in part, to environmentally induced differences in the character of the intestinal bacterial flora.

Summary. Daily oral administration of 9 and 12 g of acid PAS to 7 patients in 9 experimental periods of 6 to 19 weeks duration resulted in significant reductions of the average total serum cholesterol concentration in each subject by 15 to 35%. The average fall for the group was 29%. Daily oral administration of 12 g of sodium PAS to 14 patients for 5 to 14 weeks was followed by a significant fall of average total serum cholesterol levels in only 7 patients by 11 to 26%. The average fall for these subjects was 19%. The remaining 7 patients showed no statistically significant variation of serum cholesterol following administration of oral sodium PAS. The absorption and fecal elimination of I-131 triolein was studied during the administration of PAS in a total of 20 experiments. In 6 patients I-131 triolein absorption tests were carried out during control periods and after the reduction of serum cholesterol levels by the administration of PAS. Five patients, in whom oral administration of sodium PAS failed to alter the concentration of serum cholesterol, were subjected to I-131 triolein absorption tests during control periods and following oral administration of sodium PAS. There was no significant difference between control and PAS periods in the average whole blood radioactivity and in the 72-hour fecal elimination of radioactivity in either of the two groups. It was thus concluded that the absorption of dietary triglycerides was not significantly altered by oral administration of the drug. The possible mechanisms by which administration of PAS lowered the concentration of serum cholesterol were discussed.

The authors are indebted to Dr. Harry H. Epstein, Triboro Hospital, Jamaica, N. Y., who kindly made available patients for study.

1. Samuel, P., Steiner, A., Proc. Soc. Exp. Biol. and Med. 1959, v100, 193.

2. Samuel, P., ibid., 1959, v102, 194.

3. Samuel, P., Waithe, W. I., Circulation, 1961, v24, 578.

4. Samuel, P., Shalchi, O. B., Holtzman, C. M., Proc. Soc. Exp. Biol. and Med., 1964, v115, 718.

5. Riska, N., Acta Tuberc. Scand., 1955, v30, 134.

6. Tygstrup, N., Winkler, K., Warburg, E., Lancet, 1959, v1, 503.

7. Tygstrup, N., Winkler, K., Jorgensen, K., Ugesk.-Laeger, 1961, v123, 255.

8. ———, Symposium on drugs affecting lipid metabolism, Elsevier, Amsterdam, 1961, p493.

9. Bogen, E., Loomis, R. N., Will, D. W., Am. Rev. Tuberc., 1950, v61, 226.

10. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., J. Biol. Chem., 1952, v195, 357.

11. Kaplan, E., Edidin, B. D., Fruin, R. C., Baker, L. A., Gastroenterology, 1958, v34, 901.

12. Walker, W. F., Stewart, W. K., Morgan, H. G., McKie, J., British Med. J., 1960, v1, 1403.

13. Grossman, M. I., Jordan, P. H., Gastroenterology, 1958, v34, 892.

14. Isley, J. K., Sharpe, K. W., Baylin, G. J., Sanders, A. P., Am. J. Roentgen., 1963, v89, 797.

Received October 2, 1964. P.S.E.B.M., 1965, v118.

Effects of X-Irradiation on Susceptibility of Neonatal Rat Brain and Muscle to Coxsackie B1 Virus Infection.* (29932)

LARRY SCHNECK AND SUMNER BERKOVICH† (Introduced by Bruno W. Volk)

Departments of Medicine and Pediatrics, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

Unlike adult mouse or rat brain, the immature neural tissues of the newborn animal are highly sensitive to ionizing radiation and susceptible to Coxsackie B virus infection. Susceptibility of mature brain is increased following ionizing radiation. This increase

* Supported in part by Health Research Council of City of New York under contract #U-1151 and U.S.P.H.S. under General Research Support Grant IGS-101.

† Career Scientist of Health Research Council of City of New York under contract 1-173.

has been related to the effects of radiation on antibody production and/or adrenal cortical secretion(1,2). However, the effects of X-rays on infectivity in similar tissues from the same immature animal have not been studied. If radiation was primarily injurious to the immature neural cells capable of supporting viral proliferation, viral yield should be decreased, whereas a predominant disturbance in vascular permeability of neural tissue should result in an increased yield of virus from the same tissues. Since there are metabolic similarities between muscle and brain(3), both tissues were studied concomitantly to detect changes that might have occurred in a tissue distal from the site of maximum X-irradiation.

We have found that infection of the newborn rat with Coxsackie B1 virus following large doses of X-irradiation primarily to the head resulted in an increased multiplication of virus in the tissues of the brain. Although the increase was measured initially only in brain, secondarily a delayed rise was also detected in skeletal muscle. These primary and secondary effects of X-irradiation on viral infectivity in the newborn rat will be described.

Materials and methods. Test animals and matched controls were obtained from litters born to rats from an inbred Wistar albino strain. The newborn animals at 2 to 3 days of age were inoculated intraperitoneally with Coxsackie virus type B1 24 hours after irradiation. Control animals were treated similarly but were not irradiated. One day or 6 days following virus inoculation surviving animals were guillotined and aliquots of blood and samples of skeletal muscle and brain were prepared for viral assay as 10% suspensions in Hanks' balanced salt solution. Infectivity titrations were done in monkey-kidney cell cultures‡ by techniques similar to those previously described(4). Results were reproducible to 0.6 of a log.

The strain of Coxsackie B1 virus used was originally recovered in monkey-renal cell cultures from stool from a patient with acute infectious myelitis. The agent was put through 2 suckling mouse passages and one passage in suckling rats. A suspension of rat brain was used to prepare the stock virus. All inoculations consisted of 0.05 cc of this stock virus representing approximately 50,000 $TCID_{50}$.

X-rays were given with a General Electric Maximar 250 Kv therapy unit. Radiation factors employed were as follows: 250 Kv; 15 ma; 1 mm Cu and 1 mm Al added filtration. X-rays were directed at the head of the rat through a 1 cm opening in a steel shield. The target to head distance was 17.9 cm. The dose rate measured in air with a Victoreen r-meter was 120 r/min. Measurements indicated that 1080 r were delivered to the head and 430 r to the body.

Results. The dose of X-rays or Coxsackie B1 virus used during these studies was uniformly lethal to the newborn rat. Seventy-five per cent of a test group of irradiated non-infected animals died between the 7th and 9th post-irradiation day. Non-irradiated infected controls and irradiated virus infected animals had a similar mortality rate. Because of this low salvage rate, observations were discontinued on the 7th post-irradiation day.

The histologic changes in the central nervous system of virus infected and non-infected newborn rats 1 and 7 days after irradiation were qualitatively similar. Such radiation effects have been described(5). However, the damage to the cerebral neurons and loss of both the external granular and Purkinje cell layers of the cerebellum was more extensive than previously described. The brain of the non-irradiated infected animal showed no histologic change until the 6th post-inoculation day. There was then a detectable loss of cortical neurons with scattered infiltrates of mononuclear cells.

In Table I are summarized data obtained from virologic study of irradiated and non-irradiated newborn rats sacrificed one day after inoculation with Coxsackie B1 virus. Note the increased yield of virus recovered from the brain of irradiated animals, especially from the cerebral hemisphere. The difference between the infectivity of irradiated and non-irradiated brain does not reflect an elevated blood titer, since the yield of virus

‡ Obtained from Microbiological Associates, Bethesda, Md.

TABLE I. Comparison of Coxsackie B1 Virus Titers in Tissues from Irradiated and Non-Irradiated Newborn Rats.

2 days post-X-irradiation—1 day post-virus.

	No. of rats	Cerebral hemispheres	Cerebellum and brain stem	Muscle	Blood
Irradiated	9	3.1*	2.4	1.8	2.4
Control	10	1.6	1.4	1.7	2.3
Irradiated/control		32†	10	1	1

* Average of reciprocal log 10 titers $TCID_{50}$/0.1 cc of a 10% suspension.
† Expressed as the numerical ratio.

TABLE II. Comparison of Coxsackie B1 Virus Titers in Tissues from Irradiated and Non-Irradiated Newborn Rats.

7 days post-X-irradiation—6 days post-virus.

	No. of rats	Cerebral hemispheres	Cerebellum and brain stem	Muscle	Blood
Irradiated	6	5.8*	4.8	4.2	2.0
Control	6	4.7	4.2	2.5	0.8
Irradiated/control		13†	4	50	16

* Average of reciprocal log 10 titers $TCID_{50}$/0.1 cc of a 10% suspension.
† Expressed as the numerical ratio.

TABLE III. Arithmetic Differences in Yield of Coxsackie B1 Virus Recovered 1 day and 6 Days Following X-Irradiation.

	Cerebral hemispheres	Cerebellum and brain stem	Muscle	Blood
Irradiated: 6 day/1 day	500*	250	250	3
Control: 6 day/1 day	1300	630	6	32

* Expressed as numerical ratio of average log 10 titers $TCID_{50}$/0.1 cc.

from blood was almost identical in both groups. The values obtained for muscle were also identical and unlike those for brain. The results obtained 6 days after viral inoculation are summarized in Table II. The titer of virus in all tissues except blood had increased. The yield from blood from non-irradiated animals has actually decreased. Now 6 days after virus the titers detected in the brain from irradiated animals, especially in the cerebral hemisphere, were still considerably elevated (13-fold difference) as compared to controls. However, unlike one-day animals the greatest difference was now measured in muscle harvested from the irradiated animals (a 50-fold increase).

Table III provides an arithmetic comparison of the ratios of the average log titers on day 6 and day 1 of both irradiated and control animals. Between 1 and 6 days post-infection, virus had continued to multiply in brain tissue from both irradiated and non-irradiated animals. Yet the difference between the initial and final viral concentrations measured was greater for the control animals. In contrast, for skeletal muscle this difference was greater in the irradiated group. Blood titer is decreased in both groups after 6 days but to a far greater extent in the control group.

In order to further evaluate the results presented in Table I, an additional group of newborn rats was studied. Thirteen hundred r were delivered only to the head or the body; non-irradiated areas were carefully protected by a thick (1½ in.) lead shield. Virus titers in the various tissues tested one day after the inoculation of the Coxsackie B1 test virus were similar to those measured in 6 matched non-irradiated controls (Table IV). It is likely, therefore, that the combination of both head and body radiation was necessary for the immediate viral effects that occurred.

TABLE IV. Comparison of Coxsackie B1 Virus Titers in Tissues from Irradiated and Non-Irradiated Newborn Rats.

2 days post-X-irradiation—1 day post-virus.

	No. of rats	Cerebral hemispheres	Cerebellum and brain stem	Muscle	Blood
Head irradiated	9	2.2*	2.1	2.0	3.3
Body "	8	1.9	1.9	1.7	3.0
Control	6	2.2	1.8	2.0	2.7

* Average of reciprocal log 10 titers $TCID_{50}$/0.1 cc of a 10% suspension.

Discussion. Large doses of ionizing radiation to the head increase the permeability of the "blood-brain barrier"(3) and cause extensive damage to neural tissues(5). These effects could have influenced virus multiplication in the newborn rat brain. Yet head irradiation alone did not result in an immediate increase in the yield of virus (Table IV); furthermore, body irradiation alone was similarly ineffective (Table IV). The early increase in susceptibility of irradiated newborn rat brain to Coxsackie B1 virus infection was probably dependent upon the effects of combined head and body radiation.

The delayed increase in the concentration of Coxsackie B1 virus recovered from skeletal muscle from irradiated animals is an example of a secondary effect in tissue distal from the site of maximum radiation. Other such effects have been related to X-ray stimulation of the hypothalamic-adrenal axis. No such adrenal response has been demonstrated in the newborn rat(2).

It has been shown that X-irradiation impairs antibody production in weanling and adult mice with a resultant increase in Coxsackie virus proliferation in various tissues (1). These findings do not adequately explain the elevated virus titers measured in our irradiated animals, since neutralizing antibodies were not even demonstrable in the control animals.

Summary. Large doses of X-irradiation primarily to the head of the newborn rat 24 hours prior to infection with Coxsackie B1 virus resulted in an immediate increase in the titer of virus recovered from brain. Six days later a delayed rise was also detected in skeletal muscle harvested from a site distant from the point of maximum irradiation. In brain the increased viral yield was probably related to the simultaneous effects of ionizing radiation on both the head and the body. There is no satisfactory explanation for the delayed effect noted in muscle.

We are indebted to Doctors Ludwig W. Eichna, Donald H. Ford and Jonathan T. Lanman for helpful suggestions, and to Mrs. Lydia Taranko and Mr. Donald L. Thompson for technical assistance.

1. Smith, L. W., Cheever, F. S., Proc. Soc. Exp. Biol. and Med., 1959, v100, 817.

2. Leblond, C. P., Segal, G., Am. J. Roentgen., 1942, v47, 302.

3. Dobbing, J., Physiol. Rev., 1961, v41, 151.

4. Scott, T. F. McNair, in Diagnostic Procedures for Virus and Rickettsial Diseases, 2nd Ed., Am. Public Health Assn., New York, 1956, 313.

5. Clemente, C. D., Yamazaki, J. N., Bennett, L. R., McFall, R. A., Neurology, 1960, v10, 669.

Received October 10, 1964. P.S.E.B.M., 1965, v118.