SPECIAL ARTICLES

AGE SUSCEPTIBILITY OF DUCKS TO THE VIRUS OF THE ROUS SARCOMA AND VARIATION OF THE VIRUS IN THE DUCK[1]

THE duck so far considered as a resistant species[2] can be infected with the Rous virus as long as two conditions are fulfilled: (*a*) that newborn individuals are injected, preferably by the intravenous route; (*b*) that large amounts of virus are used. So far, it has not been possible to infect Pekin ducks older than one day with any dose of virus or newborn ducklings with amounts of virus lower than 4 cc of filtered or 2 cc of non-filtered extract of tumor at 1:20 dilution.

The intravenous infection of the duckling by the Rous virus manifests itself in two ways: (*a*) Development of a hemorrhagic disease, fatal within a few weeks with blood blebs and extravasations in viscera much as in newborn chicks similarly infected with the virus.[3] As in the latter host the hemorrhagic lesions both grossly and microscopically are or are not accompanied by tumor formation. (*b*) Development of one or a few sarcomata in varied locations several weeks or even months after injection when the bird is full grown. These two sorts of results have also been obtained when newborn ducklings were injected in the breast with large amounts of tumor filtrates, but not enough to induce local growths: blebs or tumors were found in viscera when the birds died or were killed several weeks or months later.

Once tumors have been induced in ducks by the chicken virus, the disease can easily be transmitted by grafts or filtrates to other ducks without much regard for the age of the host. The disease in the duck injected in the vein with the passage virus is characterized by the development of wide-spread tumors, mostly in the skin and digestive tract, but also in the skull, ribs and muscles, and less frequently in other locations. If cells are grafted, then rapidly growing local tumors develop, and if the host lives long enough the same wide-spread disease results as when filtrates are injected in the vein. Either after vein injections or after grafting there develop hemorrhagic lesions accompanied or not by tumor cells, and its incidence as well as that of generalized tumors is in an inverse relation with the age of the host. Tumors in the duck are typical in many respects and can easily be distinguished from the original chicken tumor.

But in the same way that the virus has acquired this capacity to infect ducks, it has lost its original one to infect adult chickens, as shown by many experiments where extract of the duck-passed virus was injected in large amounts into these hosts. Apparently, this change takes place suddenly as soon as the chicken virus has infected the duck cells, for extracts of hemorrhagic and neoplastic lesions from ducks infected with the original Rous virus have consistently failed to induce tumors in adult chickens. Also injection of large amounts of duck tumor cells into chickens resulted either in no tumors or in tumors that regressed after an initial period of growth. Three of such chickens reinjected with cells and two with filtrates of the original Rous tumor proved to be as susceptible as normal controls.

Identical phenomena were observed after the chicken tumor was passed through eight generations of ducks by means of cell suspensions. In the course of these experiments the observations of Purdy[2] concerning the age susceptibility of ducks to Rous tumor cells were amply confirmed. Therefore, whatever the defensive forces of the host are, they work equally well against both the causative virus and the result of its action, the malignant cell.

When young chicks are injected intravenously with the duck variant of the Rous virus, they develop a disease which is wholly different from that induced in these hosts by the original Rous virus similarly injected.[3] All of 20 chicks from 1 to 3 days old injected intravenously with filtrates of duck tumors from the 4th and 6th virus passage died in from 20 to 40 days of a condition characterized by the development of multiple sarcomata mostly in both flat and long bones, but also in skeletal muscles, and only occasionally in viscera. The bone tumors proved to arise from the periosteum and endosteum, and rapidly invaded the marrow cavity. In the non-invaded part pronounced non-malignant formation of new bone was the rule.[4] Analogous results have been obtained with the duck virus at its 9th and 13th passage with the difference that the incidence of tumors induced has so far been very low, and then they were not manifest until from 50 to 100 days after the injection. Filtrates or cell suspensions of the periosteal tumors injected into the vein or breast of young ducks have induced the typical duck disease, and if injected into chicks and pullets have reproduced the osteo-muscular disease in 3 successive passages.

In conclusion, it has been shown that a virus inducing sarcoma in one species (chicken), can infect another species (duck) if injected within a short period after birth, and sarcomata resulting from this infec-

[1] This investigation was aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

[2] W. J. Purdy, *Brit. Jour. Exp. Path.*, 13: 473, 1932.

[3] F. Duran-Reynals, *Yale Jour. Biol. and Med.*, 13: 61 and 77, 1940; F. Duran-Reynals and E. Estrada, *Proc. Soc. Exp. Biol. and Med.*, 45: 367, 1940.

[4] Histological study of these tumors as well as of other lesions in chicks and ducks was carried out by Dr. Henry Bunting.

tion may not be noticeable until later in life when the animal has almost attained maturity. The infection is attended by a sudden variation[5] in the virus manifesting itself by its inability to infect adult individuals of the original species; by the sort of cells attacked and characteristics of resultant tumors in the new species; and possibly by changes in its antigenic make-up. Duplicating the same sequence but in an inverse order, infection of the original species by the virus variant is only accomplished in the very young individual, which weeks or months later may develop a neoplastic disease radically different from that induced in it by the original virus. But this back infection is not attended by any other obvious variation in the virus which now attacks young individuals of both species.

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INOSITOL AND SPECTACLED EYE IN RATS

IN the biological assay for vitamin B_6 employing the diet of Halliday and Evans,[1] it was observed that denudation around the eyes could be induced in almost 100 per cent. of the fifteen animals when nicotinic acid was given at weaning in addition to thiamin, flavin, pantothenic acid and choline. This syndrome was similar to that reported by Oleson, Bird, Elvehjem and Hart,[2] except that the spectacled eye was not complicated by the exudation and final closure which Unna[3] reports could be cured by pantothenic acid.

Apparently the spectacled eye, as previously reported by other workers, is a multiple deficiency requiring at least two factors, one of which is pantothenic acid and the other a factor concerned in the growth of hair.

It is known that filtrate factor concentrates of liver, yeast and cereal grains can cure both the denudation around the eyes as well as the exudation condition. Since the true spectacled eye might be thought of as a type of alopecia, and since Woolley[4] has shown the mouse alopecia factor to be inositol, this compound was fed to rats in an attempt to cure the spectacled eye as produced under our conditions. 10 mg of Eastman's inositol were administered per rat per day. The results were quite dramatic. The swelling around the eyes disappeared within 24 hours and in three days definite signs of hair restoration were evident. By 10 to 14 days the halo around the eyes was completely overgrown with hair and the rats could not be distinguished in this respect from normal animals.

In harmony with Woolley's[4] report, a definite response in growth accompanied the above changes. On the Halliday and Evans diet supplemented with crystalline thiamin, flavin, nicotinic acid, pantothenic acid, pyridoxin and choline, these control animals showed an average weekly gain of 10 grams. When 10 mg of inositol were given in addition, the average weekly gain was 15 grams.

We believe the evidence demonstrates inositol to be the factor concerned with the regeneration of hair in the condition referred to as "spectacled eye" in rats. In addition, inositol has been shown to have a function in the growth of the rat.

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[5] The word *mutation* is purposely avoided until an agreement is reached as to whether or not it is permissible to use it in fields other than genetics.

[1] N. Halliday and H. M. Evans, *Jour. Nutrition*, 14: 45, 1937.

[2] J. J. Oleson, H. R. Bird, C. A. Elvehjem and E. B. Hart, *Jour. Biol. Chem.*, 127: 23, 1939.

[3] K. Unna, *Jour. Nutrition*, 20: 565, 1940.

[4] D. W. Woolley, SCIENCE, 92: 384, 1940.

THE DEVELOPMENT OF EARLY MOUSE EMBRYOS IN THE EXTRAEMBRYONIC COELOM OF THE CHICK[1]

IN preparation for an experimental study of the development of certain hereditary abnormalities affecting early embryonic stages in mice, a new method for cultivating entire early mouse embryos has been developed. Mouse embryos at the ages of 7 to 8 days, *i.e.*, egg cylinder to six somite stages, were removed from the uterus within their decidua and transferred into warm sterile Ringer's solution. They were then dissected out of the decidua and Reichert's membrane was pulled off the egg cylinder. For the transplantation of stages older than the egg cylinder all membranes were removed from the embryo as completely as possible. The embryos were transplanted into 72 to 80-hour chick embryos in such a way that they came to lie in the extraembryonic coelom. A window was cut into the egg shell, then a slit was made into the vitelline membrane and the serosa; the mouse embryo was transferred onto the membranes with a Spemann pipette and pushed through the slits into the extraembryonic coelom next to the allantois with the help of glass needles.

At examination after 24 or 48 or 75 hours the mouse embryos may be found floating freely in the extraembryonic coelom, or attached to any of the extraembryonic membranes; in some instances they are attached to the allantoic stalk. Some operations were performed in which the mouse embryo was pushed partly into the coelom at the place of open communication between intraembryonic and extraembryonic coelom next to the allantoic stalk. Such embryos con-

[1] These studies were aided by a grant to Professor L. C. Dunn from the Fund for Research of Columbia University and from the Josiah Macy, Jr., Foundation.