Response of the Central Nervous System of the Chicken to Methylcholanthrene: Failure To Induce a Neoplastic Process after 56 Months*

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INTRODUCTION

Tumors derived from nervous tissue have been induced with methylcholanthrene (8, 7), benzyrene (8, 9), and dibenzanthracene (1) in mice and in rats when the carcinogen was used in a high concentration and when sufficient time was allowed for it to act. Methylcholanthrene has been the most successful of the three carcinogens employed when used in at least a 30 per cent concentration fused with cholesterol (5, 6). A dietary factor consisting of a periodic deficiency of thiamine and riboflavin was found to reduce significantly the length of the induction period in the methylcholanthrene tumors in rats (5, 6). Alteration of the intracellular metabolism of the cells from the riboflavin and thiamine deficiency was suggested as the factor causing the cells to respond more readily to the carcinogen. To study further the observed dietary effect on the induction period, it was decided to repeat the experiment with the use of another species. Chickens were selected, because intracranial tumors have not been previously induced in fowl, and their susceptibility to thiamine deficiency offered an opportunity to pursue further the reported effect of diet on the induction of intracranial tumors in rats.

The following communication reports these experiments on chickens, with the same experimental procedure and concentration of carcinogen that had proved successful in the production of gliomas in rats and mice.

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EXPERIMENTAL PROCEDURE

Eighty-six pedigreed white leghorn pullets about 4 months of age and weighing approximately 700 gm. were employed.

Carcinogen.—Pellets of methylcholanthrene were made in a 30 per cent concentration prepared according to the technic of Peers (9). The methylcholanthrene was obtained from the Eastman Kodak Company. The technic of fusing the carcinogen with the chemically pure cholesterol has been described elsewhere (5, 6). When prepared, the pellets were 2 mm. in diameter, approximately 3 mm. in length, and weighed approximately 25.0 mg.

Operation.—The pellet was implanted in the right cerebral hemisphere without the use of anesthesia. The skin of the right superior aspect of the head was incised to the periosteum. A hole was made in the calvarium with a dental bur approximately 3 mm. in diameter. The pellet was inserted into the brain through the bur hole following an opening made in the dura with a pointed scalpel. After insertion the pellet was pushed laterally so that it would not be extruded from the wound. The skin margins were approximated with a silk suture.

Dietary history.—Since it was planned to subject the chickens to periodic deficiency of thiamine, the danger of losing animals in the experimental periods would be lessened if mature animals were used. The periods of deficiency, therefore, were started following a 188-day growth period after the operation. During this time, the chickens were fed, ad libitum, Purina Broiler Chow, an all-sustaining ration for growing chickens. At the beginning of the experimental period there were 48 chickens surviving the operation and growth period. These chickens, averaging 1,625 gm., were divided into four groups of 12. Several chickens died of fowl leukosis and prolapsed intestine.
It was determined that the thiamine in the Purina Broiler Chow could be inactivated by autoclaving at 50 pounds pressure for 3 hours. Chemical analysis for thiamine, done through the courtesy of the Ralston Purina Company of St. Louis, for varying time periods was as follows:

- Purina Broiler Chow (1 hr. autoclaving) — 0.85 p.p.m.
- Purina Broiler Chow (2 hr. autoclaving) — 0.15 p.p.m.
- Purina Broiler Chow (3 hr. autoclaving) — 0.14 p.p.m.

It was felt that the reduced value of thiamine obtained in the 3-hour autoclaving would be sufficient to induce thiamine deficiency in the chickens. On this autoclaved food the chickens showed symptoms of thiamine deficiency characterized by leg, neck, and general body weakness beginning at about the fourteenth day. The chickens were subjected to three 25-day periods of deficiency in 6 months. Recovery periods of 30–40 days were allowed. In order to lengthen the period of deficiency with less risk of losing animals, thiamine hydrochloride was given intramuscularly to the experimental chickens when the deficient diet was discontinued. A dramatic clinical response was uniformly seen with the chickens, all resuming their normal eating habits. It was necessary to remove the chickens to a laboratory in another state where it was not possible to carry on the deficiency periods.

Necropsy and histologic technic.—A complete autopsy was performed on the chickens dying during the experimental period, and the six chickens that survived 4 years and 8 months after the implantation of the pellets. The calvarium was opened and the brain fixed in neutral 4 per cent formaldehyde (U.S.P. Formaldehyde 1:10). Following adequate fixation, the brain was sectioned with a razor blade, and a section 2 mm. in thickness was taken containing the pellet for microscopic study. Hematoxylin and eosin stains were employed.

RESULTS

Fifteen chickens survived for over 2 years; ten survived 3 years, and 6 were killed 4 years and 8 months after implantation of the pellet. None of the chickens dying during the experiment showed any clinical signs of intracranial tumor. No tumors were observed in the gross or microscopic examination of the brains. Study of the microscopic sections prepared from the brain at the site of the insertion of the pellet showed in all instances a broad zone of gliosis surrounding the pellet. Frequently, the area of gliosis contained small foci of calcification. The pellets were in contact with brain tissue and, usually, the overlying leptomeninges. Foci of macrophages, lymphocytes, and plasma cells were found in the glial scar and frequently in the subarachnoid space close to the inserted pellet. In those brains where the gliosis was prominent, the blocks were serially sectioned to be certain that the gliosis was not a neoplastic change.

COMMENT

The results of these experiments indicate that, under the conditions produced, the central nervous system of the chicken is resistant to carcinogenic stimulation by methylcholanthrene in a concentration that regularly produced tumors in mice and rats. Peers (3) reported glial tumors produced with pellets of methylcholanthrene in mice in 10 per cent concentration fused with cholesterol. The experiment was terminated at the end of 183 days, and 15 of the 32 tumors produced were of glial origin. Zimmerman and Arnold produced glial tumors in mice with pellets of pure methylcholanthrene (8, 9). The average exposure period for mice developing the 25 gliomas reported by these authors was 279 days. One of us (W. O. R.), using pellets of methylcholanthrene in 30 per cent concentration fused with cholesterol, as was used in the experiments reported here, induced fourteen tumors of glial origin in rats with an average exposure period of 299 days (5, 6). In those experiments, periods of riboflavin and thiamine deficiency were found to reduce significantly the induction period of the tumors (5, 6). It is not likely that the three periods of thiamine deficiency given the chicken in our experiments could be regarded as an adequate test to influence tumor development. The length of the experiment, however, is particularly significant.

The six chickens that lived 1,580 days following the implantation of the pellet represents 54 times the longest average exposure necessary for rats and mice to develop tumors. The fifteen chickens surviving for over 2 years were exposed for nearly 3 times as long as the highest average period necessary to produce tumors in rats and mice. It is unfortunate that the period of thiamine deficiency could not have been continued longer, since the altered metabolism of the brain due to the deficiency might have assisted the carcinogen in inducing tumors.

SUMMARY

Pellets of methylcholanthrene in 30 per cent concentrations fused with chemically pure cholesterol were implanted into the right cerebral hemisphere of 54 chickens. Thirty-one chickens survived for more than 2 years and six for 4 years
and 8 months. No tumors were induced. It may be concluded from these results that the central nervous system of the chicken is resistant to neoplastic change by carcinogenic stimulation of greater duration than will regularly produce it in rats and mice.

REFERENCES

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