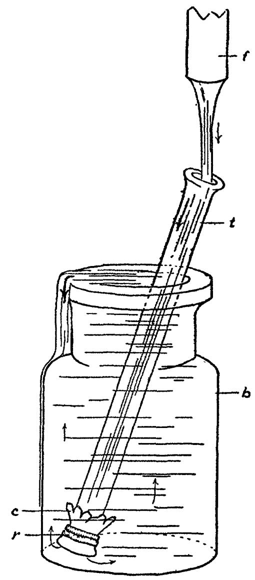
bottle a small stream of water is allowed to flow into the tube, directly from the faucet (f) if the bottle can be placed within a few centimeters of it, or via



an extension of rubber tubing, the end of which is supported a short distance above the glass tube. This procedure maintains a steady flow of fresh water over the material being washed as the column of water between the mouth of the bottle and the upper end of the tube exerts just enough pressure to cause a gentle flow through the tube. The waste escapes over the mouth of the bottle.

Modifications may easily be made to suit special needs. For example, if the tap water contains much foreign matter it may be strained by leading it through bolting cloth of finer mesh than that holding the organisms, before it is allowed to pass into the tube.

After washing, the material may be transferred to centrifuge or settling tubes for further manipulation. If preferred, it may be stained and dehydrated before removing from the apparatus by merely pipetting in the various fluids to be used, or by transferring the tube containing the organisms from one bottle to another containing the required reagents.

JOHN P. TURNER

UNIVERSITY OF MINNESOTA

SPECIAL ARTICLES

X-RADIATION AND REGENERATION IN AMBLYSTOMA

IT has been demonstrated within the past few years, especially by the work of Curtis[1] and his students, that x-radiation affects very markedly the regenerative activity in certain invertebrates. Coelenterates, planarians and annelids, for example, which under normal conditions readily regenerate lost parts, lose their capacity for regeneration when subjected to the influence of x-rays. Although considerable work has been done on the influence of x-radiation on regeneration in invertebrates, so far as the writer is aware no observations have been published on the effects of x-rays on the regenerative activity in vertebrates.

During the past two years the writer has conducted a series of experiments which deal with the effects of x-radiation on regeneration in embryos of Amblystoma, especially on regeneration of the fore limb. The fore limb of this animal is a convenient structure for use in experimental studies on regeneration, for the reason that under ordinary conditions it is very readily regenerated, and in embryonic stages of Amblystoma the regeneration takes place rather rapidly. The results of all experiments to date show conclusively that x-radiation in proper dosage will prevent normal regeneration of the limb in any stage of limb development.

When the limb bud is amputated at a very early stage of development, the amputation consists in the removal of merely the small mesodermal bud and its ectodermal covering. The wound heals quickly and, under normal conditions, regeneration of the limb bud and subsequent normal limb development takes place. However, the limb will fail to regenerate after amputation, if the embryo be given daily dosages of x-rays. Some growth takes place and a short spur-like appendage develops; but differentiation into upper arm, forearm and hand does not occur. Radiation appears to prevent differentiation during regenerative growth.

It is especially striking that a dosage of x-radiation which very definitely prevents limb regeneration has no apparent effect on normal limb growth and differentiation. If, for example, the limb bud on one side of the body is amputated at an early stage of limb development, and the embryo is radiated daily, the limb will fail to regenerate except for the development of a short spur-like appendage. In the same embryo, however, the unharmed limb bud on the opposite side of the body will grow and develop into a normal limb, apparently unaffected by the radiation. Other experiments, also, demonstrate this same phenomenon. For example, daily radiation of Amblystoma embryos has been begun in the blastula

[1] W. C. Curtis, "Old Problems and a New Technique," SCIENCE, 67: 141–149, 1928.

stage and continued during a period of over seven weeks. Embryos so radiated appear to develop normally, and limb growth and differentiation takes place in an apparently typical manner. However, if at any time during the course of the experiment a limb is amputated, it will not be regenerated; at the same time the unharmed limb on the opposite side of the embryo will continue normal development.

By means of x-radiation, regeneration can be as effectively prevented in late stages of limb development as in early stages. If an embryo, the fore limb of which is well developed into upper arm, fore arm and hand, be given daily dosages of x-rays no portion of the limb will be regenerated after amputation. This failure to regenerate under the influence of x-radiation occurs, moreover, regardless of the level at which the limb is amputated. The wound at the point of amputation heals as quickly as in normal unradiated controls, and there is a slight amount of growth at the cut end. However, there is no regeneration of the lost part such as takes place rapidly in unradiated embryos. The effect of x-radiation in preventing regeneration, therefore, appears to bear no relation to the age of the embryo, the stage of limb development, or the level at which the limb is amputated.

One of the significant results of this investigation is that x-radiation affords a method of studying experimentally the differentiation process in regeneration as compared with differentiation in normal embryonic development. It would appear that the differentiation during regeneration is in some important respect unlike the differentiation during normal development; the former is prohibited by a dosage of x-radiation that has no externally visible effect on the latter. Histological studies of non-regenerating limbs of Amblystoma embryos compared with normally regenerating limbs are now in progress.

The source of radiation for these experiments was a Coolidge medium focus tube. The factors governing the dosage of radiation were as follows: 65 kilovolts, 7 milliamperes, distance from target to embryo 25 cm., exposure from 3 to 5 minutes.

ELMER G. BUTLER
LABORATORY OF COMPARATIVE ANATOMY,
PRINCETON UNIVERSITY

EFFECTS ON THE GONADS OF CORTICO-ADRENAL EXTRACT[1]

AN apparent interrelationship between the adrenal cortex and the sex glands has frequently been noted by clinicians.[2,3] The fact that both tissues arise from a common mesodermal origin, and that hypertrophy of the adrenal cortex occurs in pregnancy and in sexual precocity, has strengthened this view. No adequate experimental evidence in confirmation of the hypothesis appears, however, to have been brought forward.

In an attempt to secure data relative to this possible interrelationship, immature albino rats were injected intraperitoneally with cortico-adrenal extract prepared in this laboratory according to the method of Swingle and Pfiffner.[4] This extract has been shown to contain the cortical hormone in sufficient amount to maintain adrenalectomized cats in good health for considerable periods of time, and to revive animals showing marked symptoms of adrenal insufficiency.[5] Litter mates which were used as controls were injected with corresponding amounts of normal saline solution. All animals used were of Wistar stock. They were fed on a standard meal diet.

Following one and two-week periods of treatment, the animals were killed and the gonads fixed in Bouin's solution. Identical methods of fixation, clearing and staining were employed in order that the observations might be in every way comparable. The results of the treatment are summarized in the accompanying table.

When histological preparations of the gonads were compared in all animals from the 25-day stage onward, it was observed that maturation of the experimental gonad, considerably beyond that seen in controls of the same age, had taken place. In the female this was evidenced by a greater amount of fluid within the follicles, and by increased follicular diameter. In the male, the difference was not so striking. The tubules of the experimental animals presented a more closely packed appearance, and a reduction and increased differentiation in the cells of the germinal epithelium was apparent. The general appearance was quite similar to that seen in young rats, following the injection of macerated pituitary gland.[6] The cortico-adrenal extract used, it should be mentioned, was in all cases sterile and protein free, and contained approximately 1 in 4,000,000 parts of adrenalin.

In several cases marked uterine enlargement was produced by cortico-adrenal extract injection. The

[1] Reported in brief at the meeting of the Federation of American Societies for Experimental Biology, Montreal, April 11, 1931.

Grateful acknowledgment is made of aid in the above investigations by the Grants-in-aid Committee of the National Research Council.

[2] W. Bullock and J. Segueira, *Trans.* Path. Soc. London, 56: 189, 1905.

[3] L. Guthrie and W. d'E. Emery, *Trans.* Clin. Soc. London, 40: 175, 1907.

[4] W. W. Swingle and J. J. Pfiffner, *Anat. Rec.*, 44: 225, 1929; *Am. Jour. Physiol.*, 95: 153, 164, 1931.

[5] S. W. Britton and Herbert Silvette, SCIENCE, 73: 322, 373, 1931.

[6] E. L. Corey, *Physiol. Zool.*, 3: 379, 1930.