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Received December 12, 1955. P.S.E.B.M., 1956, v91.

Hyperglycemia and Increased Liver Glycogen in Rats After X-Irradiation. *† (22192)

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It is well established that whole-body x-irradiation of rats results in an increase in level of liver glycogen(1-4). On the other hand it has been reported that liver glycogen of rabbits is markedly reduced following x-irradiation(5). Although x-irradiation raises liver glycogen level of rats and decreases that of rabbits, blood sugar levels in both animals are increased by exposure to x-irradiation. Kohn(6) found that in rats irradiated with 600-900 r, plasma glucose level rises within 2 to 3 days and McKee(4) has found, on the first day after irradiation, an increase in blood sugar levels of rats exposed to 1500 r. Steadman and Grimaldi(7) observed a hyperglycemia in rabbits following x-irradiation. This hyperglycemia increased with increasing dose and at every dose (500 to 2000 r) reached a maximum 4 hours after irradiation, thereafter declining to normal levels within 24 hours. Hyperglycemia was completely prevented by insulin. Therefore, these authors postulated that irradiation causes a mobilization of liver glycogen into glucose and that the normal supply of insulin in the rabbit is not sufficient to prevent hyperglycemia. Since irradiation increases liver glycogen levels in the rat, the question arises as to the reason for hyperglycemia. Before an attempt was made to answer this question, it appeared that a detailed study of the relationship of liver glycogen and blood sugar levels to dose and time after irradiation would be of value. Data on these points are presented in this paper.

Methods. Animals. Sprague-Dawley female rats about 10 weeks of age were used. In each experiment, animals of uniform weight were divided into groups of either 4 or 5 rats each. *Radiation technics.* Radiation source was Westinghouse X-ray machine of 250 KVP and 15 ma, with filter of 0.5 mm copper and 1 mm aluminum. All doses were delivered at 24.5 per minute at a target-to-skin distance of 40 inches. During irradiation rats were restrained in acetate-plastic boxes and slowly rotated under the beam to insure uniform exposure(8). Non-irradiated, control animals were caged in the same manner for the same period of time. *Experimental design.* In Exp. 1 only liver glycogen was determined. In Exp. 2 both liver glycogen and blood sugar levels were determined, whereas in Exp. 3 only blood sugar levels were measured. Rats subjected to whole-body x-irradiation were fasted for 24 hours, exposed to single x-ray dose and the fast continued. Doses administered were 500,

* This work was supported, in part, by funds provided by the Bureau of Medicine and Surgery, U. S. Navy Department.

† The opinions or assertions contained herein are private ones of the authors and are not to be construed as official, or reflecting the views of the Navy Department.

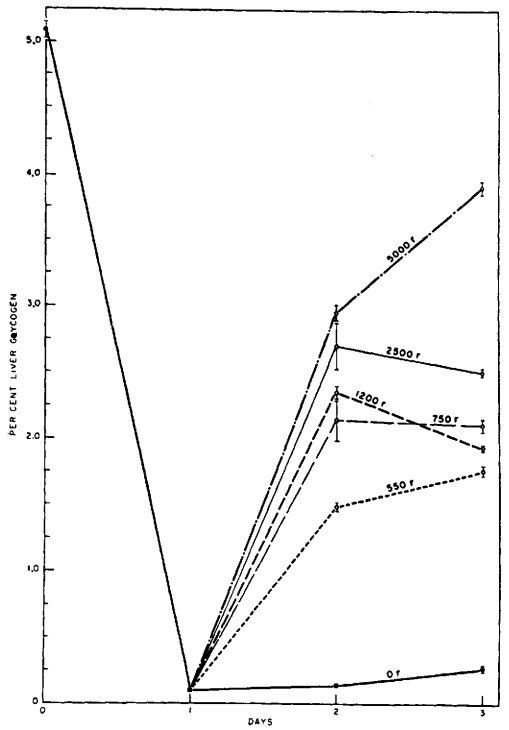

FIG. 1 (Exp. 1). Liver glycogen levels as a function of time in fasted, x-irradiated and fasted, normal rats. Fast was begun on day 0, and rats were exposed to x-radiations on day 1. There were 5 rats in each group of control animals and 4 rats in each group of fasted, x-irradiated animals. Means and stand. errors of means are plotted. Glycogen was determined by method of Good *et al.*(9).

750, 1200, 2500, and 5000 r. Eight rats were used at each dose level. Four rats from each group were sacrificed 24 and 48 hours after x-irradiation. Non-irradiated control animals were sacrificed in groups of 5 after 0, 24, 48, and 72 hours of fasting. *Analyses.* Rats were anesthetized with pentobarbital. Chest cavity was cut open and heart blood was withdrawn into a heparinized syringe. When liver glycogen was determined the liver was removed, blotted free of adhering blood and weighed. A liver sample of appropriate size was obtained, weighed and immediately analyzed for glycogen. In Exp. 1 glycogen was determined by the method of Good *et al.*(9). In subsequent experiments liver glycogen was determined by the method of Kemp(10) and blood sugar level by the method of Mendel *et al.* (11). The results are presented in Fig. 1 to 3.

Results. Liver glycogen levels. It is evident from Fig. 1 and 2 that fasting produces, within the first 24 hours, a sharp drop in percentage of liver glycogen. This drop is followed by a slight increase in liver glycogen during the next 48 hours. Whole-body x-irradiation, on the other hand, produces, in the fasting rat, a sharp rise in liver glycogen within 24 hours after time of exposure. The percentage of glycogen found in livers of irradiated rats increases with increasing dose. In each experiment the glycogen content of livers of rats exposed to 5000 r was considerably greater 48 hours after time of irradiation than at 24 hours. On the other hand, the percentage of glycogen found in livers of rats exposed to the other dose levels was about the same at both 24 and 48 hours after irradiation. At no time during the course of any experiment was the percentage of glycogen in the liver of the fasting, irradiated rat equal

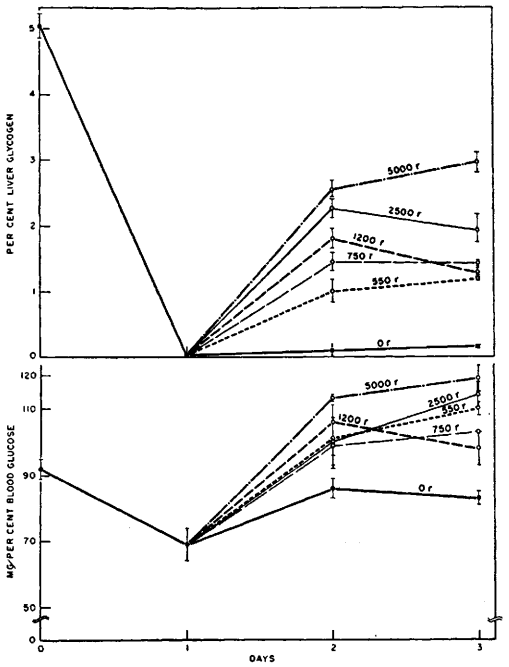

FIG. 2 (Exp. 2). Blood glucose and liver glycogen levels as a function of time in fasted, x-irradiated rats and normal control rats. Fast was begun on day 0 and rats were exposed to x-radiations on day 1. Five rats in each group of controls and 4 rats in each group of fasted, x-irradiated animals. Means and stand. errors of the means are plotted. Liver glycogen was determined by method of Kemp(10).

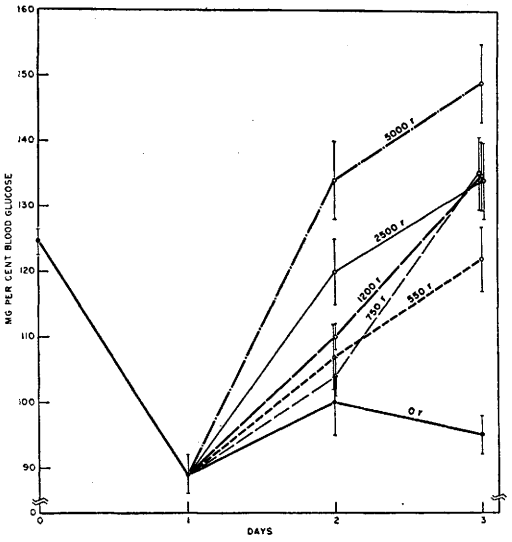

FIG. 3 (Exp. 3). Blood sugar levels as a function of time in control and fasted, x-irradiated rats. Fast was begun on day 0 and rats were exposed to x-radiations on day 1. Five rats were used in each group of controls and 4 rats in each group of fasted, x-irradiated animals. Means and stand. errors of means are plotted.

to or greater than that found in the normal, fed animal.

Blood sugar. From Fig. 2 and 3 it can be seen that during a 72-hour fast the blood sugar level drops by about 20% during the first 24 hours, rises slightly during the next 24 hours and remains at this new level over the final 24 hours. On the other hand, within 24 hours after exposure to x-irradiation the blood sugar level of fasting, x-irradiated rat increases and, at every dose (with the exception of 1200 r in Exp. 2), it continues to increase during the next 24 hours. Forty-eight hours after exposure to x-irradiation, concentration of sugar in blood of irradiated rats exceeds that found in normal, fed rats. There appears to be no clear relationship between the x-ray dose and blood sugar level. However, in each experiment the highest blood sugar levels, at both 24 and 48 hours after irradiation, are found in rats exposed to the highest dose (5000 r).

Discussion. The data presented indicates that exposure of fasting rats to whole-body x-irradiation results in an increase in liver glycogen and blood sugar levels. If the increased glycogen levels are a result of increased gluconeogenesis, then it is obvious that x-irradiation must cause appropriate glucose precursors to be made available. That glucose precursors are made available from mobilized protein is indicated by the finding of increased urinary excretion of urea in fasted x-irradiated rat(12). However, it appears that the availability of glucose precursors is not the only factor that determines amount of glycogen found in the liver following irradiation. This is indicated by the fact that an increased dose of x-irradiation over the range of 550 to 5000 r results in increased liver glycogen, while daily excretion of urea, the major end product of protein metabolism, is not altered by increasing the dose over the range of 500 to 2500 r(12). Furthermore, since it has been found that hypophysectomy prevents deposition of glycogen in the liver of irradiated rat(3), it seems that stimulation of the pituitary-adrenal system is necessary for the conversion of glucose precursors to liver glycogen. Since glycogen deposition increases with increasing dose, whereas availability of glucose precursors apparently does not, it seems possible that, once glucose precursors are available, the amount of glycogen deposited in the liver is determined by strength of stimulus delivered to the pituitary-adrenal system.

The fact that the fasted, x-irradiated rat is hyperglycemic clearly shows that x-irradiation does not, as was concluded by McKee(4), merely prevent the fall of blood sugar due to fasting. The hyperglycemia of the x-irradiated rat does not appear to be necessarily related to the accumulation of glycogen in the liver. This is indicated by the fact that, at all doses, blood sugar level of irradiated rat continues to rise 48 hours after irradiation, whereas (with the exception of the rats exposed to 5000 r) maximum levels of liver glycogen are obtained within 24 hours.

Summary. 1. Whole-body x-irradiation increases the percentage of liver glycogen in the fasting rat. At 24 hours after time of irradiation glycogen deposition was shown to increase with an increase in dose. 2. In rats exposed to 5000 r the percentage of liver glycogen was greater 48 hours after the time of irradia-

tion than at 24 hours. In animals exposed to 550, 750, 1200 or 2500 r the percentage of liver glycogen was not greatly different at 48 hours and 24 hours after the time of irradiation. In no case did the liver glycogen levels in the irradiated rats equal or exceed that found in normal, fed rats. 3. Whole-body x-irradiation increased the blood sugar levels in fasting rats. Larger increases were noted at 48 hours after the time of irradiation than at 24 hours. At 48 hours after the time of irradiation the blood sugar level was, in every case, greater than that of fed, normal rats.

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Received December 19, 1955. P.S.E.B.M., 1956, v91.

A Winter Isolation of Western Equine Encephalitis Virus from Hibernating *Culex tarsalis* Coquillett. (22193)

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The means of survival of Western equine encephalomyelitis virus (WEE) during the winter months has been a perplexing question since the disease was first recognized. Several explanations have been proposed for reintroduction or reappearance of the virus every year in certain endemic areas. The 3 most common theories are: 1. Migrating birds bring the virus northward each spring. 2. Arthropods, other than mosquitoes, harbor the virus during winter and infect susceptible birds during the most active stages of the arthropod life cycle. 3. Infected mosquitoes hibernate over the winter and infect susceptible birds and mammals during the following spring. It is the purpose here to present our findings and discuss the last possibility. As pointed out by Eklund(1,2) the conditions under which a mosquito-vertebrate cycle fails to account for maintenance of virus must be better understood before alternative mechanisms can be explored intelligently.

During the summer months WEE virus has been recovered from *Culex tarsalis* Coquillett frequently and from other mosquitoes occasionally in endemic areas(3-8). Hibernating *C. tarsalis* have not previously been reported in such numbers, and few of these have been tested for the presence of WEE virus. Although winter isolations of WEE virus from this species have been made in California during November and January(9) it is felt that these mosquitoes did not represent true hibernators. We consider the following factors to be indicative of true hibernation: the presence of fat bodies, lack of feeding activity of mosquitoes, absence of male mosquitoes, quiescence of female mosquitoes, and temperatures prohibitive for mosquito survival outside of the hibernating sites.

Methods. Mosquito hibernation sites. C. tarsalis mosquitoes were found hibernating in abandoned mines during winter months. These mines were in the foothills in Boulder and Larimer Counties, Colo., between elevations of 5000 and 8000 feet above sea level. The temperature from Dec. 22 through Feb. 20, was between 12.2°-13.3°C in a mine near