curred after Rose Bengal and ultraviolet light. The 5 and 15 mg doses, however, did not completely inhibit the effect. In general, vit. B_{12} in a dose from 5 to 35 μg almost completely prevented the increase of urinary coproporphyrin, while the combination of folic acid and B_{12} exhibited no special advantage.

Nicotinamide has a significant effect in reducing the coproporphyrinuria (type III) caused by the photodynamic action of ultraviolet light. Nevertheless, it is evident that the increases are only partially prevented.

Summary and Conclusions. The administration of large amounts of folic acid inhibited the increase of the urinary coproporphyrin in rabbits given Rose Bengal and exposed to ultraviolet light. The administration of small amounts of vit. B_{12} had the same effect. The combination of folic acid and vit. B_{12} had no advantage over either substance given separately. The administration of nicotinamide in large amount produced a significant but only partial inhibition.

In view of the fact that nicotinamide, folic acid, and vit. B_{12} are all intimately related to cellular metabolism, it seems highly likely that the marked increases of urinary coproporphyrin under these experimental conditions are likewise so related, but whether to the metabolism of the bone marrow, or some other system of cells is not clear. Further studies of these relationships are in progress.

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Effect of Total Body X-Irradiation on Glutathione Levels in Rats.* (18914)

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Recently the theory has been advanced that a relationship exists between x-irradiation effects and sulfhydryl groups. Barron(1) reported that irradiation decreases the metabolism of substances requiring sulfhydryl enzymes and other investigators that changes in glutathione levels affect the sensitivity of animals to irradiation,(2,3). Therefore, it appeared desirable to investigate glutathione levels following irradiation.

Method. Male and female Sprague-Dawley rats weighing from 170 to 275 g were used. Reduced glutathione in blood and liver was determined(4). Total glutathione was measured by treating the filtrates with H_2S at pH 8, acidifying, removing H_2S with CO_2, and determining total reduced glutathione. The difference between the glutathione before and after H_2S reduction was considered to represent oxidized glutathione. Known solutions gave good recoveries. Hematocrit values were obtained using 0.01 cc of blood(5). Somogyi's method was used to determine blood glucose(6). Reduced glutathione values on 0.2 cc of whole blood were determined one week before irradiation. Analyses one week apart showed no significant variation. No glutathione was found in rat plasma before or after irradiation. Red blood cell glutathione concentrations were calculated by

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dividing the whole blood glutathione value by the hematocrit and multiplying by 100.

Duplicate determinations were done on 160 mg or less of liver (light nembutal anesthesia). No significant change was found when control biopsies were obtained at 2 week intervals. Eight series of 10 rats each were irradiated. Initial liver biopsies were taken in series 4, 5, and 6. Two weeks after biopsy initial whole blood glutathione determinations were made, and the rats were irradiated a week later. Only one biopsy was taken following irradiation; no blood glutathione values were determined after biopsy. The animals were subjected to 500 r total body irradiation in a round plywood box holding 10 rats in separate V-shaped compartments. Radiation factors were 200 KV., 15 ma., 0.5 mm Cu. + 1 mm Al. filters, 80 cm target distance, 1.28 mm Cu. half value layer, 24 r per minute dose rate.

Results and Discussion. The average initial reduced glutathione value in the blood of 88 control rats was 44 ± 5.0† mg %. The average initial calculated red blood cell glutathione was 104 ± 11 mg %. In 20 rats no significant deviation from initial values in blood glutathione levels were found 10 minutes to 24 hours post-irradiation. Thirteen rats picked at random 48 to 72 hours after x-irradiation showed a rise in blood glutathione values (t = 3.5). The average hematocrit value in these animals after irradiation was identical with that of the control animals. The calculated red blood cell glutathione value rose as high as 177 mg %. Since a relationship between adrenal glands and glutathione metabolism has been indicated(3,7,8) it is possible that this increase in glutathione is due to adrenal cortical activity. Rats confined in the irradiation box for the same length of time as irradiated animals showed no change in glutathione levels (n = 18, t = 0.02) or hematocrit values

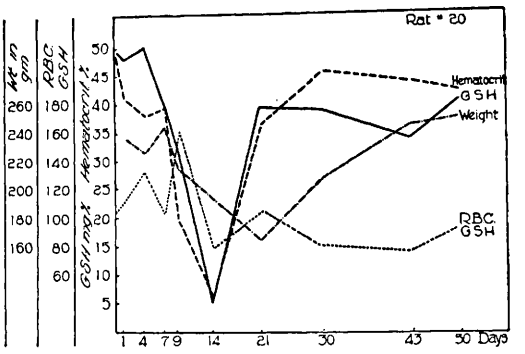

Figure I

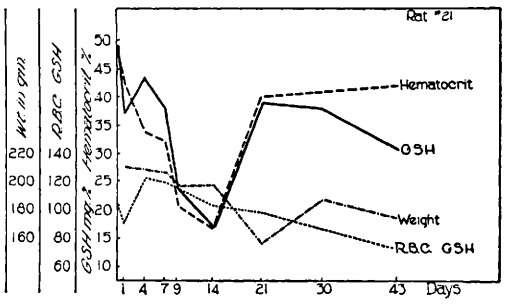

Figure II

FIG. 1 and 2. Showing the effect in rats of 500 r irradiation on hematocrit levels, blood glutathione values, calculated red blood cell glutathione values and weights.

(n = 17, t = 0.04).

Between 6 and 19 days post-irradiation, 37 of 45 rats showed reduced glutathione levels in blood more than twice the standard deviation below the control mean and 29 more than 3 times the standard deviation below the control mean. The blood glutathione values of 11 animals fell to 10 mg % or lower. However, even this low glutathione level was not necessarily fatal (Fig. 1). Our data are compatible with those of Schacter and Fishler(9).

Hematocrit values ordinarily decreased post-irradiation, at times irregularly in relation to blood glutathione. As blood glutathione fell, the RBC glutathione often rose above 142 mg % (more than 3 x the standard deviation above the control mean), but

† Standard deviation.

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in some instances fell below 70 mg % (more than 3 x the standard deviation below the control mean). Some values continued to remain significantly low during recovery.

Fig. 1 and 2 are representative of 6 animals followed for over a month. Rat 21 was sacrificed on the 43rd day, at which time the liver glutathione concentration was significantly low (85 mg %).

Total glutathione levels were done on 3 rats with blood glutathione concentrations below 10 mg %. Only a small amount of oxidized glutathione was found, an average of 5 mg % for blood and 6 mg % for liver. Therefore, the low reduced glutathione values were not due to an increase in oxidized glutathione.

Initial values on 40 rats for reduced glutathione in liver averaged 184 ± 19 mg %. No change occurred before the 6th day following irradiation. Nine low liver glutathiones (47 to 116 mg %) were found in a total of 23 rats biopsied between 6 to 19 days post-irradiation. Reduced glutathione levels in the blood of these rats were 10 mg % or less.

Inanition was considered as a possible factor in this experiment(10). Forty-eight hours following irradiation a typical series of 10 animals (average weight 183 g) had lost 12 g. However, by the seventh day the average weight had returned to 185 g. Body weights then slowly decreased again. Nine rats with significantly low liver glutathiones (47 to 116 mg %) lost the same amount of weight as 7 rats with no change in liver glutathione values (162 to 222 mg %). At biopsy the stomach was palpated and food particles could be felt. When rats succumbed at biopsy food was always found in the stomach and small intestine. Six rats were starved until their average weight loss was 47 g; yet no decrease was found in liver reduced glutathione values (162 to 196 mg %). Paired feeding experiments were considered useless without determining intestinal absorption(11). However, 9 rats with blood glutathione concentrations from 9 to 20 mg % maintained their average blood glucose level at 120 mg %. Thirteen irradiated animals starved for 24 hours had an average blood glucose level of 76 mg %. Therefore, it was concluded that inanition does not explain the decrease in glutathione levels in liver.

Glutathione may function in the tissues to maintain enzymes in their active SH form. Therefore, it is reasonable to assume that low glutathione levels may interfere with metabolic processes.

Summary. A study has been made of blood and liver glutathione after 500 r total body x-irradiation. The data show first, that irradiation generally causes a decrease in blood glutathione; and second, in some cases, a decrease in liver glutathione.

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Crystal Structures in Dried Smears of Gastric Mucus.* (18915)

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In the course of our studies on acid-free canine mucus, we have examined a large number of specimens microscopically. Air-dried smears were stained with toluidine blue, by a technic previously described(1)†; Wright's stain was also used occasionally.

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