Our previous experiences(1), in which a protective spleen extract was obtained from organs of irradiated animals which were in an active stage of regeneration and reduced to only one-half or less of the average normal spleen size, support the last assumption.

Summary and conclusions. 1. Continuing previous studies, employing improved bioassay methods, the protective effect of cell-free saline extracts made from spleens of normal mice against X-ray-induced mortality in this species has been demonstrated. 2. Extracts made from the spleens of donor animals exposed to an X-ray dose which killed 55% of the donor animals in 14 days failed to show a protective effect, which was in agreement with previous observations. 3. A certain amount of parallelism between the protective effect of various extracts made from normal mouse spleens and the failure to protect of those made from irradiated mouse spleens was found in studies of the total protein and NPN content of such extracts. 4. It appears that within a wide range of concentration of total protein and NPN content such protection can be achieved. 5. However, reduction of average content of total protein and NPN below one-half of lowest average value for normal spleens, ascertained in samples made from spleens of irradiated donor animals, was found to be associated with a lack of protection. Whether this failure is due merely to quantitative or also to qualitative differences in these extracts is not clear.

Since the writing of this paper, an article by Allen *et al.* appeared in *Science,* 1956, v123, 1080, in which postirradiation protection of rabbits with plasma obtained from the splenic vein has been reported. Presence of a humoral spleen factor has thus been indicated in another animal species with a different method.

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Anaphylaxis in the Mouse: Possible Relation of the Schultz-Dale Reaction to Serotonin Release.* (22579)

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The evidence against the release of histamine in mouse anaphylaxis has been summarized(1). The purpose of the experiments reported here is to determine, using the Schultz-Dale technic, whether or not the substance responsible for contraction of the sensitized mouse uterus in the presence of antigen is serotonin (5-hydroxytryptamine).

Materials and methods. The virgin female mice were of the DBA or BALB/c strains, and were over 6 months of age when used. The mice were immunized intramuscularly in the hind legs with alum-precipitated egg white, as previously described(1). This technic of immunization is sufficient to render the mice anaphylactically sensitive to egg albumin as evidenced by the occurrence of a characteristic shock reaction on the intravenous injection of egg albumin(2); and by a contraction of the uterine smooth muscle of the sensitized mouse upon the addition of egg albumin *in vitro,* using the classical Schultz-Dale technic(1). Mice which had not received a prior injection of antigen never reacted by either technic when egg white was

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added. Further evidence of a real anaphylactic sensitivity was the observation of desensitization of the sensitized uterus after the addition of antigen(1). Circulating antibodies have been demonstrated in mice after immunization under parallel conditions(3); and the passive transfer of sensitivity has been effected by the administration of antiserum from similarly immunized mice to normal mice(1). Testing for intracutaneous sensitivity in the immunized mouse has repeatedly given negative results.

The technic used in the Schultz-Dale reaction, as previously described(1) consists, in brief, of attaching one-half of one uterine horn from the mouse to a lever in a water bath, and recording the contractions on a kymograph prior to and during the addition of the antigen.

Because this study is specifically intended to demonstrate drug inhibition of the anaphylactic reaction on *known* sensitized uteri, the criterion selected for determining sensitivity to egg white was the demonstration of a positive Schultz-Dale reaction on one or 2 of the 4 uterine strips. Only those mice were used which fulfilled this criterion. When normal mice were used to test drug sensitivity, it is so noted in the text.

Results. Reactivity of mouse uteri to serotonin. Serotonin† was dissolved in Ringer's solution, and the reactivity of normal and of sensitized uteri determined. It was found that a dose of 0.05γ/ml of bath was always sufficient to produce a good contraction in normal or sensitized uterine strips. Some strips reacted equally well to a smaller quantity. This amount is similar to that required to stimulate the mouse jejunum(4) and the rat uterus(4,5,6). *Inhibition of serotonin contraction with lysergic acid diethylamide (LSD) and with reserpine.* The addition of LSD‡ in a concentration of 3γ/l was sufficient to prevent the reaction to 0.05-0.1γ/ml of serotonin when LSD was added immediately before the serotonin; 1γ/l was sufficient when the serotonin was added after 5 minutes had elapsed. These findings are comparable to those of previous workers(5-7) for the rat uterus. Reserpine§ in a concentration of 5 mg/l was sufficient to antagonize the reaction to 0.05-0.1γ ml of serotonin when in contact with the uterus for 5 minutes prior to addition of serotonin. This amount is 5 times greater than that reported recently by Costa(7) as being necessary to antagonize the serotonin contraction of the rat uterus; smaller concentrations were not tested here. *Inhibition of anaphylactic contraction with LSD and with reserpine.* A total of 16 sensitized mice were used: 6 were tested by showing positive reactions to egg white on 2 of the 4 uterine strips, and complete inhibition of anaphylaxis in the presence of 1-3γ/l of LSD on the other 2 strips; 6 were tested similarly, using 5 mg/l of reserpine; one strip from each of the remaining 4 mice was tested to demonstrate the anaphylactic contraction, one strip was tested in the presence of LSD, and one in reserpine. Following each test in the presence of drug, the reaction of the strip to serotonin and to 10γ/ml of acetylcholine(1) was tested. Uterine strips tested in the absence of drug gave uniformly positive Schultz-Dale reactions on the addition of egg albumin. In all of the 10 mice tested with LSD, 6 in duplicate, the reaction to egg albumin was completely inhibited; in all of the 10 with reserpine, identical inhibition was shown. The drugs inhibited the reaction to serotonin in every case, and never inhibited the reaction to acetylcholine.

Discussion. The evidence against the importance of histamine in mouse anaphylaxis consists primarily of the fact that the intact mouse and the isolated uterine strip of the mouse are sensitive only to enormous doses of the drug, that the body of the mouse probably does not contain sufficient histamine to produce a reaction, and that anaphylaxis in the intact mouse or on the isolated uterine

† Serotonin creatinine sulfate, Nutritional Biochemicals Corp., Cleveland. Concentration calculated on weight as 5-hydroxytryptamine.

‡ L.S.D.25, Sandoz Chemical Works, Inc., Hanover, N. J.

§ Reserpine, Nutritional Biochemicals Corp., Cleveland. Dissolved in equal parts ethylene glycol and ethyl alcohol with 0.02% HCl 1-500 dilution in tissue bath.

strip cannot be inhibited by antihistaminic drugs.

It has been shown in this study that the isolated uterine strip is sensitive to 0.05γ/ml of serotonin, while a concentration of histamine of 50γ/ml(1) is necessary to produce a reaction. Thus the isolated tissue of the mouse is 1,000 times more sensitive to serotonin than it is to histamine. This sensitivity is completely abolished with the serotonin antagonists LSD and reserpine; and the anaphylactic contraction is completely inhibited in the presence of these drugs. Serotonin is the only substance known to be inhibited by these two drugs. It has also been shown(6) that the tissues of the mouse contain a quantity of serotonin easily sufficient to produce the anaphylactic response. These findings support the hypothesis that the anaphylactic reaction in the mouse is dependent on the release of serotonin.

Summary. (1) The egg white sensitized or normal mouse uterus is 1,000 times more sensitive to serotonin than it is to histamine. (2) This sensitivity is completely abolished in the presence of two known serotonin antagonists, LSD and reserpine. (3) LSD and reserpine, which as far as is known inhibit only serotonin, always and completely prevented the anaphylactic contraction on strips of demonstrably sensitized uterus in the ten trials with each drug.

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Teratogenic Effects of Herpes Simplex, Vaccinia, Influenza-A (NWS), and Distemper Virus Infections on Early Chick Embryos.*† (22580)

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The discovery of a causal relationship between maternal rubella and fetal pathology in human beings(1) has led to an experimental investigation of the role played by infectious agents in the production of congenital malformations. Recent studies concerning viral teratogenesis have resulted in the finding that characteristic developmental abnormalities and death follow infection of early chick embryos with the PR8 strain of influenza-A virus (2,3). Moreover, the induction of these teratogenic effects has been prevented by neutralization of the virus with specific antiserum, thus providing evidence that the virus was the cause of the syndrome(3). In addition, Newcastle disease virus has also been found to produce specific malformations in this embryonic host(4-6). In view of these results, experiments were conducted in this laboratory to determine whether the induction of teratogenic and lethal effects may be a property of other viruses as well. It is the purpose of this paper to describe the effects produced by infection of early chick embryos with herpes simplex, vaccinia, the NWS strain of influ-

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