Propagation of Frog Renal Carcinoma in Embryonated Hens' Eggs.* (22332)

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The frog renal carcinoma (Lucké) is readily propagated in tissue culture and by intraocular implantation in frogs; homo- and heterotransplants can be made(1). It can also be grown by implantation of tadpoles (2). In the salamander the character of the tumor is reported to be altered(3). Propagation of the tumor in embryonated hens' eggs was attempted in the present study.

Materials and methods. The tumors used were from experimental frogs kindly sent by Dr. B. Lucké from Philadelphia. Naturally occurring renal tumors were also obtained from *R. pipiens* received from J. M. Hazen Co., Alburg, Vt. Embryonated eggs (White Leghorn) 8 to 12 days of age were obtained from local commercial dealers. Minced tumor tissue was placed on the dropped chorio-allantoic membrane. The window previously cut in the shell was closed and sealed with paraffin. After incubation at 35°C for 8 to 12 days the eggs were opened and the membranes were examined for growth of tumor. Histologic examination of the implanted tumor tissue and membrane was made periodically. Egg passaged tumor tissue was tested for host stability by intraocular implantation of frogs.

Results. In one experiment tumor was carried through 13 serial passages. Tumors appeared as discrete growths on the membrane and attained a size of 2-5 mm diameter. Sections of the chorio-allantoic membrane showed infiltration of the mesoderm by the tumor either in the form of cystic, papillary growths or as loosely infiltrated nests of cells. The series of passages was terminated by failure of the tumor to grow. In other attempts tumor was passed through 4 passages on 3 different occasions and through 5 and 6 passages in separate experiments. It was felt that eventual failure to grow might be associated with a tendency of the implanted tissue to become desiccated before it could become established. An attempt to avoid this difficulty was made by imbedding minced tumor fragments in chick embryo blood or adult chicken plasma clots before implantation to the egg membrane. A few drops of modified amphibian Ringer's solution (containing phosphate buffer and antibiotics) also was sometimes added onto the tissue immediately after implantation. This addition was not essential since equally good growth was obtained without it. By use of the clot technic tumor was carried through 20 serial passages. Although some tumors were as large as 10 mm in diameter the average size was 2-3 mm. Egg passaged tumor could be stored, minced in modified amphibian Ringer's solution at 4-6°C for 2 to 10 days between transfers.

Two of 4 frogs given ocular implants of tumor tissue from the 20th egg passage developed local tumor growths and had kidney tumors when examined 2 to 6 months after inoculation. Attempts to grow egg passaged tumor in the yolk sac of 5-day embryos failed. Microscopic examination of sections of the yolk sac made 13 days after implantation revealed only few, necrotic tumor cells.

Frog tumor grown at 36-37°C in roller tube, tissue cultures containing chicken plasma clot, and modified amphibian Ringer's solution grew readily in eggs after culture, *in vitro*, for 3 to 5 weeks(4).

Discussion. Heterologous transplants of amphibian tumor tissue to hens' eggs grew irregularly in serial passages. Vigorous growth of tumor in one passage might be followed by poor growth or failure to grow in the next passage or *vice versa*. It is not clear to what extent this is due to failure of the tissue to establish contact with the chorio-allantoic membrane suitable to permit nourishment of the tumor. It seems unlikely that the relatively high environmental temperature is in-

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hibitory since the tissue, *in vitro,* survived a temperature of 36-37°C for a prolonged period. The relatively slow rate of growth of the tumor tissue possibly contributes to the difficulty of implantation on the membrane. There was no evidence of alteration in the type of growth of tumor tissue during the course of 20 heterotransplants, such as was noted elsewhere during passage of frog renal carcinoma in newts(3) and during passage of a murine carcinoma in eggs(5).

Summary. The frog renal carcinoma (Lucké) has been propagated on the chorioallantoic membrane of hens' eggs for 20 serial passages. Growth was irregular.

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Glycogen Stores in Glucagon-Treated Rats. II. Effect of Alloxan Diabetes.* (22333)

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Chronic treatment with small doses of glucagon causes no change in liver glycogen(1), while large doses may cause a significant increase(2). Further study of this seemingly paradoxic effect of the "glycogenolytic" hormone demonstrated that, although single intraperitoneal injections of large doses of glucagon into normal rats cause an immediate decrease in liver glycogen, this is followed 24 hours later by a marked increase above normal levels(3). When the animals are sacrificed 24 hours after the last glucagon injection, as is done in chronic experiments, the short-lived glycogenolytic effects are no longer detectable because glycogen has been resynthesized. This resynthesis of glycogen may be due to 3 causes: 1) reversal of the phosphorylase reaction(3); 2) increased secretion of adrenal cortical hormones, in a manner similar to that which follows the injection of epinephrine(4); or 3) increased secretion of insulin, as a result of glucagon hyperglycemia (5). The purpose of this work was to investigate the third hypothesis by studying the effect of glucagon and epinephrine on liver glycogen, muscle glycogen and adrenal ascorbic acid in alloxan-diabetic rats.

Materials and methods. Seventy-two adult Sprague-Dawley male albino rats were used. The animals were kept on a diet of Purina checkers *ad libitum* and fasted for 24 hours before the experiments. Alloxan diabetes was produced according to the method of Sturtevant(6). Since glycogen deposits of alloxanized animals vary with the severity of the diabetes(7-9), only rats with established diabetes of more than 6 weeks' duration and with severe (4+) glycosuria and polyuria were used.‖ Glucagon (Eli Lilly and Co., lot no. 208-158B-197; 2.5 or 5.0 mg/kg),¶ epinephrine (0.15 mg/kg) and glucose (1 g/kg) were injected intraperitoneally. After 2 or 24 hours, the animals were decapitated, samples of liver and of gastrocnemius muscle and both

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¶ Glucagon was a gift of Dr. O. K. Behrens of the Lilly Research Laboratories.