Thirdly, we cite the finding of Gilson[9] that there is a very great increase in accommodation of turtle auricular muscle following vagal stimulation. If one defines accommodation in the most general terms: namely, as an active resistance of the tissue to depolarization, then this phenomenon may be the excitation counterpart of the decrease in spike voltage and accelerated repolarization seen in the electrograms.

Finally, insofar as the duration of the monophasic action potential curve and the absolute refractory period are measures of the length of the depolarized zone,[10] we should expect this length to be considerably reduced by vagal stimulation. Some idea of the order of magnitude of this reduction may be calculated as follows. From diphasic records of linear strips in air the speed of conduction may be taken, roughly, as 0.5 m./sec., though this may be a slight underestimate. At room temperature (30°C) the duration of the monophasic curve of the uninhibited muscle is about 0.8 sec. The depolarized area, by far the greater part of which is in the state of repolarization, is therefore 40 cm long. The duration of the shortest monophasic curve following vagal stimulation is 0.04 sec. (Fig. 1), and, since there is no apparent change in conduction speed, its length is 2 cm.

Now, if the decrease in spike amplitude is real, the degree of depolarization of the inhibited muscle, as well as its length, must be very much less than that of normal, uninhibited muscle. If the extent of depolarization is not different, then, leading from the injured to the uninjured surface, a decrease in the diameter of the electrode on the uninjured surface should give a considerable increase in the percentage amplitude of the spike of the inhibited muscle. The fact that no such increase was found may be taken as further evidence that the decrease in spike height following vagal stimulation is associated with a partial depolarization of the individual heart muscle fiber.

Summary. The effects of vagal stimulation on the form of the monophasic action potential of auricular muscle are listed along with the technics for demonstrating them. Evidence is presented for the viewpoint that auricular muscle, following vagal stimulation, acts like a single cell capable of giving graded electrical, as well as mechanical, responses.

8 Asher, L., and Scheinfinkel, N., *Z. Biol.*, 1929, **88**, 540.

9 Gilson, A. S., Jr., *Am. J. Physiol.*, 1939, **127**, 333.

10 Lloyd, D. P. C., in Fulton's revision of Howell's Textbook of Physiology, Philadelphia, 1941.

16847

Failure of Rutin to Decrease the Mortality of Acute Ionizing Radiation Illness in Mice.*

E. P. CRONKITE, D. C. ELTZHOLTZ, C. R. SIPE, W. H. CHAPMAN, AND F. W. CHAMBERS, JR.† (Introduced by J. Sendroy, Jr.)

From the Naval Medical Research Institute, Bethesda, Md.

Rutin, a crystalline rhamnoglucoside of quercetin, has been reported to influence favorably the hemorrhagic syndrome of acute ionizing radiation illness in dogs.[1] It has been found to accelerate the healing of the skin lesions of rats induced by localized radiation injury.[2] It seemed desirable, therefore, to study the effect of rutin on the mortality of

* Project NM 007 039, Report No. 16, Naval Medical Research Institute, Bethesda, Md., August 19, 1948.

† The opinions or assertions contained herein are the private ones of the authors and are not to be considered as official or reflecting the views of the Navy Department or the naval service at large.

1 Rekers, P. E., and Field, J. B., *Science*, 1948, **107**, 16.

acute irradiation illness in mice.

Materials and methods. White, Swiss, female mice, obtained from this Institute's colony 30 days after weaning, were fed ground Purina mouse pellets to which had been added 35 mg of ascorbic acid and 16 g of Brewer's yeast per 1000 g of food. They were approximately 7 to 8 weeks old when irradiated, having been isolated and observed for a period of at least 10 days before use in any experiment.

Rutin administration was commenced either 2 weeks prior to, or on the day of, irradiation. In one group receiving rutin *before irradiation,* the drug was given in the following doses: One hundred were given a saturated solution of rutin in water to drink (13 mg/100 cc). Ninety-nine were given a solution of 10 mg/100 cc in drinking water. One hundred received the saturated solution of rutin in the drinking water plus 20 mg of rutin per 100 g of ground food. In another group 99 animals received rutin beginning *on the day of exposure* (saturated solution in water plus 20 mg/100 g of food). All animals received the drug continuously for the 28 day post exposure observation period. The 196 control animals for the above groups received the same amount of radiation.

All irradiated control and treated mice of a given group were exposed simultaneously. The source of radiation was the angular beam of a 1000 KV, 3 ma, G.E. Industrial X-ray machine, the characteristics of which have been previously described.[3] The animals were exposed in specially designed cages constructed of 3/16" plywood. Each cage consisted of two tiers, each holding 25 mice, and conformed in shape to a segment of a circle with a one-meter radius. The tops and bottoms of the cages were made of window screening for ventilation. Details of the construction of the cages and of their placement are reported elsewhere.[4] Animals from each group were marked and uniformly distributed in the cages around the tube so that each segment of the circle contained a representative number from each group. By this method it was possible to irradiate 400 mice simultaneously.

The average output of the tube was 31.2 r/min. at the geometric center of the cages, which was 104.4 cm from the center of the target. The variation in irradiation output as measured at the center, front, back, top and bottom of each cage was not more than ± 0.5 r/min. The exposure period was 22.5 minutes for all mice (a calculated dose of 702 r plus an estimated 3 r from the warm up of the tube, or a calculated total dose of 705 r $\pm$ 11 r.

All mice were weighed daily before and after the exposure to radiation. Cages were inspected in the morning and the evening for dead animals. A few gross pathologic examinations were performed. No microscopic examinations were made. In general the study consisted exclusively of survival and weight recordings.

Results. The administration of rutin alone to mice resulted in no evidence of toxicity. The usual clinical signs of radiation illness in both the control and rutin-treated *groups of irradiated* animals were identical. By the end of the 28-day observation period all surviving animals appeared well and were gaining weight.

In the first experiment there were 96 control mice and 99 experimental mice that received rutin for 2 weeks before irradiation. Mice began to die in the control group on the ninth day after exposure and in the rutin group on the eighth day. After the nineteenth day there were only scattered deaths. From this initial experiment it appeared that the mortality was increased from 40.6% in the control animals to 52.5% in the rutin-treated animals. The probability of these data being from the same distribution is between 0.10 and 0.20 as determined by the Chi

[2] Griffith, J. Q., Jr., Anthony, E., Pendergrass, E. P., and Perryman, R., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 332.

[3] Tullis, J. L., Tessmer, C. F., Cronkite, E. P., and Chambers, F. W., Jr., Naval Medical Research Institute, Project NM 007-039, Report No. 3, Dec. 1947, in press, *Radiology,* 1949.

[4] Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Cronkite, E. P., Lawrence, G. H., and Chambers, F. W., Jr., Naval Medical Research Institute, Project NM 007-039, Report No. 14, July 1948.

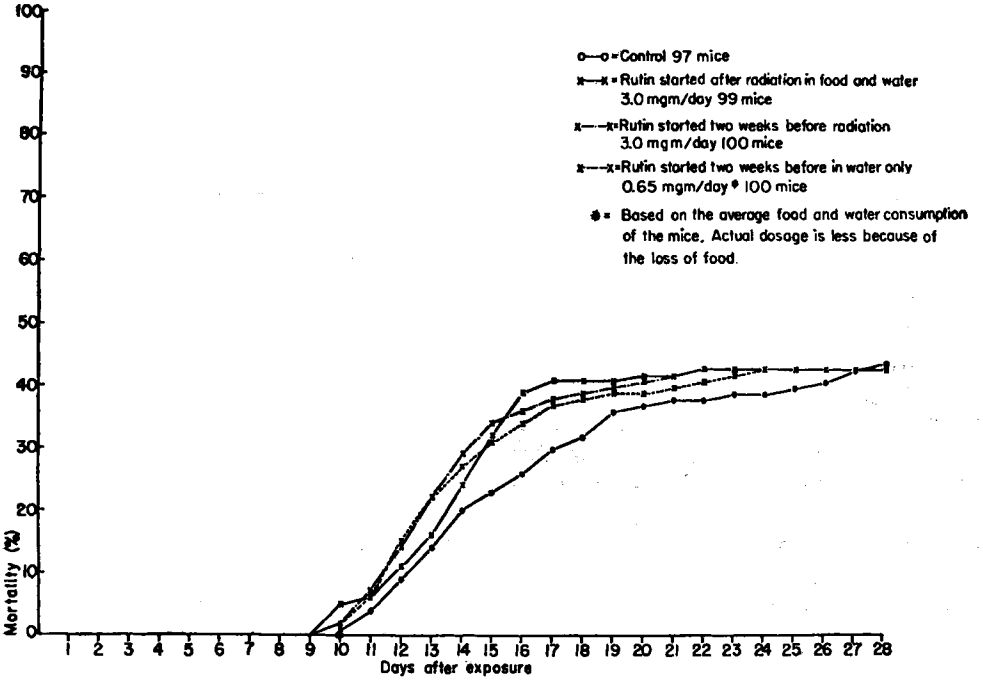

Figure 1.— Mortality curves of the control mice and the three rutin groups

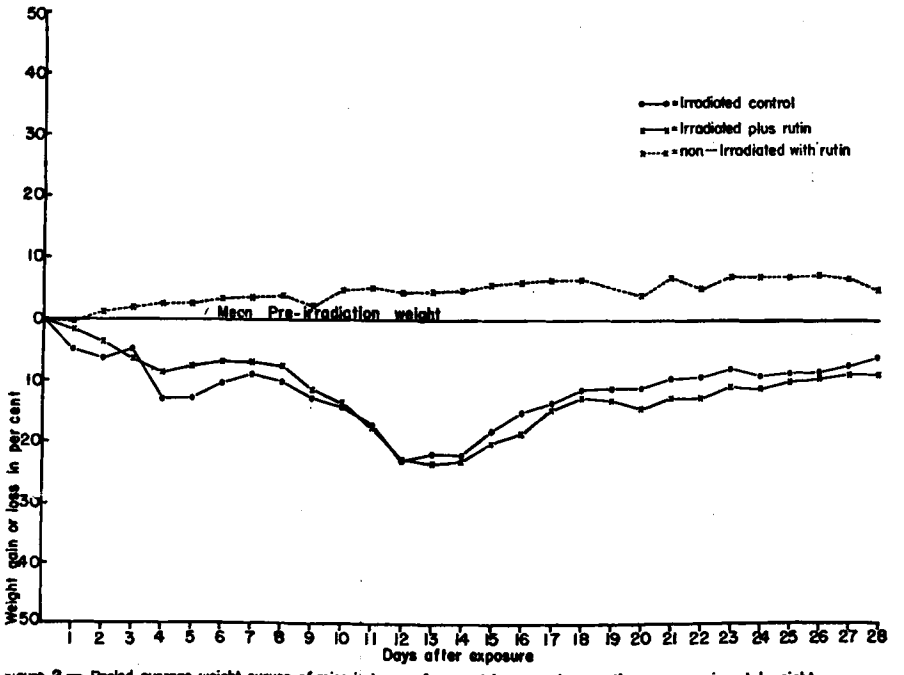

Figure 2.— Pooled average weight curves of mice in terms of percent loss or gain over the pre-experimental weight.

square method. Hence, the difference in results is not significant.

Fig. 1 shows that dosage and the time of administration did not significantly affect the results. The ultimate pooled mortality of the treated and non-treated mice on the 28th day was not significantly different ($0.70 > P_{diff} > 0.50$); however, on the 17th day the mortality of the rutin-treated groups was significantly greater than that of the non-treated

groups ($0.05 > P_{diff} > 0.02$). This is further emphasized by the shorter average survival time of the combined rutin-treated animals that died (14.1 days) as compared to 15.4 days for the non-treated.

In Fig. 2 the pooled weights of all irradiated control mice, rutin-treated mice, and rutin-treated non-irradiated mice are plotted in terms of per cent gain or loss of original pre-irradiation weight. There was no significant difference between the irradiated control and irradiated rutin-treated mice. Maximum total weight loss was obtained by the 12th day after exposure when the mortality rate was at a maximum. The weight loss and mortality decreased simultaneously. However, scattered deaths did occur after the average weight began to increase. At the end of the observation period the average weight was still below that of the pre-irradiation period. The rutin, non-irradiated mice maintained a normal growth rate.

Autopsies of selected animals demonstrated the usual picture of irradiation illness in mice that has been adequately described in the past.[5]

Comment. The original attempt of Rekers and Field[1] to control the hemorrhagic syndrome of irradiation illness was based on the favorable clinical reports of rutin in the management of increased capillary fragility.[6,7] The results reported by Rekers and Field on the use of rutin in irradiated dogs were most encouraging. However, the *modus operandi* of rutin in controlling capillary fragility in general or in producing the favorable results of Rekers and Field in dogs is not known. It has been emphasized that rutin is less effective in the presence of an ascorbic acid deficiency.[7] Szent-Györgi and his co-workers[8] demonstrated that the crude glucosides of citrin could control increased capillary fragility of guinea pigs. It is probable, although not proved, that the active part of the citrin is rutin. The relationship of ascorbic acid and rutin in the maintenance of capillary fragility is not known. Apparently only man, monkey and guinea pig, in marked contrast to dogs and mice, are dependent upon diet for ascorbic acid. On this basis it is probably desirable to repeat this work on guinea pigs, an animal whose dietary requirements for maintenance of capillary integrity are more nearly like that of man. However, there are many factors which influence survival following exposure to radiation, and capillary integrity may not be the critical one. Many animals die with only minor evidence of hemorrhage.[9,10] The control of the coagulation defect with protamine and toluidine blue in dogs by Allen[11] prevented hemorrhage to a great extent but the animals *died nevertheless.* The extensive use of blood and penicillin by one of us did not significantly influence the survival of goats exposed to the atomic bomb.[9] The present work conclusively demonstrates that rutin in the doses used was of no value in improving the survival of mice. The data presented suggest that rutin may have been harmful inasmuch as all rutin-treated groups died at a significantly faster rate.

Summary and conclusions. 1. Rutin not only was of no value in improving the survival of mice simultaneously exposed to a dose of ionizing radiation in the lethal range, but significantly increased the rate at which the mice died.

2. It is considered desirable to repeat this type of investigation on animals with ascorbic acid requirements similar to man.

The authors wish to acknowledge their appreciation for the rutin kindly supplied by J. F. Couch, Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, U. S. Department of Agriculture, Chestnut Hill Station, Philadelphia, Pa.

5 Ellinger, F., *Radiol.*, 1945, **44**, 125.

6 Shanno, R. L., *Am. J. Med. Sci.*, 1946, **211**, 539.

7 Couch, J. F., Krewson, C. F., Naghski, J., and Copley, M. J., U. S. Department of Agriculture, Bureau of Agricultural and Industrial Chemistry, April 1946, AIC-115.

8 Armentano, L., Bentsath, A., Benes, T., Ruszynak, I., and Szent-Györgi, A., *Deutsche med. Wchnschr.*, 1936, **62**, 1325.

9 Cronkite, E. P., Naval Medical Research Institute, Project NM 007 039, Report No. 10, July 1948.

10 Prosser, C. L., *et al.*, *Radiology*, 1947, **49**, 299.

11 Allen, J. G., personal communication.