The results of cross-hemagglutination-inhibition reactions between PR8, 965 and the variant strains derived from them on passage with heterologous immune serum are shown in Fig. 2. For comparison, the results of 4 successive experiments during the passage series are presented. As in the case of Fig. 1, the data for Exp. I and II are taken from a previous paper(1), whereas those for Exp. III and IV derive from this investigation. The passage history of the strains employed in Exp. I to IV is described above. The sera were identical with those used in the cross-neutralization reactions.

It appears from the data shown in Fig. 2 that the antigenic differences between PR8 and $PR8_9$ disappeared after 12 passages in the absence of immune serum. However, the differences between 965 and 965_p remained demonstrable, by the *in vitro* procedure, through 24 passages without serum although they were less marked than after 6 such passages. This evidence supports that obtained in the cross-neutralization experiments.

Discussion. It was demonstrated previously that it is possible to select antigenic variants of influenza A virus strains by passage *in ovo* in the presence of immune serum against a somewhat different strain. The new antigenic patterns persisted for 6 serial passages in the absence of immune serum(1). On increasing the number of serial passages without serum to a total of 24, the antigenic differences between one parent strain (965) and its variant persisted, while those between the other parent strain (PR8) and its variant disappeared. The variant (965_p) which showed the persistence of antigenic properties different from those of the parent strain was that which manifested such differences both in neutralization and in hemagglutination-inhibition experiments. On the other hand, the variant ($PR8_9$) which appeared to revert on prolonged passage without serum was that which had shown antigenic dissimilarity only in hemagglutination-inhibition experiments.

Summary. Antigenic differences in influenza A virus strains which appear during serial passage *in ovo* in the presence of heterologous immune serum may remain demonstrable for at least 24 passages without serum.

1. Archetti, I., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1950, v92, 441.

Received April 25, 1952. P.S.E.B.M.. 1952, v80.

Pharmacological Studies on Irradiated Animals. II. Effect of Cortisone on X-Ray Mortality of Mice.* (19574)

FRIEDRICH ELLINGER. (With the assistance of J. Blagg and W. Brooks.)

From the Pharmacology Division of the Naval Medical Research Institute, Bethesda, Maryland.

In continuation of our systematic pharmacological studies on irradiated animals, the effect of cortisone on the lethal effect produced by X-rays in total body irradiation of mice is here reported.

Materials and methods. Swiss male white mice of the Institute strain, 22 g ± 15% of body weight. were used. Irradiation was done as described previously(1). The radiation factors were: 200 kv, 15 ma, .25 Cu + 1.0 mm Al filtration, HVL = .8 mm Cu. In agreement with previously outlined rules for pharmacological studies on irradiated animals (2), the effect of a standard dose of cortisone of .25 mg (Cortone (Merck) 1 cc = 25 mg) was administered intramuscularly to mice exposed to various X-ray doses namely those of 410, 350, and 200 r/air, representing the LD 60 and 25/14 days and minimal lethal dose, respectively. Hormone injections were given immediately after exposure and continued to the 14th day after irradiation. For graphic

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

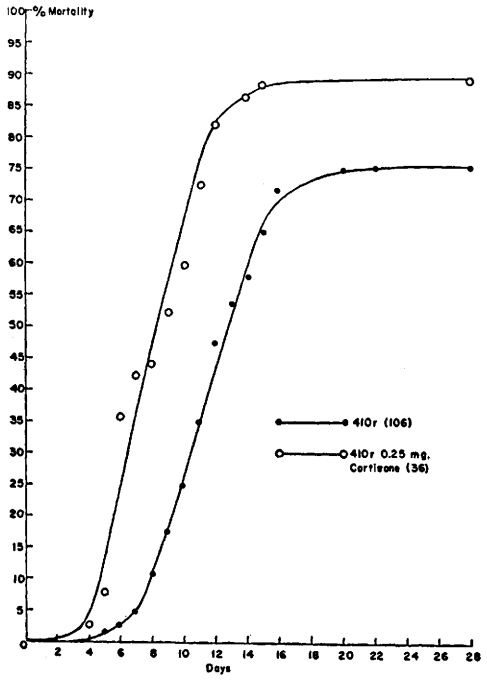

FIG. 1. Comparison of percentage mortality in mice irradiated with 410 r, total body irradiation; cortisone-treated group (open circles) and non-medicated group (solid circles).

presentation of results the mortality rate of hormone treated animals is compared with that of the non-medicated irradiated control group. Figures in parenthesis indicate the numbers of animals in the different groups.

Results. 1. *Effect of cortisone on radiation induced mortality.* Fig. 1 and 2 show the influence of cortisone on the mortality rates produced by the 3 X-ray doses. At all 3 doses death started earlier in the cortisone-treated and irradiated animals than in their non-medicated irradiated controls. There was a definite increase in the mortality rates produced by the use of cortisone in animals exposed to the X-ray doses of 410 and 350 r respectively. These differences in mortality rate are statistically significant at the end of the first week after irradiation ($P < 0.01$ for the 6th day for 410 r, and $P = 0.02$ for the 7th day for 350 r) although not throughout the entire period.† No increase in mortality was noted at 200 r. (Fig. 2B.)

† We are indebted to Dr. J. P. Flynn for the statistical analysis.

2. *Effect of cortisone and ascorbic acid treatment on the lethal effect of total body irradiation.* In view of the demonstrated increase of the radiation induced mortality rate of total body irradiation by cortisone administration, an attempt was made to ascertain whether additional treatment with ascorbic acid might influence the cortisone effect on irradiated mice. This investigation was based on the premise that cortisone in large doses affects the blood forming tissues similarly to X-rays (3,4). There are reports indicating that large amounts of ascorbic acid speed up the restoration of the white blood count after x-irradiation (5) and also after benzol administration (6). Even though ascorbic acid seems not to influence radiation induced mortality (7), it was thought that this substance might modify the influence of cortisone on the radiation effects. Consequently, two sets of experiments were carried out to test this hypothesis. Animals exposed to doses of 410 and 200 r/air received in addition to the standard dose of .25 mg cortisone, daily intramuscular injections of 15 and 5 mg of

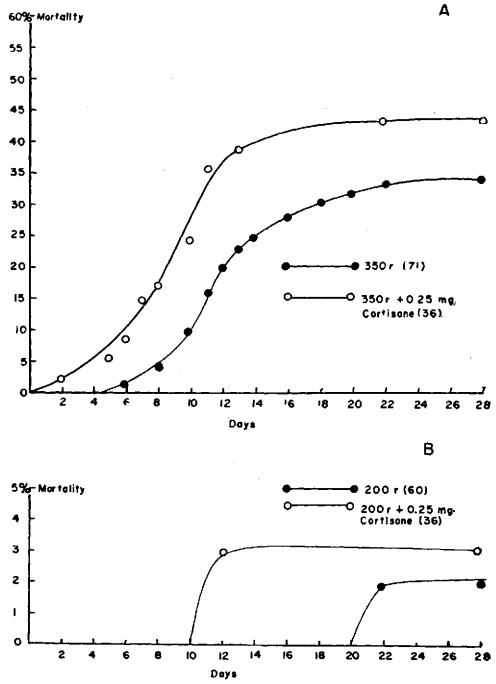

FIG. 2. Comparison of percentage mortality in mice irradiated: A, with 350 r and B, with 200 r, total body irradiation; cortisone-treated group (open circles) and non-medicated group.

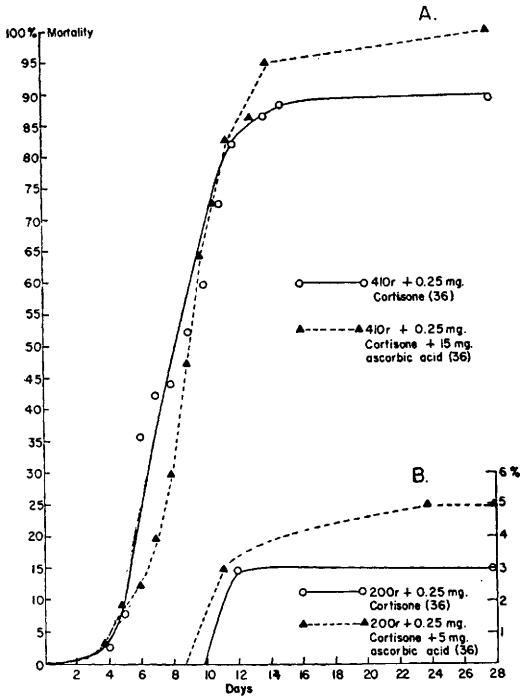

FIG. 3. Comparison of percentage mortality in mice irradiated: A, with 410 r and B, with 200 r, total body irradiation; cortisone-treated group (solid line), cortisone and ascorbic acid-treated group (broken line).

ascorbic acid respectively for 14 days after irradiation. The pertinent results summarized in Fig. 3A and B indicate that administration of ascorbic acid in the doses used did not change significantly the course of the cortisone effect.

Discussion. Our results concerning the effect of cortisone administration on the lethal effect of total body irradiation with X-rays clearly indicate an enhancement of the radiation induced mortality in this animal species. The effect was most pronounced at the end of the first post-irradiation week. These data confirm and supplement previous observations of Smith, Smith, and Thompson(8) who noticed "failure of cortisone or ACTH to reduce mortality in irradiated mice." Our observations also emphasize the suggested procedure in pharmacological studies on irradiated animals(2), to use a standard dose of a pharmacological agent on at least 2, preferably 3, X-ray dose levels in order to clarify more completely such drug action. The enhancement of the effect of total body irradiation by cortisone, in contra-distinction to the previously reported beneficial effects of DCA (9), is not surprising on the basis of the known physiological actions of these two substances. Edelmann(10) recently demonstrated that not only DCA but also whole extract of the adrenal cortex has a beneficial effect on radiation induced mortality of adrenalectomized rats. This seems to indicate that the effects of cortisone within the gland may be balanced by others of the numerous substances elaborated by the adrenal cortex. Thus the effect of irradiation on the adrenal cortex may well consist not only in stimulation and eventual exhaustion of secretion, but also result in a disturbance of the balance of these various adrenal cortical hormones in the secretion.

The unfavorable effect of cortisone on irradiated animals appears noteworthy from a clinical point of view: the simultaneous use of cortisone and ionizing irradiation as well as the use of this substance shortly after an acute exposure to this type of radiant energy must be considered contra-indicated.

Summary. 1. Administration of 0.25 mg cortisone intramuscularly to mice daily for 14 days after their exposures to the LD 60 and 25/14 days of x-rays resulted in a definite increase of the mortality rates produced by these x-ray doses. 2. There was no increase in mortality in the cortisone-treated mice exposed to the minimal lethal dose of x-rays but as in the higher x-ray doses, cortisone treatment produced a shortening of the latent period between irradiation and onset of death, indicating an adverse effect even at this low radiation level. 3. Simultaneous treatment of irradiated mice with cortisone and ascorbic acid did not modify the enhancement of the radiation induced mortality rate produced by the cortisone administration. 4. Some clinical implications of these animal experimental data are briefly mentioned.

1. Ellinger, F., Morgan, J. W., Chambers, Jr., F. W., Naval Medical Research Institute Report NM 006.012.04.43 & 44.

2. ———, Naval Medical Research Institute Report NM 006.012.05.04.

3. Antopol, W., PROC. SOC. EXP. BIOL. AND MED.,

1950, v73, 262.

4. Quittner, H., Ward, N., Sussman, L. N., Antopol, W., *Blood,* 1951, v61, 513.

5. Carrie, C., *Strahlentherapie,* 1938, v63, 183; Carrie, G., Schnettler, O., *Strahlentherapie,* 1939, v66, 149; Szenes, T., *Strahlentherapie,* 1940, v71, 463; Smithers, D. W., *Brit. J. Radiol.,* 1942, v15, 233; Claussen, A., *Acta Radiol.,* 1942, v23, 95; Ellis, F., *Brit. J. Radiol.,* 1942, v15, 194; Simmons E. L., Jacobson, L. O., Pearlman, N., Prosser, C. L., Manhattan District Declassified Document No. 1277.

6. Feller, A., *Strahlentherapie,* 1937, v60, 393.

7. Patt, H. M., Smith, D. E., Tyree, E. B., Straube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 18.

8. Smith, W. W., Smith, F., Thompson, E. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 529.

9. Ellinger, F., *Science,* 1946, v104, 502; PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 31; *Radiology,* 1945, v44, 125; 1948, v50, 234; v51, 394.

10. Edelmann. A., *Am. J. Physiol.,* 1951, v167, 545.

Received May 1, 1952. P.S.E.B.M., 1952, v80.

Phage Multiplication on Two Hosts. Isolation and Activity of Variants of Staphylococcus Phage P_1.* (19575)

DORIS J. RALSTON† AND ALBERT P. KRUEGER.

From the Department of Bacteriology, University of California, Berkeley, Calif.

By simultaneously titrating phage on two susceptible hosts, the authors have been able to detect and isolate variants from a stock staphylococcus phage P_1. The dual host assay system has provided a sensitive means of studying certain unusual activities of one of the phage variants.

Experimental procedures and results. I. *Isolation of phage variants from stock P_1.* When aliquots of stock P_1, prepared on host strain Staphylococcus K_1 in tryptose-phosphate both, were titrated by plaque count(1) on two hosts—strains K_1 and WF 145‡—a slightly but consistently higher titration value was found with the latter. This difference could not be ascribed to rates of adsorption, latent periods, or burst sizes of the phage for the two hosts. Stock phage P_1 was then produced on strain 145 in trypticase soy broth. At intervals aliquot samples were titrated on the two hosts. The ratio of titer per ml on 145 to titer per ml on strain K_1 (hereafter designated $145/K_1$) was found to increase to approximately 4 during the growth period of the phage. Plaque isolates were made from samples exhibiting this high ratio. Virus from plaques on strain 145 was suspended in saline and aliquots plated on the two hosts. Four isolates of 32 exhibited distinct differences in titration ratio $145/K_1$, and, in 2 cases, formed small sized plaques on K_1 cells. Growth in broth on 145 cells resulted in characteristic curves of phage titratable on the two hosts. Two variants, No. 15 and 16, showed very similar curves and titration ratios $145/K_1$ = ca 5. Since they also formed small plaques on K_1 cells, they were considered identical isolates. One variant, No. 5, exhibited a growth curve much like that of stock phage and proved to have a titration ratio of 1. The lysate of the fourth, phage 14, exhibited a ratio $145/K_1$ = ca 40. This variant was selected for detailed investigation to determine the factors involved in producing this high ratio.

II. *Observations on phage 14.* A. *Attempted strain separation by plaque isolation.* The high plaque ratio $145/K_1$ of P_{14} lysates of 145 cells pointed to a possible mixture of virus strains, one of which produces no plaques on K_1 assay cells. A series of 10 successive serial passages of P_{14} on host K_1 was made by plating saline suspensions of virus from individual plaques. On the tenth transfer the saline suspension of virus assayed on the 2 hosts in a ratio $145/K_1$ = 1.3. A similar

* This investigation was supported in part by grants from ONR Task 21, and the Board of Research, University of California. The authors wish to express appreciation to Dr. John H. Northrop, for his generous advice during the course of this study.

† Edith Claypole Memorial fellow in Bacteriology.

‡ Obtained through the courtesy of Dr. John E. Blair, New York Hospital for Joint Diseases.