

with external symptoms (vertigo) of avitaminosis-B₁. (One male survived a test period of 56 days without supplementary vitamins, showing no loss or gain on his starting weight.) Here again, replacement of rice starch by wheat starch produced no demonstrable effect.

We have, however, found wheat starch unsuitable for diets used in work on the rat 'filtrate factor'. Our animals never reach a true growth plateau on a filtrate-factor-free diet, but their weight-increase slows down to such an extent that statistically significant differences in weight-increases can be produced by supplements of filtrate factor in the ratio of 2 : 1, provided suitable response levels are chosen. This is an adequate basis for reasonably accurate vitamin assay. When, however, wheat starch replaces rice starch in our routine diet used for these tests, many of the animals show no slackening of growth after 4 weeks: discrimination between negative controls and animals receiving filtrate factor concentrate made from 32 gm. of fresh liver is seldom possible. With the rice starch-containing diet, groups of 3 animals are sufficient to detect the presence of such a concentrate in amounts made from only 8 gm. of liver. It would appear that the wheat starch we used had adsorbed or otherwise retained appreciable quantities of filtrate factor from the wheat berry.

We have no information about the effect of wheat starch on diets used for work on vitamin B₆ (adernin, 'eluate factor').

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Tumour Induction in *Rana pipiens* Tadpoles

DURING the normal development of Amphibians the well-known 'fields' of organization operate to induce morphological differentiation¹. Furthermore, adult Urodeles and larval Anurans are known to retain local fields which are active in the regeneration of parts². Therefore, problems in abnormal growth may well be studied in those Amphibians which retain power of regeneration. One of the commonest present-day methods of experimentally producing abnormal growth is by means of carcinogenic substances, but apparently these substances have been little used for such studies in Amphibia. Recently, however, Koch, Schreiber and Schreiber³ have induced tumours in adults of *Triton cristatus* and *Triton taeniatus* with 1 : 2 benzpyrene and with carcinogenic tar. The present report deals with induction experiments with *Rana pipiens* tadpoles using methylcholanthrene-choleic acid as the carcinogenic agent.

During February and March of 1939, 154 young tadpoles (20-30 mm. total length) were each injected subcutaneously near the base of the tail or behind the ear with about 0.2 mgm. of methylcholanthrene-choleic acid in crystalline form or in solution in lard. Healing occurred satisfactorily immediately after the operation, but during the subsequent three or four weeks the skin above the injected mass perforated and the carcinogen was lost in most cases. 94 control tadpoles were injected with lard alone or with paraffin. All animals were reared at room temperature (c. 22°). Eleven experimental and four control tadpoles died and were autopsied before metamorphosis; they had developed no growths. All of the surviving animals were autopsied during June, July and August of



TUMOUR INDUCTION IN *Rana pipiens* TADPOLES (× 61).

1939 at about the time of metamorphosis. Thirty-nine of the ninety-four control tadpoles had retained the lard or paraffin; no growths were found. Of the original 154 experimental tadpoles only 12 retained the injected mass and in 3 of these subcutaneous tumours had developed in tissue surrounding the carcinogen. In two cases (*MC* 512 and *MC* 711) observed 95 and 158 days after injection respectively the tumour remained localized, forming a firm, non-pigmented mass of tissue about 2 mm. × 1 mm. × 0.5 mm. in size in hosts of about 22 mm. body length. In the third case (*MC* 62-119 days) the induced tumour showed signs of malignancy. From the injection site it had spread subcutaneously, penetrating the body wall musculature and invading the posterior part of the left lung. At the time of autopsy it covered an area of 5 mm. × 1.8 mm. and was 1 mm. to 1.5 mm. thick. This is a large tumour in proportion to the size of the host—a metamorphosing tadpole of 18 mm. body length.

The accompanying photograph illustrates some of the structural features of this growth. From the original site of injection in the loose subcutaneous tissue (*S*), the tumour has grown outward, spreading along the base of the dermis (*E*). Growing in the opposite direction, it has penetrated the body wall musculature (*M*), expanded as a sizable mass in the coelom (*T*) and invaded the lung wall (*L*). Histologically it appears to be a connective tissue tumour of mixed cell type. Cells are spindle-shaped, round, or intermediate; also they are quite variable in size. Generally the cells are closely packed and give no evidence of differentiation.

One of the two localized tumours (*MC* 711) presents the same histological picture as that described above for *MC* 62. The second localized tumour (*MC* 512) was transplanted subcutaneously to five tadpole hosts. Apparently the tumour tissue did not take in this small number of cases, for no abnormal growths have appeared in six months.

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¹ Spemann, H., "Embryonic Development and Induction" (New Haven, Conn.: Yale University Press, 1938).

² Weiss, P., "Principles of Development" (New York: Henry Holt and Co., 1939).

³ Koch, C., Schreiber, B., and Schreiber, G., *Bull. de l'Association française pour l'Étude du Cancer*, 28, 852 (1939).