Studies in Stress Glycosuria

I. Prolonged Glycosuria in Chinese Hamsters after Repeated Stress

Otto F. Ehrentheil, M.D., Leo J. Reyna, Ph.D., George Yerganian, Ph.D., and Edith T. Chen, M.S., Boston

SUMMARY

Chinese hamsters, unfamiliar to each other, were briefly placed together daily for several weeks. This resulted in fighting and stress. The stress groups were arranged in such a way that no animal met the same animal within any five-day period. A gradual increase in the incidence of positive urine glucose occurred in the experimental animals but not in the nonfighting controls. Glycosuria continued for two to twelve weeks following the termination of the prolonged regimen of daily repeated stress periods. These findings are an extension and amplification of those reported by Cannon and his group. The results may have significance in exploring the relationship of emotional stress to diabetes mellitus.

The occurrence of glycosuria of a few hours' duration in cats following a brief episode of stress is well known, dating back to the reports of Böhm and Hoffmann[1] in 1877 and of Cannon and his group[2,3] in 1911. These investigators found glycosuria on the day of stress. The promptness with which glycosuria developed was directly related to the emotional state of the animal. The average time required to bring about glycosuria was less than an hour and a half. In no instance was glucose found in the urine passed on the day after the excitement. Similar experiences were repeatedly noted in humans, for example college students during examinations.[4] The above studies were, however, confined to the use of a single, brief, emotional episode or a relatively small number of them. Barnett, Eaton and McCallum[5] have subjected wild rats to "social stress" by letting them fight with animals of the same species. They observed low liver glycogen and high blood glucose after acute stress of five or twenty-four hours' duration and similar though less outspoken findings after low-intensity social stress of two weeks' or eight weeks' duration. . .

From the Psychiatry Research Laboratory, Veterans Administration Hospital, Boston, the Department of Medicine, Tufts University School of Medicine, and the Department of Experimental Psychology, Boston University, Boston, Massachusetts.

MATERIALS AND METHODS

The present study reports the results during and following a chronic stress regimen on urine glucose lasting several months. The experimental animal was the Chinese hamster, *Cricetulus griseus*, selected because of its tendency to develop hereditary spontaneous diabetes.[6,7] Two groups of fifteen adult hamsters, each matched as closely as possible for litter origin, age, sex, and weight, were randomly designated as control and stress groups. The animals were fed Purina Laboratory Chow and water ad libitum. The stress condition consisted of removing animals from their individual home cages and keeping three or four of them, each of a different litter origin, for several hours in a single cage measuring 7 inches wide, 10 inches deep and 7 inches high. These stress groups were arranged in such a way that no animal met the same animal within any five-day period. This procedure consistently led to fighting, particularly during the first half-hour after hamsters were placed together. The space in the cage was narrowed by a movable partition when only two or three animals were put together because it was observed that intensity of fighting was (within certain limits) inversely related to the available space. To prevent serious injuries, the incisor teeth of the upper and lower jaws of animals in the experimental groups were cut before the experiment started and recut twice weekly. This represented, of course, an additional stress but was limited to stress weeks. To reduce injuries further, animals were observed closely during the first hour and, particularly, when they were extremely pugnacious, at which time they were separated for a few moments.

The stress regimen began on July 2, 1962, and between that date and July 22 the urine of each animal was examined for glucose at five different times, using a paper enzyme test (Clinistix, Ames Laboratories). The advantages of this method were (1) that only a very small amount of urine is needed for this test, and (2) that it nevertheless compares favorably with, for example, the Benedict test in sensitivity and specificity.[8]

We have compared a few samples by using a microchemical method (Technicon AutoAnalyzer[9] and Clinistix) and found a satisfactory agreement. Later on urines were tested five times a week and the findings were recorded according to the Clinistix scale as negative, trace, or 0.25 or 0.5 (or more) per cent.

These examinations were done routinely each morning, and in the majority of instances seventeen to twenty hours after the previous day's stress. Tests for acetone were made only when the Clinistix indicated the presence of at least 0.5 per cent glucose. The weights of the animals were recorded weekly.

RESULTS

Both the control and experimental animals maintained a relatively constant weight throughout the study. A significant loss of weight occurred only in the animals which died. Acetone tests were, except for occasional traces, always negative.

The four main findings were: (1) Stressed animals showed positive urine glucose more frequently than the controls. (2) During the first four weeks the only evidence of experimental glycosuria occurred immediately following the daily stress period. (3) However, after six weeks of stressing, definite recurrent glycosuria was often found on the day following stress in every animal still alive. (4) Glycosuria persisted for several weeks after cessation of the stress. Figure 1 shows the findings of this experiment, which was continued for eight months. In this figure, as well as in figure 2, "positive glucose" refers only to those urine tests showing 0.25 per cent or more. The percentages indicated in the ordinate pertain to numbers of positive urine tests independent of numbers of animals. During the first four weeks neither the experimental nor the control animals showed glucose on the day following stress. During the fifth and sixth weeks the positive tests numbered less than 10 per cent, but rose to more than 20 per cent in the seventh week for the stressed group. The eighth week was a staff vacation and no animal was stressed. As soon as the stress was reinstated in the ninth week, the percentage of positive tests rose to 30 per cent. During the following three weeks stress was not induced either by fighting or by teeth cutting, yet the percentage of positive glucose tests remained constant for the first two weeks and then fell in the third week (the twelfth week of the experiment) to the control level. From the thirteenth to the seventeenth week, inclusively, the animals were stressed once again and the percentage of occurrence of positive glucose tests rose rapidly to approximately 50 per cent. After the seventeenth week the stress was terminated, but the urines continued to show positive tests for almost three additional months.

There were no fatalities in the control group; in the experimental group, however, five animals died in the fourth week, one each in the sixth, seventh, ninth and fourteenth weeks, and finally three animals died in the seventeenth week, so that at the end of seventeen weeks only three animals out of fifteen remained in the experimental group. All twelve animals which died showed a marked weight loss and the fur became scrubby during the last weeks prior to death. Four hamsters had been injured, but the majority was not. While glycosuria was found in the last week before death in all the eight nonwounded animals which died, none of these animals showed definite signs of diabetes. As the trials were then terminated, twelve of the control animals were used for other purposes and three litter mates of the three surviving animals continued to serve as controls. Glycosuria was never detected among these three control animals during the eight months of observation. Among the fifteen control animals, which comprised the original control group, two showed sporadic positive glucose tests (figure 1). Every stressed animal which survived the first six weeks of the experiment showed glycosuria at one time or another.

To check the outcome of this study another experiment was initiated on Nov. 5, 1962. Because of the high fatality rate in the initial stress groups, we started the second experiment with twenty-eight animals in the experimental group and ten animals in the control group. During the first four weeks of the second experiment, fighting animals were separated sooner than in the first experiment to prevent serious injuries. However, since the intensity of fighting decreased as time went on, it was necessary to introduce new animals to stress the experimental hamsters. The findings of this second experiment are shown in figure 2. The results are similar to those of the first experiment, though less striking. The occurrence of positive urine glucose in this group increased to only 32 per cent in the fifteenth and sixteenth week of the experiment, as compared to 50 per cent in the first experiment. The control animals failed to show glucose throughout the seven-month period of observation. While in the first experiment glycosuria was present for twelve weeks after the stress period, the second experiment resulted in glycosuria persisting for only two weeks after termination of stress. In this second experiment the stressed animals once again showed a high mortality. Fifteen of twenty-eight experimental and two of ten control animals died during the seven-month period. Three of the experimental hamsters were found dead in the morning after the room

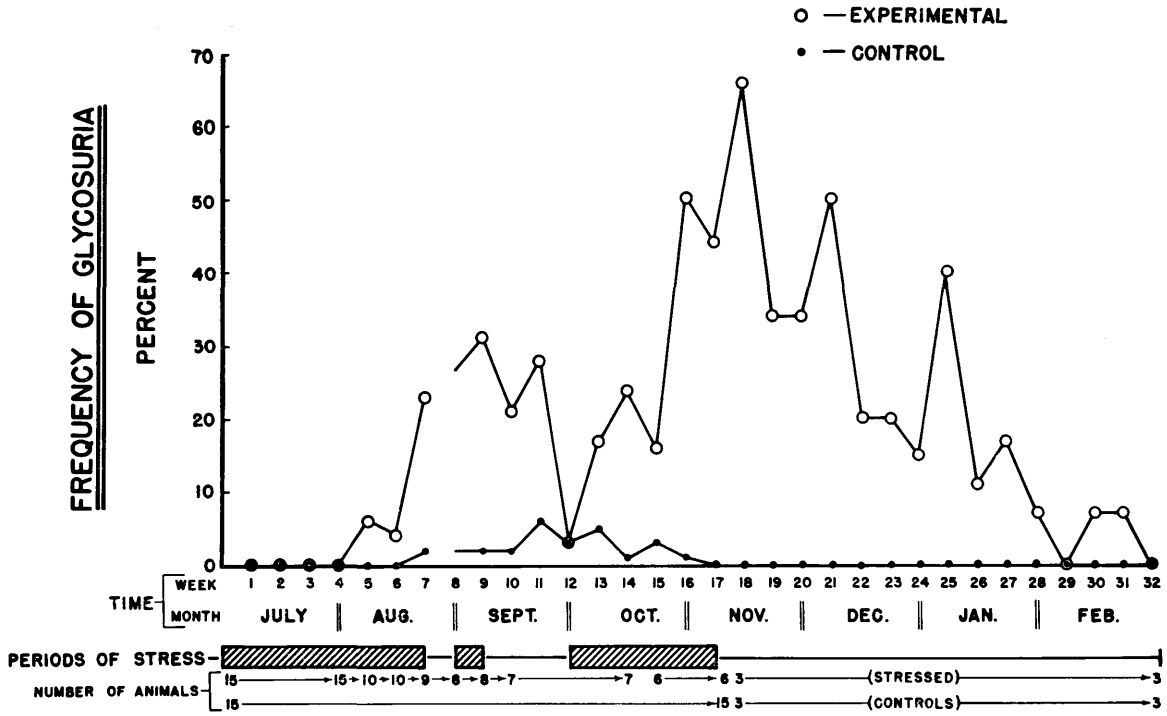

FIG. 1. The relationship of frequency of glycosuria to the presence and termination of stress in Chinese hamsters (results of study started July 2, 1962). The percentages in the ordinate pertain to numbers of positive urine tests independent of numbers of animals.

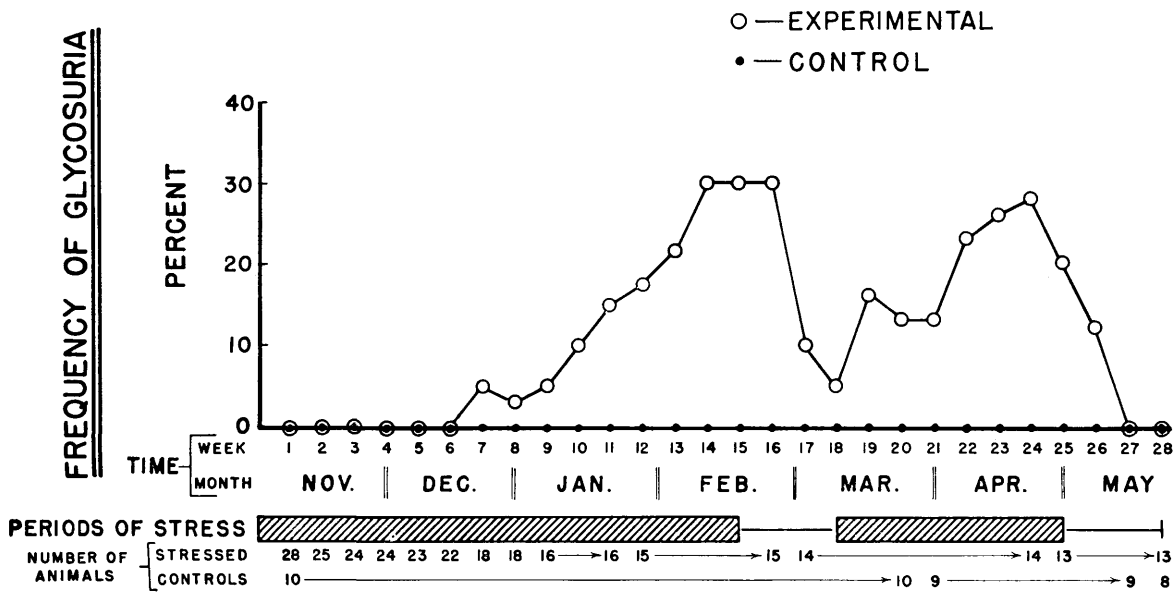

FIG. 2. The relationship of frequency of glycosuria to the presence and termination of stress in Chinese hamsters (results of study started Nov. 5, 1962, with a group of hamsters different from that shown in figure 1).

temperature, due to a heating system failure, had fallen to 53° F., but none of the controls died on that night. Of the other twelve animals which died in this experiment, eight were wounded, the others were not. Barnett has reported that when adult male wild rats come into conflict some die without being wounded. Death of obscure origin following stress has been reported in other mammals by various authors cited by Barnett. We may assume that death in the unwounded Chinese hamsters was similarly related to the stress situation.

DISCUSSION

The differences in the results of the two experiments are probably due to two factors: (1) The intensity of the stress was less in the second experiment, a fact which is independently documented also by the lower mortality in this second group (figure 2). (2) While the start of the first experiment was in early summer the second was begun in November. It is the experience of one of us (G.Y.) that the incidence of spontaneous diabetes in the Chinese hamster colony (in the Children's Cancer Research Foundation) is greatly decreased in winter.

This paper presents our first report of studies which are intended to examine by animal experiments the relation of stress (especially emotional) to carbohydrate metabolism, particularly the possible relationship to diabetes mellitus. Our findings are an extension and amplification of those reported by Böhm and Hoffmann[1] and by Cannon[2] and his group. In a separate study we examined the urines of Chinese hamsters twice daily, before and after the stress experience. In this species glycosuria was frequently found one hour after stress, a result similar to that found by Cannon in cats. However, the important difference in the results of the present study compared to the classical investigations of Cannon is the persistence of glycosuria for several weeks after cessation of a long-term repeated stress regimen.

The following two possible hypotheses, which are not mutually exclusive, may explain these findings. (1) The continuation of glycosuria for significant periods after cessation of fighting may have been due to the establishment of a conditioned reflex, and the later observed gradual disappearance of the glycosuria could be due to the gradual extinction of the conditioned reflex. The physiologic mechanism of the glycosuria could be consistent with a continued outpouring of adrenalin as suggested by Cannon. (2) Outpouring of corticoids is regarded by Selye[10] as an important part of the stress reaction. The continuation of glycosuria after the stress was discontinued could be due to the diabetogenic effect of these corticoids[11-13] in a species (Chinese hamsters) which has a known constitutional tendency to develop spontaneous diabetes mellitus.[7] However, none of the animals in the experimental or the control groups developed diabetes mellitus.

SUMMARIO IN INTERLINGUA

Studios in Glycosuria de Stress. Prolongate Glycosuria in Hamsters Chinese post Repetite Stresses

Hamsters chinese, non cognoscite le unes al alteres, esseva ponite insimul, breve- sed diurnemente e durante plure septimanas. Le gruppos esseva arrangiate de maniera que nulle animal unquam incontrava le mesme altere animal intra un periodo de cinque dies. Isto resultava in combattos e stress. Un augmento gradual occurreva in le incidentia de urinas positive pro glucosa inter le animales experimental sed non inter le non combattente animales de controlo. Glycosuria continuava durante inter duo e dece-duo septimanas post le termination del prolongate curso de diurnemente repetite periodos de stress. Iste constatationes es un extension e amplification de illos reportate per Cannon e su gruppo. Le resultatos es possibilemente de interesse in le exploration del relation inter stress emotional e diabete mellite.

ACKNOWLEDGMENT

This study was supported in the main by Grant A-5408 and, in part, by Grant A-4468 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and Grant G-21307 from the National Science Foundation. The authors wish to acknowledge the assistance of Mr. Henry J. Gagnon.

REFERENCES

[1] Böhm, R., and Hoffmann, F. A.: Beiträge zur Kenntnis des Kohlehydratstoffwechsels. Arch. exp. Path. u. Pharm. *8*: 271-308, 375-445, 1877/1878.

[2] Cannon, W. B., Shohl, A. T., and Wright, W. S.: Emotional glycosuria. Am. J. Physiol. *29*:280-87, 1911.

[3] Cannon, W. B.: Bodily Changes in Pain, Hunger, Fear and Rage. New York, Appleton, 1915, and idem, Newton, Mass., Branford, 1953.

[4] Folin, O., Denis, W., and Smillie, W. G.: Some observations on "emotional glycosuria" in man. J. Biol. Chem. *17*: 519-20, 1914.

[5] Barnett, S. A., Eaton, J. C., and McCallum, H. M.: Physiological effects of "social stress" in wild rats. II. Liver glycogen and blood glucose. J. Psychosom. Res. *4*:251-60, 1960.

[6] Yerganian, G.: The striped-back or Chinese hamster Cricetulus Griseus. J. Nat. Cancer Inst. *20*:705-27, 1958.

[7] Meier, H., and Yerganian, G.: Spontaneous hereditary diabetes mellitus in Chinese hamster (Cricetulus griseus). I. Pathological findings. Proc. Soc. Exp. Biol. Med. *100*:810-15, 1959.

[8] O'Sullivan, J. B., Kantor, N., and Wilkerson, H. L. G.: Comparative value of tests for urinary glucose. Diabetes *11*: 53-55, 1962.

[9] Hoffmann, W. S.: Rapid photoelectric method for determination of glucose in blood and urine. J. Biol. Chem. *120*:51-55, 1937.

[10] Selye, H.: A syndrome produced by diverse nocuous agents. Nature *138*:32, 1936.

[11] Ingle, D. J.: The production of glycosuria in the normal rat by means of 17-hydroxy-11-dehydrocorticosterone. Endocrinology *29*:649-52, 1941.

[12] Ingle, D. J., Sheppard, J., Evans, J. S., and Kuizenga, M. H.: A comparison of adrenal steroid diabetes and pancreatic diabetes in the rat. Endocrinology *37*:341-56, 1953.

[13] Buse, J., Gundersen, K., and Lukens, F. D. W.: Steroid diabetes in the cat. Diabetes *6*:428-32, 1957.