TABLE II. Showing a "Box-titration" of Antigen in Graded Dilutions Tested with Serum in Graded Dilutions to Determine Titer of Antigen.

MEF1 antigen (dilutions)	Serum: Cotton rat, in dilutions (reciprocal)								Serum: Mouse, in dilutions (reciprocal)							
	2	4	8	16	32	64	128	256	2	4	8	16	32	64	128	256
Undiluted	4	4	4	4	3-4	3	±	0	4	4	4	4	4	3	±	0
½	3	3	3	2-3	2	2	2	±	2	1	1	1	1	±	0	0
¼	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
⅛	1	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normal brain, undil.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

included in each test. It is emphasized that all sera included in each test were tested against 1 or 2 control antigens. In addition, 2 different lots of MEF1 antigen have been prepared and 2 different groups of mice were immunized. The test could be repeated 2 and 3 times with the different sets of materials. Table I shows the results of such tests.

With the exception of sera that were anticomplementary, the specificity of the reaction was manifested. Furthermore, in a test with the Brunhilde and Leon types of hyperimmune monkey serum, it appeared that the result was type specific, *i.e.*, MEF1 antigen only fixed complement in the presence of the Lansing-type antibody. Additional tests with convalescent sera, to be reported, will concern the possibility of cross-reactions between the serological types.

A titration in which serial dilutions of serum were tested against serial dilutions of antigen is shown in Table II.

Table II indicates that the antigen as prepared has a low titer, that is, undiluted antigen reacted with mouse serum, and 1:2 dilution with cotton-rat serum. Incomplete reactions were observed in higher dilutions. Methods for increasing this titer are now being investigated.

Summary. The results of experiments here reported show that the MEF1, a Lansing type of poliomyelitis virus, can be adapted to infant mice in which the virus develops an LD_{50} titer in excess of that found, as a rule, in adult mice. In the medium of newborn mouse brain, and in such an increased titer, the virus now lends itself to the preparation of a suitable antigen for specific and reproducible complement fixation.

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Further Studies of the Beneficial Effect of Glutathione on X-irradiated Mice.* (18185)

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The beneficial effect of glutathione and cysteine on the course of radiation illness has been previously reported(1-3). Glutathione

* The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department.

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injected subcutaneously 5-30 minutes prior to irradiation improves the survival rate and decreases the weight loss in X-irradiated mice (1). Cysteine has a similar effect in rats and mice when administered either intravenously or orally before irradiation(2,3). In addition, Smith, *et al.*(4), have shown that the protective action of cysteine is related to the dosages of cysteine and X-radiation. The present report consists of a series of experiments in which glutathione-injected and non-treated mice were irradiated and compared with respect to X-ray dosage-mortality, weight change, and the effect of controlled trauma on the surviving irradiated animals.

Methods. A total of 2343 male, inbred, Swiss Albino mice was used in a series of 4 experiments. Mice were obtained at weaning from the Institute's stock colony, housed in individual cages, and irradiated when they weighed 20-25 g. The methods for housing and irradiating mice in individual cages have been reported(5). Irradiation consisted of a single total-body exposure to the radial beam of a 2 Mev. GE Industrial X-ray unit (6). The radiation factors were: 2000 KVP, 1.5 ma; no added filter; intensity of the radial beam: 15.0 ($\pm$0.15) r/minute in air at 2 meters; HVL 4.3 mm Pb. Lethal dose curves were determined simultaneously in each experiment by initially placing all animals of the experiment in the radial beam of the 2 Mev. unit, and then removing cages of animals as they received their prescribed dose of X-rays(5).

In the first 3 experiments the following doses of X-ray were employed: 550, 650, 750, 850, 950, and 1050 r (air). There were at least 36 mice for each dose of X-ray in each experiment, of which 12 or more received 1.6 mg glutathione per g of mouse in a single subcutaneous injection before irradiation, 12 received an injection of normal saline equal in volume to the glutathione injections, and 12 were non-treated. For each of these experiments there were also 60 non-irradiated controls, 20 of which received glutathione injections, 20 received saline injections, and 20 were non-treated. In the fourth experiment the following doses of X-ray were employed: 550, 650, 720, 775, 850, 950, 1050, and 1150 r. At each dose of X-ray, at least 24 mice received 4.0 mg glutathione per g of mouse in a single subcutaneous injection before irradiation; 12 mice received a corresponding volume of normal saline; 12 mice received no treatment. In addition, there were 96 non-irradiated controls; 24 received glutathione injections, 24 received saline injections, and 24 were non-treated.

A 10% solution of glutathione was used in each of the 4 experiments. Glutathione was dissolved in distilled water and adjusted to pH 6.5 with 10% sodium hydroxide. The control saline solution was adjusted to the same pH. All injections were given 5-30 minutes before irradiation in the backs of the animals, with tuberculin syringes and 22 gauge needles. Mortality rates were obtained during the 28-day post-irradiation observation period of each experiment. All mice were weighed individually each day. The change in weight of the survivors from each dose of X-ray was calculated daily and expressed in per cent of their pre-irradiation weight.

For the study of the influence of trauma, the following procedure was employed: On the 29th day of each experiment, all surviving animals were subjected to the same degree of trauma by use of a Noble-Collip drum(7), which was operated at 42.5 rpm for 8 minutes (3 mice per drum). The immediate and 24-hour mortalities were recorded. The 24-hour mortality data of an additional 891 male mice, similarly traumatized, are presented with the data from the survivors of these 4 experiments. These mice were in 3 groups: (a) 512 were non-treated and non-irradiated,

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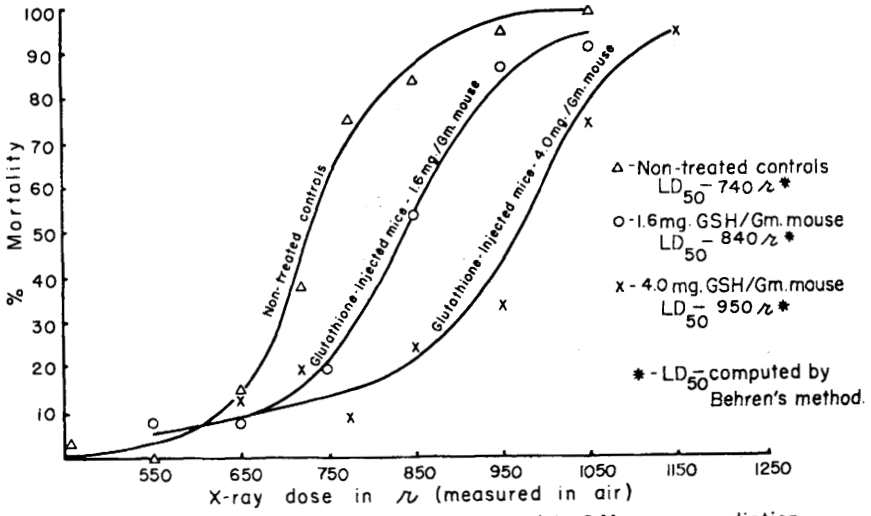

Figure 1:- Effect of Glutathione on mice exposed to 2 Mev x-ray radiation (single subcutaneous injection of Glutathione administered before irradiation.)

(b) 128 were non-treated and had survived 28 days following 800 r. and (c) 251 had received 1.6 mg of glutathione per g of mouse before irradiation and had survived 28 days following 800 r.

Results. Mortality data. The X-ray dosage-mortality curves for glutathione-injected and non-treated mice are presented in Fig. 1. The mortality data of all non-treated irradiated controls were combined, and the 28 day LD_{50}‡ for these mice was found to be 740 r. The mortality for the saline-injected control animals was not significantly different from that of the non-treated controls, consequently these data are not included in Fig. 1. The 28 day LD_{50} for the mice that received 1.6 mg glutathione per g of mouse was 840 r (experiments 1, 2, 3), and for the mice that received 4.0 mg glutathione per g of mouse, the 28 day LD_{50} was 950 r (Exp. 4). In these experiments 2.9% of 68 non-treated mice survived 950 r, while with 1.6 mg glutathione per g of mouse, 12.5% of 48 mice survived 950 r and with 4.0 mg glutathione per g of mouse, 65.6% of 30 mice survived 950 r.

It is of interest that with 4.0 mg glutathione

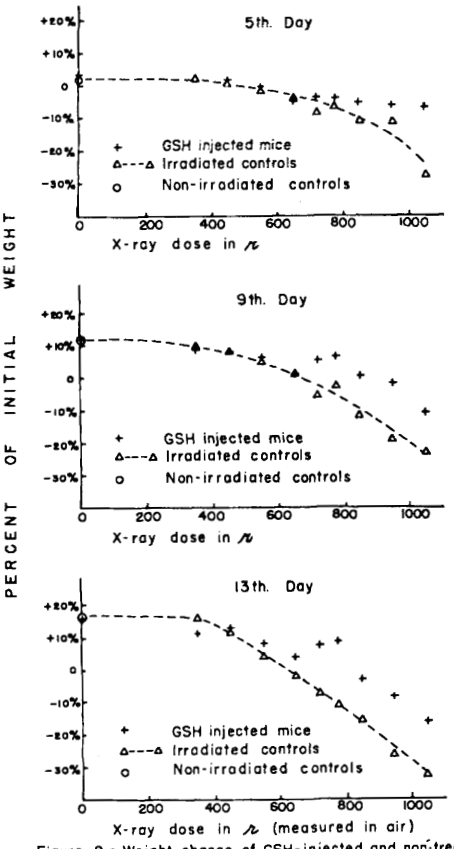

Figure 2.- Weight change of GSH-injected and non-treated mice surviving on 5th., 9th., and 13th. days, following single exposure to total body x-irradiation. (Pre-irradiation injection of Glutathione (4.0 mg./Gm. mouse)

‡ Behren's Method(8)

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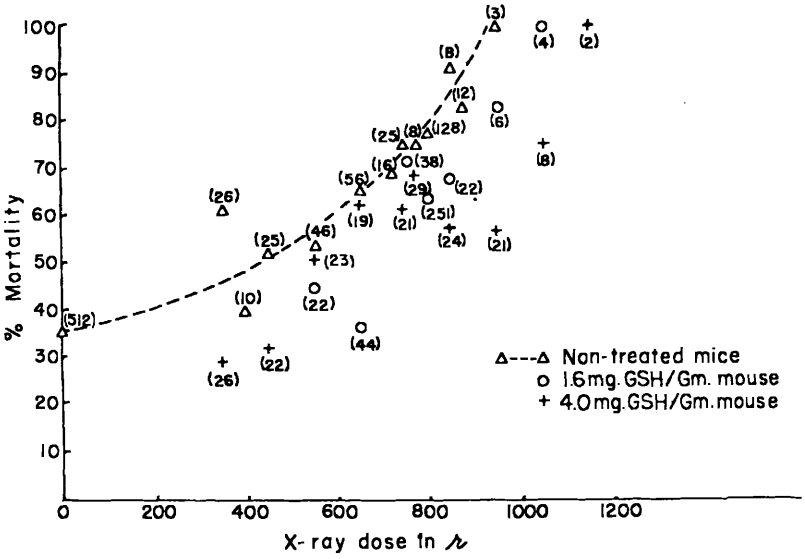

Figure 3.- 24 hour mortality produced by controlled trauma in GSH-injected and non-treated mice that survived 28 days following various doses of total body x-irradiation. The number of animals at each point is indicated in parentheses.

per g of mouse there were a few deaths within 3 hours after completion of the irradiation but, despite these early deaths, this larger dose of glutathione provided a greater over-all protection from radiation. It is also of interest that in the low lethal dose range (450-650 r), glutathione protection was not evident in these experiments. There were no deaths in the non-irradiated control animals of Exp. 1, 2, and 3. In Exp. 4, in the glutathione - injected non - irradiated control group, there was one death 24 hours after the glutathione injection.

Weight change. While weight change data were obtained daily for all animals, only those concerning Exp. 4 are presented in Fig. 2 as being representative of the entire group of experiments. The weight changes are expressed in per cent of the pre-irradiation weight of the surviving animals. It is apparent on the fifth day that with doses of X-ray above 800 r the glutathione-injected mice lost less weight; on the ninth day, the glutathione-injected mice with 720 r and above, lost less weight, and on the thirteenth day this difference in weight loss is evident in those mice with 550 r and above. At the time of maximum weight loss (13th day) there is, in the non-treated irradiated controls that received 450-1050 r, apparently a linear relationship between per cent weight change and X-ray dose.

Effect of trauma. The 24-hour mortalities of mice traumatized in the Noble-Collip drum are presented in Fig. 3. The mortality of 512 non-treated, non-irradiated mice was 36.5%, whereas, the mortalities of the non-treated, irradiated 29-day survivors varied from 40 to 100%, increasing with the X-ray dose. A curve is fitted to the 24-hour mortalities of these non-treated animals. The glutathione-injected survivors, in all cases, had a lower mortality than their non-treated controls.

Discussion. The results of these experiments confirm our preliminary observations on the beneficial effects of glutathione when this agent is injected subcutaneously in mice 5-30 minutes prior to X-irradiation(1). Patt, *et al.*(3) found glutathione effective in rats when injected intravenously before irradiation, but ineffective in mice and rats when administered orally. Two doses of glutathione were employed in the present experiments and, although the larger dose afforded more protection, the data are insufficient to quantitate the relationship between glutathione dosage

and its protective action. Furthermore, the X-ray dosage-mortality curves show that the greatest degree of glutathione protection was evident at the higher dosages of X-radiation employed. Below 650 r there was no significant difference in the mortalities of the treated and non-treated animals. Either the numbers of animals used at these points were not sufficient to establish the true relationship or glutathione has less protective action in this range (450-650 r). If the latter is true, one might infer that different lethal mechanisms are induced by high and low doses of X-irradiation and glutathione is capable of modifying only those operating at the higher doses.

Change in body weight was followed daily in all animals as an indication of the degree of radiation injury sustained at the various X-ray dosages employed. The study of the effects of trauma on the twenty-eight-day survivors was utilized as an objective index of relative recovery from radiation injury. The results of these two studies, showing less weight loss and a decreased susceptibility to trauma in the glutathione-injected mice, together with the increased survival rates of the treated animals, indicates: (a) either recovery is accelerated in the mice that were injected with glutathione, or (b) less injury is sustained by the glutathione-treated animals per unit of radiation. The mechanism of this protective action of glutathione will be discussed in a subsequent report.

Summary. Glutathione injection prior to irradiation improves the survival rate, decreases the weight loss, and reduces the susceptibility to trauma of X-irradiated mice. Four mg glutathione per g of mouse is roughly twice as effective as 1.6 mg glutathione per g of mouse. In these experiments no protection was evident in the low lethal dose range (450-650 r).

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Relation of Vitamin A and "Lard Factor" to Disease Caused by Rancid Lard.* (18186)

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The biological effects of rancid fats, particularly of rancid lard, have attracted the attention of various examiners. Reviews of the literature were given by Burr(1,2) and by Quackenbush(3). All authors found that rancid lard causes a fatal disease in rats which has been surmised to be due to a combination of the oxidative destruction of known vitamins and unknown nutritional factors by the rancid lard and of a possible toxic effect of these fats.

In these studies, we shall try to demonstrate that the effects of rancid lard on rats can be prevented by vit. A or by the "lard factor" previously described by Kaunitz and Slanetz(4,5).

Methods. The examinations were carried

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