that the agar cup method for determining lysis was neither very accurate nor very consistent for the quantitation of lysozyme, nevertheless, it was applied to the albumins which did not lend themselves to turbidimetric assays.

Petri dishes containing 15 ml of nutrient agar were seeded with 4 ml of agar containing *Micrococcus lysodeikticus* cells. Nine-millimeter cups were formed in the agar and filled with the turtle egg albumin. For comparison, lysozyme dilutions were also prepared in the same manner. After 14 hours of incubation, the zones of inhibition surrounding the cups were measured. These were indicative of the bacteriostatic nature of the turtle egg albumin. Diffusion of some component, presumably lysozyme, in the turtle egg albumin prevented the growth of *M. lysodeikticus*. To further determine the bacteriolytic effect of this material upon the test species, another series of plates was prepared. These were seeded with ultraviolet, light-killed *M. lysodeikticus* cells and cups made as before. The amount of cells added was sufficient to impart a definite turbidity to the agar. After incubation, the zones surrounding the cups were measured. The clearing of the cells adjacent to the cup indicated that the material in the turtle egg albumin was lysozyme because the lysis of the dead cells is currently considered as a specific enzymatic reaction between lysozyme and its aminopolysaccharide substrate. Using this method, it was possible to demonstrate that undiluted turtle egg albumin caused a 20 mm zone of inhibition and a 17 mm zone of lysis. A 1:10,000 dilution of crystalline lysozyme caused a 20 mm zone of inhibition and a 14 mm zone of lysis. In this manner lysozyme was demonstrated in turtle egg albumin.

These results indicate the distribution and quantitative variation of this enzyme in egg albumins. There is variation between species of birds in the lysozyme content of their eggs.

Received January 30, 1951. P.S.E.B.M., 1951, v76.

Relation of Clearance and Distribution of Injected Glutathione to Protection Against Radiation Injury.* (18521)

E. P. CRONKITE,† W. H. CHAPMAN,† AND GEORGE BRECHER.

From the Naval Medical Research Institute and the National Institutes of Health, Bethesda.

In a series of studies on the effect of glutathione (reduced) on radiation injury it has been shown that this sulfhydryl tripeptide will protect significantly when administered before irradiation(1,2), that the radiation dosage mortality curve is significantly shifted to the right above an LD_{20}(3), and that there is histologic evidence of more rapid regeneration of the hematopoietic organs in the glutathione treated mice(4). With the publication of evidence by Forrsberg(5) that bacteria, skin, and hair follicles could be protected by the local action of cysteine, the question naturally arose as to whether glutathione protection of the irradiated animal arose from selective concentration of reduced glutathione in radiosensitive tissues essential to survival. Accordingly studies on the clear-

* Opinions and assertions are those of the authors and are not to be construed as reflecting the policies or opinions of the Navy Department at large.

† Naval Medical Research Institute, Bethesda, Md., From Report No. 2, Project NM 006 012.05, dated June 27, 1950.

1. Cronkite, E. P., and Chapman, W. H., *Fed. Proc.*, 1950, v9, 329.

2. Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Chambers, F. W., Jr., and Cronkite, E. P., *Radiology*, December, 1950.

3. Chapman, W. H., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 318.

4. Cronkite, E. P., Brecher, G., and Chapman, W. H., PROC. SOC. EXP. BIOL. AND MED., in press.

5. Forssberg, A., *Acta Radiol.*, 1950, v33, 296.

ance rate and distribution of injected reduced glutathione were performed.

Materials and methods. All solutions of reduced glutathione and cysteine hydrochloride were prepared as a 10% solution in sterile distilled water, and the pH was adjusted to 6.5 with 10% sodium hydroxide. Blood clearance studies were performed on mongrel dogs. These dogs were injected intravenously through 18 gauge needles into a vein of the foreleg. The injection time was maintained constant at 15 seconds. Blood was withdrawn at stated intervals after injection and the levels of reduced glutathione in the blood were determined by the iodometric method of Woodward and Fry(6). White Swiss male mice, weighing 22-28 g, were used in studying the disappearance time of injected reduced glutathione from the whole body of the mouse. The glutathione was administered in single subcutaneous injections (5 mg/g mouse) with a 24 gauge needle into the backs of the mice. For this study the whole body was weighed and then ground with 100 ml of distilled water for 4 minutes in a standard Waring blendor. Powdered, solid CO_2 was used to maintain the temperature at approximately 2°C. Protein-free filtrates of the tissue homogenates were then made as prescribed by the method of Woodward and Fry(6). With this method, 90-95% of added reduced glutathione could be recovered from the tissue homogenates. For the tissue distribution studies, white Swiss male mice were injected subcutaneously and Sprague-Dawley rats were injected intravenously with 5 mg/g of animal. These determinations were made by cutting the organ into fragments which were placed in a micro Waring blendor containing dry ice and 50 ml of distilled water. The temperature was maintained at approximately 2°C with powdered dry ice while the tissues were being macerated. Protein-free filtrates were then made according to the method of Woodward and Fry(6). Total glutathione levels were not made because the published methods do not yield consistent results(7,8).

Results. The level of reduced glutathione in the blood of 4 dogs was determined daily under fasting conditions for one week prior to the determination of the clearance rate of intravenously injected reduced glutathione. The mean concentration of reduced glutathione in the whole blood of the 4 dogs was 35.7 ± 7.2 mg/100 ml of blood. The mean blood glutathione of each of the four dogs respectively, was 36.0, 36.1, 33.5, 34.7 mg/100 ml of blood. In Fig. 1 is plotted the rate at which intravenously injected reduced glutathione disappears from the blood of dogs. The first point on each curve is calculated by assuming a uniform distribution of the intravenously injected glutathione in a blood volume equal to 7% of the body weight.

Following the injections of glutathione and cysteine hydrochloride at pH 6.5 in the stated doses, systemic reactions occurred. Respiration became more rapid, the pulse accelerated, and the dogs became excited. The symptoms subsided within five minutes. From Fig. 1 it is apparent that the non-protein reduced glutathione and cysteine levels had returned to the normal range within 30 minutes after injection.

The disappearance of subcutaneously injected glutathione from the body of the whole mouse was studied as follows: 5 mg of reduced glutathione per gram of mouse was injected subcutaneously. This amount occasionally killed a normal mouse. Many of the mice became cyanotic and shivered for 10-15 minutes after injection. The total amount of reduced glutathione remaining in the body of the mouse was determined at various time intervals after injection. In Fig. 2 is seen the rate at which the reduced glutathione disappeared from the body of the mouse. Each point on the curve is based on the average content of glutathione in the bodies of three mice sacrificed at stated times. The first point in Fig. 2 is the calculated value based on the amount of glutathione injected plus the mean glutathione level of the body of normal mice. The whole body gluta-

6. Woodward, G., and Fry, E. G., *J.B.C.*, 1932, v97, 465.

7. Ennor, A. H., and Anderson, C. M., *Australian J. Exp. Biol. and Med.*, 1941, v19, 69.

8. Cronkite, E. P., personal unpublished observations.

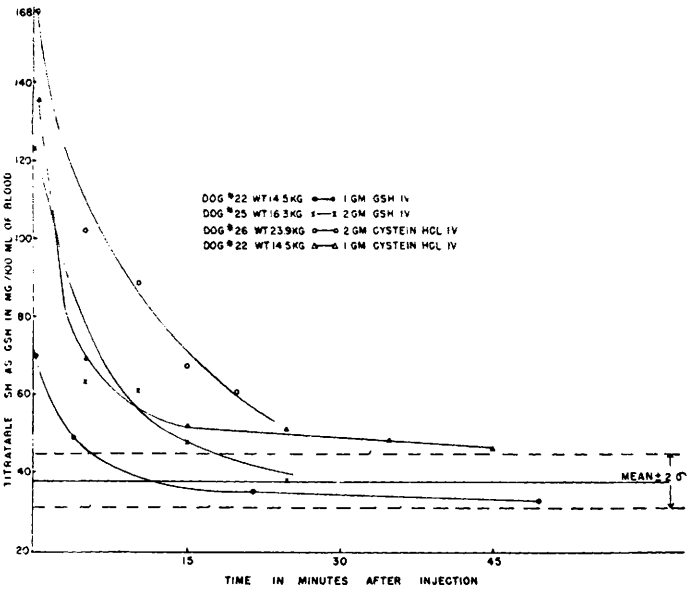

FIG. 1.

Blood levels of non-protein sulfhydryl as reduced glutathione (GSH) in mg/100 ml of whole blood following injection of both glutathione (pH 6.5) and cysteine (pH 6.5) intravenously in dogs.

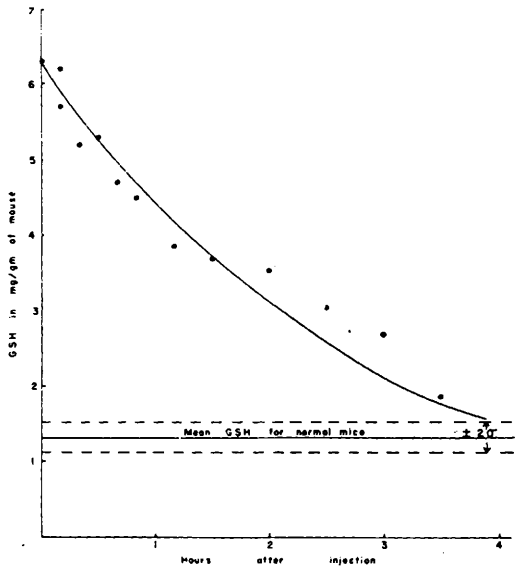

FIG. 2.

Disappearance rate of reduced glutathione (GSH) from the body of the mouse after subcutaneous injection 5 mg/g of mouse. Each point represents the average of three mice.

thione content remains two times above the normal levels for mice over two hours after a single subcutaneous injection of 5 mg per gram of mouse. It took approximately 3 hours for the reduced glutathione content of the whole body of the mouse to return to the normal range.

Following the establishment of the general period of time that the content of reduced glutathione in the bodies was elevated, the distribution of reduced glutathione in various tissues of mice and rats was determined. In mice, the concentration of reduced glutathione in the blood, liver, and spleen was determined 45 minutes after the injection of 5 mg of glutathione per gram of mouse. In rats, the distribution of glutathione in the blood, liver, spleen, testicles, small intestine, thymus, muscle, and kidney was determined 30 minutes after the intravenous injection of 5 mg/g of rat. The concentrations compared to normal concentrations in these species are tabulated in Table I. The mean value of the organs for 6 normal animals and 6 injected animals is compared in this table.

The concentration of reduced glutathione in the liver, spleen and kidneys was definitely increased. The concentration in the thymus and the small intestine was questionably increased. The measurement of glutathione in the bone marrow of the rat was attempted, but satisfactory preparations were not achieved and the great variability in the re-

TABLE I. Distribution of Injected Reduced Glutathione in the Tissue of Mice and Rats. Each value is the mean of 6 animals in mg/100 g of tissue.

Mice	Controls	45′ after 5 mg per g of mouse subcut.
Blood	39	44
Liver	344	435
Spleen	275	322
Rats		30′ after inj. 5 mg/g i.v.
Blood	41	48
Liver	338	476
Spleen	288	376
Testicles	293	292
Small intestine	278	295
Thymus	276	312
Muscle	56	63
Kidneys	205	310

sults prevented the accurate determination of differences in concentration of injected and control animals.

Discussion. The distribution and disappearance of reduced glutathione after intravenous and intraperitoneal injections has been previously reported by Braier(9). The results reported herein are in good agreement with his initial work. Both intravenously and subcutaneously injected glutathione tend to concentrate in specific tissues (liver, spleen, and kidney), rather than to be uniformly distributed throughout all tissues. In previous studies reporting the protective effect of glutathione(1-3) the period of irradiation coincided with the period of elevated glutathione concentration in the body at large, and these specific organs.

Adequate concentration of sulfhydryl compounds have been shown to protect unicellular organisms and locally irradiated skin and hair follicles(5). These findings, the ability of the animals whose spleen has been shielded to survive lethal doses of X-ray(10), and the high concentration of glutathione in the spleen, thymus and liver constitute an impressive sequence of events suggesting that glutathione may protect against whole body radiation by protection of some cellular or humoral element in the spleen, thymus, or liver which in turn can accelerate regeneration of the bone marrow which is essential for survival in the dose range where the sequelae of pancytopenia are the main causes of death. Unfortunately glutathione levels in the bone marrow are not available, but should these be high it would suggest that glutathione might protect the hemopoietic system by concentrating at the sites of hemopoiesis (bone marrow in the dog; spleen and bone marrow in the mouse).

Indirect influences cannot be excluded because glutathione has been shown to have other properties that might indirectly influence recovery from various stresses. For example, it has been shown that glutathione discharges the adrenal ascorbic acid(11) and will protect against potassium intoxication (12).

Summary and conclusions. 1. The clearance of intravenously injected reduced glutathione from the blood of dogs is rapid (< 30 min.). 2. The disappearance of subcutaneously injected glutathione from the body of the mouse is much slower (> 3 hours). 3. The distribution of injected reduced glutathione is not uniform throughout the body. Of the organs studied it concentrates to a considerable extent in the liver, spleen, and kidneys.

4. These experiments suggest that concentration of glutathione at sites vital for survival may be the prime factor in glutathione protection of the irradiated mammal. Whether the protection arises from preventing radiation death of essential cells or whether the protection is mediated through protection of humoral factors essential to orderly hemopoiesis remains unanswered.

9. Braier, B., *La Semana Med.* (Argentine), 1941, v48, 1473.

10. Jacobson, L. O., Marks, E. K., Gaston, E., Robson, M., and Zirkle, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 740.

11. Carey, M. M., Vollmer, E. P., and Spence, D. L., in press, Naval Medical Research Institute, Bethesda 14, Md.

12. Zwemer, R., Vollmer, E. P., and Carey, M. M., *Am. J. Physiol.*, in press, 1951.

Received February 1, 1951. P.S.E.B.M., 1951, v76.