cardio-vascular system might account for the hypotension. Indeed this point of view can be defended even in the face of an obvious lack of histological evidence of such damage. An inspection of the data with regard to the spinal rats, however, excludes a possible direct effect upon the cardiovascular system. The 30-hour spinal rat's blood pressure, blood volume and hematocrit are not changed by irradiation. Should there be a direct effect one would expect the irradiated spinal animal to show a more profound hypotension than his non-irradiated control. Thus it is also difficult to conceive of histamine-like substances being the responsible agents, as has been claimed by Painter(6); for again one would expect a further lowering of the blood pressure in the spinal rat following radiation.

It is the remarkable failure of the irradiated spinal rat to differ from his non-irradiated spinal control—in so far as blood pressure, blood volume, and hematocrit are concerned—that lead us to the conclusion that depression of the nervous system is the cause of the immediate hypotension produced by large doses of total body X-ray which are not accompanied by changes in the blood volume or the hematocrit. Additional support for this hypothesis is found in the observations of Prosser *et al.*(7), that the immediate hypotension in rabbits after total body irradiation may be largely guarded against by vagotomy or atropinization; and that it can be antagonized by adrenalin. We have been unable to observe any histological changes in the central nervous system of the irradiated rats by conventional technics. We do not, however, feel that this is any assurance that there has been no change in function. That the autonomic nervous system can be damaged by external radiation has been demonstrated by Suzuki(8). However, as shown by Griffith and Pendergrass(9) the dose must be high, *i.e.* in excess of 800r.

Summary and conclusions. 1. The effects of large doses of total body radiation upon the blood pressure, blood volume, and hematocrit of normal adrenalectomized and spinal adult rats have been studied. 2. From the data presented it appears that the cause of the immediate hypotension following such irradiation is depression of the nervous system.

We wish to acknowledge the technical assistance of Miss Blair Burwell.

6. Painter, E. E., Prosser, C. L., Moore, M. C, Swift, M. N., *AEC Reports,* MDDC-761.

7. Prosser, C. L., Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., Swift, M. N., *Radiology,* 1947, v49, 299.

8. Suzuki, S., *Jap. J. Obst. and Gyn.,* 1931, v14, 410.

9. Griffith, J. Q., and Pendergrass, E. P., *Radiology,* 1934, v23, 463.

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A Simple Assay of Mammalian Pineal Extracts. (18931)

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Amphibian larvae react to feeding with, or injection of, pineal substance with a transient contraction of the skin melanophores. This reaction has been described by McCord and Allan(1) and subsequently by Hogben(2), Engel(3), and others, as occurring in tadpoles only until metamorphosis. At that period the response of the melanophores to pineal extract ceased in the species observed by these authors.

In the following report experiments are described, in which extracts of mammalian

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1. McCord, C. P., and Allan, F. P., *J. Exp. Zool.,* 1917, v23, 207.

2. Hogben, L. T., Pigmentary Effector System III, *Amphibia,* Oliver & Boyd, Edinburgh, 1924.

3. Engel, P., *Endocrinology,* 1939, v25, 144.

pineal bodies were injected into tadpoles and toads (*Xenopus laevis*). The melanophores of the skin of *Xenopus laevis* contract in a specific manner following an injection of pineal extract. This reaction could not be elicited with extracts from any other brain tissue tested. Injection of pituitary extract led to the known effect of expansion of the skin melanophores. The use of Xenopus for a simple assay of mammalian pineal extract is suggested.

Materials and methods. Seventy toads and 40 tadpoles were kept in glass containers at room temperature (about 21°C). Extracts were prepared from pineal bodies of freshly slaughtered pigs† and from one human pineal. The pineals were frozen on dry ice and macerated in a mortar with water, sand, and distilled water or saline. The mixture was then centrifuged and the supernatant fluid used for injection. For most of the work reported here a standard extract (extract A) was prepared by using 500 mg of tissue per cc of distilled water. The supernatant of the centrifuged material (extract A) was injected intra-abdominally, or into the posterior lymph sac of toads. One-tenth cc of the extract was injected into a specimen weighing approximately 50 to 100 g. The amount injected into tadpoles varied with the size of the specimen. Controls were injected with similarly prepared extracts of tissues from various parts of the brain, and with saline and distilled water.

Results. Five to 7 minutes after injection of extract A, the color of the animals started to change from the normal grayish green to a pale pink-white shade. After 30 minutes the height of depigmentation was reached. The toads appeared extremely pale and somewhat translucent. The melanophores remained fully contracted for 30 minutes. After this time, the color of the skin returned gradually to normal. The entire "cycle" (McCord and Allan) from injection to return of normal pigmentation ran a course of approximately one hour in toads and tadpoles alike. The tadpoles appeared colorless and translucent at the height of the reaction, and the change of the stellate melanophores into small spherical bodies could be observed under the dissecting microscope. Animals were used repeatedly for the test, after an interval of several days. The extract from one human pineal body produced an identical reaction. This specimen had been obtained at the post mortem of a white male, 68 years of age, who died of coronary thrombosis.

In higher concentrations than extract A the blanching became more extreme, and the duration of the cycle was prolonged. The highest concentration corresponded to 4,000 mg of pineal tissue per cc of water. Dilution of extract A to 16 mg equivalent of tissue per cc of distilled water led to the characteristic one-hour cycle. With dilution of the extract to a concentration of 8 mg of tissue per cc the bleaching was incomplete and spurious; in further dilutions the bleaching effect could no longer be obtained. The reaction could also be produced by injection of pineal extract into an ablated leg, or by immersion of excised skin into the extract. However, the effect of these procedures was weaker than from injection into the intact animal.

Boiling of the extract did not abolish the activity of pineal extracts upon melanophores. Dialysis for 24 hours against distilled water resulted in loss of activity in this respect.

Summary. (1) The African toad (*Xenopus laevis*) responds to the injection of pineal extract by a contraction of melanophores, in a manner similar to that previously described for amphibian tadpoles, prior to metamorphosis. This response is suggested as the basis for a simple assay of mammalian pineal body. (2) Preliminary chemical studies suggest that the melanophore contracting substance is a relatively stable, low molecular weight compound.‡

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‡ Since completion of this report, chemical studies on the melanophore contracting agent have been in progress. Epinephrine has been ruled out. (O.B.)

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