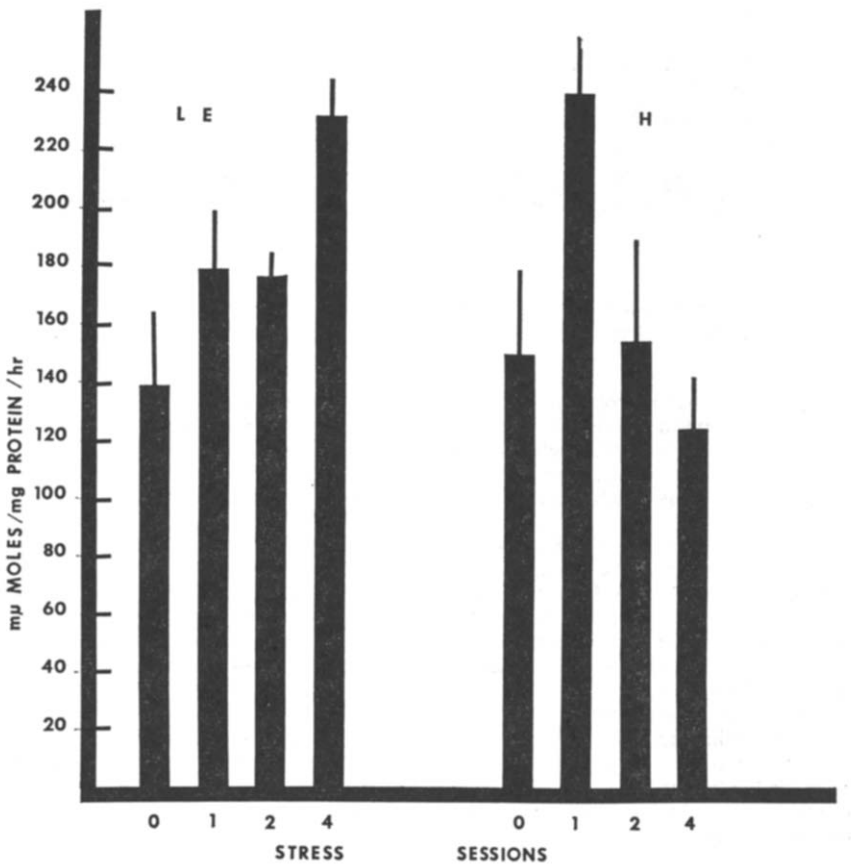

FIG. 5. Effect of cold stress on hexobarbital metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

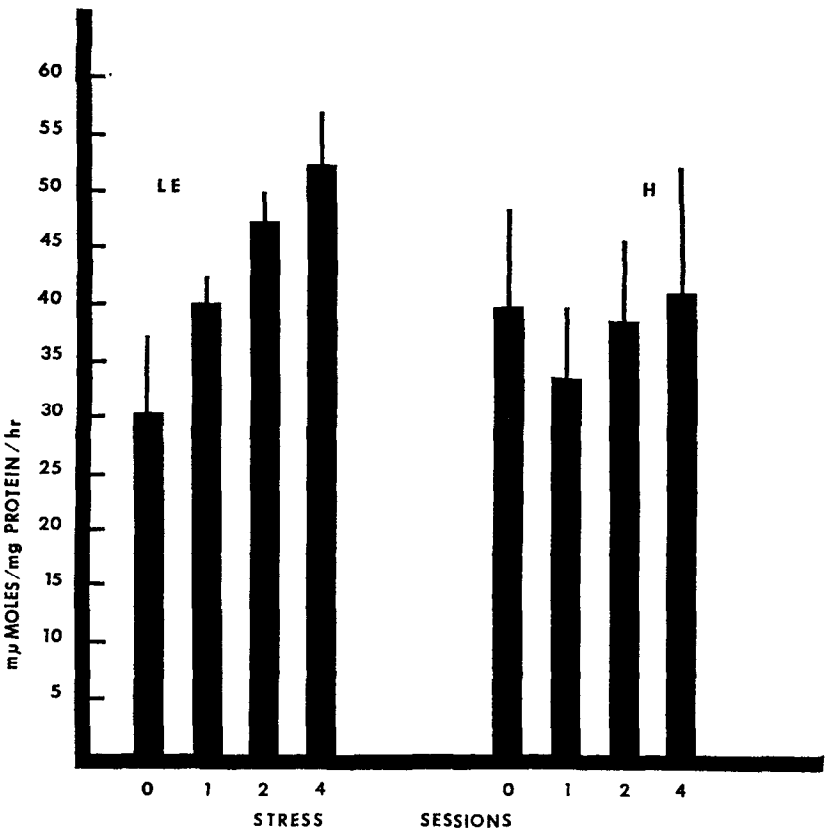

FIG. 6. Effects of cold stress on aniline metabolism. The bars represent the mean values ± S.E. obtained from five to seven animals. LE (Long–Evans), H (Holtzman).

Studies dealing with drug-caused induction of microsomal enzymes have shown that both the qualitative and quantitative nature of the microsomal response is dependent upon the strain of animal used.[9–11] It seems probable from the present studies that the microsomal response to stress is also genetically determined. Exposure of rats of the Long–Evans strain to a swim stress resulted in an elevation in their rate of hexobarbital oxidation, whereas Holtzman rats responded to the swim stress by a depression of hexobarbital metabolism. The present results, therefore, emphasize the importance of strain differences in microsomal metabolism in general, and in stress-produced induction of microsomal enzymes in particular. The choice of the rat strain used in an experiment may determine whether a particular enzyme pathway is stimulated or depressed by either drug treatment or exposure to stress.

Not all microsomal pathways are equally affected by stress. Although both Long–Evans and Holtzman rats showed changes in their rate of oxidation of hexobarbital after swim stress, aniline hydroxylation was unaffected. It may be that the time course of stress-produced alterations in microsomal activity is different for different enzymes, or perhaps a more prolonged exposure to this stress would eventually have affected aniline hydroxylation.

In addition to the particular animal strain used in stress studies, the type of stress employed also appears to affect the microsomal response. After a restriction of movement, stress Long–Evans rats showed no significant alteration in either hexobarbital or aniline metabolism while a cold stress in the same strain resulted in elevated rates of metabolism of both substrates. Holtzman animals were unaffected by the cold stress, but showed a decrease in hexobarbital metabolism after both the swim and restriction stress. When they responded to stress, Holtzman rats generally responded with decreased hexobarbital metabolism, whereas when Long–Evans rats responded they showed increases in barbiturate oxidation.

From the foregoing studies it seems that whether there is an effect on microsomal metabolism within a single rat strain depends upon the type of stress used, and the nature of that effect (i.e. increase or decrease) depends upon the animal strain used.

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