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Received January 28, 1959. P.S.E.B.M., 1959, v100.

Relation of Viral Proliferation and Antibody Formation in Mice Exposed to Roentgen Radiation.* (24788)

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Previous work showed(1) that exposure of adult mice to x-radiation prior to parenteral inoculation of Coxsackie virus resulted in an increased proliferation of the viral agent in various organs examined without a concomitant rise in mortality rates. Although the mechanism of this enhancive effect was not made clear by this study, one possible explanation suggested was the inhibition of antibody formation by x-radiation administered prior to injection of the antigen, as demonstrated by Dixon, *et al.*(2,3). The experiment reported here was, therefore, designed as a supplementary study to those previously reported in an attempt to correlate viral titers from selected organs with serum antibody levels. Further, it attempted to determine the effect of x-radiation upon secondary antibody response of mice and its relation to viral proliferation in selected tissues.

Materials and methods. Virus. The Powers strain of Coxsackie virus isolated by Cheever, *et al.*,(4) and classified by Dalldorf(5) as Group B Type 4 strain was used. The characteristic lesions produced in suckling mice have been described by Pappenheimer, *et al.*, (6). The strain had undergone 27 suckling mouse passages in our laboratory and the stock virus consisted of a 20% suckling mouse carcass suspension. All inoculations consisted of 0.10 ml of a 10^{-1} suspension of stock virus representing approximately 100,000 suckling mouse LD_{50}. Swiss white mice of the Tumblebrook and CFW strains obtained from Tumblebrook Farms, Brant Lake, N. Y., and from Carworth Farms, New City, N. Y., respectively, were employed. No differences were noted in susceptibility of these 2 strains of mice to infection with the virus or to effect of x-radiation. Roentgen radiation was delivered by Westinghouse therapy x-ray unit housed in a special chamber. Radiation was delivered by 4 milliampere current operating at 100 kilovolts and filtered through 1 mm of aluminum (HVL 1.1 mm Al). The field size was 20 cm square; individual readings taken at selected points in the target area with Victoreen r meter varied from 5.1 to 5.5 r in air/minute. X-radiation delivered as a single massive dose (400 r) was administered to the whole body of the animal at skin target distance of 75 cm at average rate of 5.3 r/minute. All radiation was administered 24 hours prior to inoculation. *Titration of virus.* Selected tissues were harvested at various intervals following inoculation and prepared as 20% suspensions in 0.2% bovine albumen broth and stored in a CO_2 chest until tested. Infectivity determinations were carried out in suckling mice less than 48 hours old. All litters were pooled and redistributed in random manner prior to inoculation. Decimal dilutions of the suspension to be tested were made in 0.2% bovine albumen broth and 0.02 ml was injected intraperitoneally into each of 16-22 mice comprising 2 litters. The inoculated mice were observed for 12 days. When possible, mice dying after inoculation of critical dilutions were autopsied and examined for gross lesions of interscapular fat pads taken as a criterion of specific death. The LD_{50} endpoint dilution was calculated by the meth-

* This study supported by U. S. Atomic Energy Comm.

od of Reed and Muench(7). *Determination of antibody level.* The constant virus-varying serum method was used for all neutralization tests. Equal amounts of virus and serum dilution were mixed and incubated at 37°C for 2 hours. The tubes were then placed in ice water bath until inoculations could be carried out. Inoculations consisted of 0.02 ml injected intraperitoneally into each of 16-22 suckling mice (2 litters) for each serum dilution. Mice were observed for 12 days and the specificity of deaths determined as previously described. All serum dilutions were made in phosphate buffered saline. Virus concentration used was approximately 10 LD_{50} (range 3.2-32LD_{50}). Actual titer was confirmed at time of each test by inoculation of appropriate dilutions into 2 litters of suckling mice. Serum controls were also included. The antibody level was recorded as the initial serum dilution protecting 50% of the mice and was calculated according to the method of Reed and Muench(7). *Procedure.* Two hundred 3-4 week old CFW mice with an average weight of 8-10 g were separated in random manner into 2 groups of 100 mice each. These were designated as control and x-ray groups. The x-ray group received 400 r whole body radiation as described. Twenty-four hours after exposure all mice in both groups were inoculated intraperitoneally with 0.1 ml of a 10^{-1} suspension of Powers virus. The mice were observed daily and on 5th, 10th and 30th days following inoculation 10 mice were sacrificed from each group. The mice were lightly etherized and exsanguinated by severing the subclavian artery. Blood was collected and pooled separately for each of the 2 groups. The serum obtained was frozen at –20°C and stored for later antibody studies. Brain, heart, spleen and pancreas were harvested aseptically using individual instruments for each organ. These were pooled for each group according to tissue and suspensions prepared for infectivity determinations as described. On 30th day following inoculation the 2 groups were each separated into sub groups. One half of control and x-ray groups were then exposed to 400 r x-radiation as before and designated "control - x-ray," and "x-ray - x-ray" sub groups, respectively. The remaining mice of both groups received no further radiation and were designated as "control-control" and "x-ray-control" sub groups according to their x-radiation experience in the first and second phases of the experiment. Twenty-four hours following radiation of the first 2 sub groups all 4 sub groups of mice were reinoculated with 0.10 ml of a 10^{-1} suspension of Powers virus. On 5th, 10th and 30th day following this inoculation 10 mice were sacrificed from each of the 4 sub groups and bled for serum antibody studies. Selected organs were harvested and viral content determined as previously described. No spontaneous deaths occurred during the period of study.

TABLE I. Viral Titers of Selected Organs and Serum Antibody Levels in Irradiated and Control Mice following Inoculation with Coxsackie Virus.

	Control			X-ray		
	5*	10	30	5	10	30
Brain	†			2.5		
Heart				2.6		
Spleen				2.6		
Pancreas	5.1‡			6.0	2.5	
Serum PD_{50}§	1:32	1:320	1:320	1:68	1:130	1:66

* Days following inoculation.
† Negative at 10^{-1} dilution.
‡ Log of reciprocal of LD_{50} endpoint dilution.
§ Dilution of serum protecting 50% of animals.

Results. The results of the first phase of this experiment are presented in Table I. During the first 30-day period only the pancreatic tissue yielded any demonstrable virus in the control (unirradiated) group. In this group an appreciable amount of virus was present on fifth day following inoculation. On the other hand, all tissues tested from the x-ray group had demonstrable levels of virus 5 days after inoculation and the pancreas remained positive through the tenth day. These findings are in keeping with those previously reported by Cheever(1). The antibody titers of the 2 groups did not differ on the fifth day but at 10 and 30 days following inoculation somewhat higher levels of neutralizing antibody were recorded for the control group.

In the second phase of the experiment no virus was recovered from the organs examined except in the control-x-ray group where on

TABLE II. Secondary Immune Response of Irradiated and Control Mice following Inoculation with Coxsackie Virus.

Group	Days after inoculation 0	5	10	30
Control				
Control	1:320*	1:2240	>1:4096	>1:4096
X-ray	"	1:66	1:1480	1:380
X-ray				
Control	1:66	1:3550	1:4096	>1:4096
X-ray	"	1:256	1:2190	1:2450

* Dilution of serum protecting 50% of animals.

fifth day following inoculation spleen and pancreas yielded titers of $10^{-2.5}$ and $10^{-5.3}$, respectively. The data concerning antibody levels are summarized in Table II. The control-control and x-ray-control groups demonstrated a clear-cut anamnestic immune response in that a marked and rapid increase of neutralizing antibody was demonstrated. The other 2 groups, control-x-ray and x-ray-x-ray, did not show so definite a secondary response, for although there was an increase in antibody level it occurred more slowly and did not attain the level noted for the other 2 groups. The control-x-ray group showed the least marked secondary response.

Discussion. These results confirm those of Cheever(1) that x-radiation administered prior to inoculation with Coxsackie virus increases the yield of virus from selected tissues, notably the spleen and pancreas, and apparently lengthens the period during which the virus may be demonstrated in these organs. They are also in accord with those reported by other investigators(8,9) and are compatible with the hypothesis that the increased viral proliferation noted in x-radiated animals is due to an inhibition of the antibody response.

The demonstration of viral proliferation as well as a lack of a true anamnestic response in those groups receiving radiation in the second phase of the experiment suggests a deleterious effect of x-ray on the secondary response. In this latter phase a retardation of the secondary immune response occurred in the irradiated groups even though a marked primary response had occurred previously. This finding is in agreement with the observation of a retarded secondary hemolysin response to sheep red blood cells in x-radiated rabbits reported by Taliaferro, *et al.*,(10). On the other hand, Dixon, *et al.*,(2,3) using bovine gamma globulin as the antigenic stimulus, reported x-ray to be ineffective in altering the secondary response. Talmage(9) has discussed this apparent discrepancy in the light of the nature of the antigen involved and has advanced the hypothesis that the character of the antigen may determine the type of antibody response.

The 2 groups not irradiated in the second phase of the experiment demonstrated a prompt, marked and approximately equal anamnestic response. This suggests that the effect of radiation on antibody formation was so transitory that the secondary response was uninfluenced when the same antigen was reinjected 30 days after radiation exposure. Similar results have been reported by Taliaferro, *et al.*,(10) in that rabbits responded normally when inoculated with sheep rbc 28 days following x-ray exposure.

This experiment suggests that inhibition of the immune response as a result of radiation exposure is the mechanism accounting for the increased proliferation of subsequently injected virus. Further work is necessary to determine whether this inhibition of antibody formation is due to a direct or an indirect effect of radiation on the antibody forming mechanisms.

Summary. 1. X-radiation administered prior to Coxsackie virus inoculation increased and prolonged the yield of virus from selected tissues, particularly the pancreas. 2. X-radiation administered prior to virus inoculation inhibited the specific immune response. 3. The deleterious effects of irradiation on antibody formation were not long lasting, in that virus reinoculated 30 days after x-ray exposure evoked a prompt secondary response equal to that of control mice. 4. X-radiation exposure prior to a second inoculation of virus inhibited the secondary response in both the previously irradiated and control mice. 5. These data are compatible with the hypothesis that increased viral proliferation in x-radiated animals is due to the inhibition of the antibody response.

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Received January 28, 1959. P.S.E.B.M., 1959, v100.

Progestational Activity of 6α-Methyl-17α-Acetoxyprogesterone. (24789)

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It is difficult to demonstrate progestational activity of 17α-hydroxyprogesterone as measured by endometrial proliferation in the rabbit whether administration is by the subcutaneous or oral route(1). In contrast, 17α-acetoxyprogesterone (AP)* is 4 to 6 times as potent as progesterone by subcutaneous injection and 2 to 5 times as potent as ethinyltestosterone orally in producing proliferative changes in the rabbit endometrium (unpublished). When assayed by the effect on endometrial carbonic anhydrase, AP was 7.3 times progesterone, and on the glandular total muscosal area (G/M ratio) it was 3.3 times as active as progesterone when administered subcutaneously(2). This compound has also been shown to exhibit a significant amount of oral activity(2). In women, 17α-acetoxyprogesterone is more than twice as potent as ethinyltestosterone as a progestin (3). A new progestin, structurally related to AP, 6α-methyl-17α-acetoxyprogesterone (MAP)†(4) has been reported to be more active than AP in G/M ratio and endometrial carbonic anhydrase studies(2). This paper reports its activity by the McPhail assay(5) and in an ovulation inhibition test.

Methods. McPhail rabbit endometrial proliferation test. Intact immature female rabbits were injected subcutaneously once daily for 6 days with 5 μg of estradiol benzoate in 0.2 cc of CMC (0.9% NaCl, 1% carboxy methylcellulose, 0.4% polysorbate 80, .042% propylparaben in water). Progestins were given in 0.2 cc of CMC per day subcutaneously or 2 cc of cottonseed oil per day orally. All doses are expressed as total amount of compound administered over a 5-day period. To eliminate bias in analysis, slides of the uterine sections were coded and identified only after grading had been completed. Because of the low oral potency of progesterone, the standard compound used was AP. Potency ratios derived from these data are not precise because of the inescapable subjective nature of the measurements and difficulty in statistical analysis of a measurement of this type. *Rabbit ovulation inhibition test.* This test was a modification of the method described by Makepeace *et al.*(6). The test animal was the adult rabbit weighing 2-4 kg. Test compounds were administered subcutaneously in 0.2 cc of CMC; 24 hours later each rabbit was caged with a male and allowed to copulate. Approximately 24 hours after copulation, laparotomy was performed and the ovaries examined for ovulation points.

Results. Endometrial proliferation. Results of these tests comparing the potency of MAP and AP on the McPhail assay are

* *Prodox*, registered trade mark, Upjohn Co., Kalamazoo, Mich.

† *Provera*, registered trade mark, Upjohn Co., Kalamazoo, Mich.