The Response of the Central Nervous System of the Rat to Methylcholanthrene

II. The Effect of a Diet Deficient in Thiamine and Riboflavin on the Induction of Tumors Derived from Nervous Tissue*

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INTRODUCTION

The work of Kinosita (7), demonstrating a dietary factor in the production of carcinoma of the liver in rats with p-dimethylaminoazobenzene, and that of Kinsler, Sugiura, Young, Halter, and Rhoads (6), who showed that riboflavin and casein can protect against this compound, suggested the possibility of influencing similarly the induction of tumors in nervous tissue by methylcholanthrene. Previous work, indicating that the cells of the central nervous system of the rat are resistant to carcinogenic stimulation (12, 15, 19, 22), offered an additional impetus to determine if this could be enhanced by altering intracellular metabolism through vitamin deficiency.

The following communication reports the effect of periodic removal of thiamine and riboflavin from the diet of rats with pellets of 30 per cent methylcholanthrene implanted in their brains. Thiamine and riboflavin were selected for study because these substances are known to be concerned with enzyme systems and intracellular metabolism (3); moreover, a deficiency of either produces pathologic change in nervous tissue (17, 18).

The morphologic and histologic study of the tumors that were induced are reported elsewhere (14).

MATERIAL

Eighty-five young male or female rats of the Rockland strain, about 3 months of age and averaging approximately 120 grams in weight, were employed. There were 55 females and only 30 males, because of the greater availability of female animals from the breeding colony in the Department of Pathology. Young growing animals were selected, rather than adults, so that the effect of the vitamin deficiency could be more readily observed in the weight curves (Fig. 1).

Pellets of 30 per cent methylcholanthrene fused with chemically pure cholesterol, each weighing approximately 25 mgm., were implanted in the right cerebral hemisphere. The details of making the pellets and the operation for implanting them, as well as the necropsy and histologic technic employed for diagnosis of the tumors, have been described elsewhere (14).

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1 Niacin was also removed, but since the rat can synthesize this substance its elimination from the diet is probably not significant.
would have to be maintained on them for a long period of time, it was thought advisable to place more rats in the deficient group, since unavoidable losses in this group would probably be heavier than in the control groups that received adequate amounts of vitamins.

The stock formula used for the incomplete preparation of the complete and deficient diets was:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>73.0%</td>
</tr>
<tr>
<td>Commercial casein*</td>
<td>16.0</td>
</tr>
<tr>
<td>Salt mixture No. 185, McCollum's</td>
<td>5.0</td>
</tr>
<tr>
<td>Cod liver oil (U.S.P.)</td>
<td>5.5</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Thoroughly washed with acidulated water.

The complete diet, for the control rats, was made by adding the following amounts of synthetic vitamins to 100 gm. of the mixture above:

- Thiamine ............... 0.75 mgm.
- Riboflavin ............ 1.1 "
- Niacin ................ 9.4 "
- Pyridoxine ............ 1.13 "
- Pantothenic acid ...... 2.82 "
- Choline ............... 94.0 "

The deficient diet for the experimental rats contained all the ingredients given alone for the complete diet except thiamine, riboflavin, and niacin.

The diets were prepared by mixing the corn starch, casein, salt mixture, cod liver oil, and wheat germ oil with water to a thick consistency, and adding appropriate amounts of the synthetic vitamins dissolved in water. When prepared, the mixtures were kept in a refrigerator and fed daily to the animals in small dishes. Fresh diet was prepared every 3 days to avoid possible contamination of the food by molds.

**EXPERIMENTAL PROCEDURE**

Following a 43 day recovery period after the operation the rats were placed on the diets. After 5 weeks those receiving the deficient diet failed to gain as rapidly as those given the complete diet, appeared less active, had ruffled and poorly kept fur, and were irritable when handled. Following a 3 week period in which the animals on the deficient diet failed to gain weight comparably with the controls, one drop of a solution containing approximately 18 μgm. of thiamine, 45 μgm. of riboflavin, and 18 μgm. of niacin was given orally to each rat for 2 days. Following the administration of these vitamins the animals promptly gained weight and their general physical appearance improved remarkably. The improvement generally lasted for 3 to 4 weeks, when the same symptoms reappeared and the average gain of weight again was reduced and finally converted into a loss. This routine of successive periods of deficiency was successfully carried out for 435 days, when the last rat in the deficient group died. During the course of the experiment the control rats gained on an average about 75 gm. less per animal than the deficient ones (Fig. 1). Both the deficient and control groups of rats attained their maximal growth and weight approximately 227 days after the experiment was started. This weight was maintained as a well defined plateau for about 70 days, when both groups simultaneously lost an average of approximately 60 gm.; this second level was maintained for the remainder of the experiment. The loss of weight noted after the plateau had been maintained for 70 days was probably the result of some other deficiency factor, since both groups showed a nearly identical response.

**RESULTS**

Twenty-one of the 42 rats that survived past the time of appearance of the first tumor developed an intracranial neoplasm. The incidence in the deficient rats as compared to the controls was approximately the same: 46 per cent (11 of 24) in the deficient group and 50 per cent (9 of 18) in the controls. Fourteen of the rats developed tumors derived from nervous tissue, and 10 had tumors derived from connective tissue. Three had both types.

The incidence of tumors derived from nervous tissue was nearly the same in the deficient and control groups: 39 per cent (7 of 18) in the controls, and 35 per cent (7 of 20) in the deficient group. The tumors derived from connective tissue likewise showed no significant difference in their incidence in the two groups. Twenty-five per cent (6 of 24) of the rats in the deficient group developed this type of tumor and 22 per cent (4 of 18) of the controls.

The induction period for tumors derived from nervous tissue was notably less in the deficient rats than in the controls. Six of those derived from nervous tissue arose in the deficient group before any developed in the rats receiving the control diet (Table I). Moreover, there was an interval of 41 days between the time of appearance of the last of the first 6 tumors derived from nervous tissue in the deficient rats, and the appearance of the first tumor from nervous tissue in the control group. Only 1 other growth derived from nervous tissue appeared in the deficient group, and that was late in the experiment, the induction period in this rat having been 366 days. The average induction period for this type of neoplasm was 230 days for the rats receiving the deficient diet, and 372 days for those on the control diet. The average difference in the induction period for tumors derived from nervous tissue was 142 days.

There was no significant difference in the length
of the induction period for tumors derived from connective tissue. The first 2 fibrosarcomas developed in deficient rats that also developed a tumor from nervous tissue, but 32 days later a fibrosarcoma developed in a control rat, and for the remainder of the experiment this type of tumor appeared as frequently in the deficient rats as in the controls (Table II). The average induction period for the fibrosarcomas was 292 days in the deficient rats and 315 days in the controls. The average difference in the induction period for tumors derived from connective tissue was 23 days.

Table I: Tumors Derived from Nervous Tissue Arranged in Order of Appearance

<table>
<thead>
<tr>
<th>Necropsy no.</th>
<th>Experimental period, days</th>
<th>Deficient rats</th>
<th>Control rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>19D</td>
<td>153</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>27D</td>
<td>194</td>
<td>+*</td>
<td></td>
</tr>
<tr>
<td>52D</td>
<td>206</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43D</td>
<td>227</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>50D</td>
<td>228</td>
<td>+*</td>
<td></td>
</tr>
<tr>
<td>60D</td>
<td>237</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>71C</td>
<td>278</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>43C</td>
<td>303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>73C</td>
<td>303</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21C</td>
<td>321</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80C</td>
<td>360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20D</td>
<td>366</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>70C</td>
<td>488</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>59C</td>
<td>553</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates rat also developed a fibrosarcoma.

The number of animals in these experiments is too small to justify a detailed statistical analysis. It should be noted, however, that while the average difference of 23 days in the induction period for tumors derived from connective tissue is statistically insignificant, there is a high probability that the average difference of 142 days for tumors derived from nervous tissue may prove statistically reliable if the number of observations is increased.

COMMENT

The results of these experiments indicate that vitamin deficiency in rats significantly altered the susceptibility of the cells of the nervous system to the carcinogenic action of methylcholanthrene, while the connective tissue cells were apparently unaffected. The development of fibrosarcomas, which was not affected by the deficiency, actually afforded a control experiment to emphasize further the observed effect of the deficiency on the origin of tumors from nervous tissue. This result is noteworthy in view of the known effects of thiamine (18) and riboflavin (17) on nervous tissue, and the apparent indifference of connective tissue elements to a deficiency of these vitamins. It is impossible to say at this time without additional experimental work which deficiency altered the susceptibility of the nervous tissue to the action of the carcinogen. The statement can be made, however, that certain processes of intracellular oxidation of the nervous system were probably altered significantly as a result of the removal of thiamine and riboflavin from the diet.

Mention should be made here of the fact that niacin also was removed from the deficient diet. It is most unlikely, however, that its absence produced any significant alteration in the intracellular metabolism of the nervous tissue, since the rat apparently can synthesize this substance and hence does not develop the signs of deficiency noted in other animals when niacin is withheld (2, 16). Nevertheless, it was decided to remove niacin from the diet so that the amount of this vitamin in the tissues would be restricted at least as far as possible. Moreover, there is reason to believe that thiamine, riboflavin, and niacin are all collectively concerned with biological oxidation, and that any investigation of one demands consideration of the other two (1).

The thiamine deficiency in the rats of this experiment interfered with the normal oxidation of carbohydrate by the cells of the nervous system that is so vital to their metabolism and function. It has been demonstrated that a phosphoric acid ester of thiamine acts as a coenzyme necessary for the oxidation of pyruvic acid, an intermediate in carbohydrate oxidation. In thiamine deficiency there is first a decrease in free thiamine in the tissue and then a decrease in the phosphoric acid ester of thiamine (cocarboxylase). As a result of this decrease in cocarboxylase, pyruvic acid accumulates in the tissues and oxidative mechanisms in the brain are inhibited. Although less well understood, it has been suggested that the symptoms of thiamine deficiency may be correlated with a decreased acetylcholine content in certain tissues of
the body (3), since it has been demonstrated that thiamine inhibits the action of cholinesterase (4). The work of Zeller and Birkhäuser (21) would indicate, however, that alteration of this enzyme probably is not a factor concerned in thiamine deficiency of nervous tissue, since they demonstrated that cholinesterase is not reduced in the brain in thiamine deficient rats when significant reductions of this enzyme are demonstrable in the liver.

The deficiency of riboflavin produced in the rats of this experiment would appear significant, since this substance is related to more enzyme systems than any of the known vitamins and plays an important role in the intracellular oxidative mechanisms of the entire animal organism (3). The possibility that the riboflavin deficiency may have been responsible for the results observed in these experiments is suggested by the work of Kensler, Sugiura, Young, Halter, and Rhoads (6), who have demonstrated that this substance can protect the liver of rats fed \(p\)-dimethylaminoazobenzene from developing cancer. Kensler, Sugiura, and Rhoads (5) have shown that both riboflavin and coenzyme I were decreased in the livers of rats fed \(p\)-dimethylaminoazobenzene presumably as the result of a toxic metabolite produced by the carcinogen. It has been further demonstrated that the production of cancer in the liver paralleled the inhibition of the coenzyme I system (13). Since the niacin-containing coenzyme (coenzyme I) functions in biologic oxidations by the transfer of electrons to riboflavin enzymes, this observation suggested a niacin deficiency. Because the rat can synthesize niacin, and the addition of large amounts of this substance to the diet failed to prevent the lowered coenzyme I content of the liver, it was concluded that the lack of riboflavin interfered in some way with the normal mechanism of the body that protects against the carcinogenic effect of the \(p\)-dimethylaminoazobenzene, the interference causing the formation of an abnormal metabolite that prevented the synthesis of coenzyme I. It was further demonstrated that the toxic metabolite was harmful to normal liver cells, but had no effect upon hepatic tumor cells. From these observations arose a new idea on the genesis of the neoplasia effected in liver cells by \(p\)-dimethylaminoazobenzene: The liver cells were forced to develop a new system of intracellular oxidation not dependent upon either riboflavin or niacin and not interfered with by the toxic metabolite. This new method of respiration apparently represented an irreversible change, which might be the mutation said to be present in malignant tissue.

It is difficult to imagine any parallel mechanism at play between the production of intracranial tumors in the experiments reported here and experimental hepatic tumors produced with \(p\)-dimethylaminoazobenzene, since an adequate diet failed to protect the control rats from developing intracranial tumors. However, there was a common factor of riboflavin deficiency in both cases. All that it is possible to say now of the intracranial tumors is that the deficiency so altered the nervous tissue that it responded sooner to the carcinogenic stimulation of the methylcholanthrene. If there was a reorganized cellular metabolism in response to a conditioned deficiency produced by the carcinogen such as has been suggested for the hepatic tumors, and it accounts for neoplasia of the cells of the nervous system in these experiments, it must be assumed that the new systems of metabolism were induced by factors other than the dietary factor, which was not present in the control rats, where there was the same tumor incidence.

These experiments are open to criticism because there was no group of animals to control the inanition effect produced by the deficient diet. It was originally planned to run such a control group, but after the pellets had been implanted, and before the experimental groups were selected, it was necessary to kill a significant number of animals because they had developed "middle ear disease." It was then decided that it would be better to have more animals in two larger groups than to divide them into three small groups.

Generally, however, investigation has shown that underfeeding and inanition lengthen the period of induction for tumors induced with carcinogenic agents. Tannenbaum (2), studying the effect of inanition on the induction and growth of spontaneous breast and lung tumors, and skin tumors induced with benzpyrene, reported that fewer tumors developed, and at a later period, in underfed animals than in those receiving an adequate diet. The rate of growth of the tumor once it was initiated in the underfed animal was the same in both the spontaneous and the induced tumors. McCay, Ellis, Barnes, Smith, and Sperling (11) have observed a lower incidence of spontaneous tumors in rats with retarded growth. Lavik and Baumann (8), studying the effect of diet on the incidence of tumors induced with methylcholanthrene in the skin of mice, reported an increased number of tumors with a high fat diet. In another study these authors (9) observed that the animals eating the high fat diet consumed more calories, and that the increased caloric intake correlated roughly with the rate of tumor production. It would seem fair to conclude from these experiments concerned with inanition that the uncontrolled inanition factor in the experiments reported here would not be important, since the influence of inanition and a lowered caloric intake would have tended to lengthen the induction period rather than to decrease it.
SUMMARY

Under the conditions of the experiment, the periodic removal of thiamine and riboflavin from the diet of white rats with intracerebral pellets of methylcholanthrene produced a significant reduction in the length of the induction period of tumors derived from nervous tissue. Their average induction time was 230 days for the rats given the deficient diet, and 372 days for those fed the control diet. The incidence of tumors derived from nervous tissue was the same on both deficient and adequate diets. The deficiency had no effect upon either the incidence or the length of the induction period for tumors derived from connective tissue.

It is suggested that the altered metabolism of the cells of the nervous system resulting from the deficiency of thiamine and riboflavin caused the cells to respond more readily to the carcinogen. It is not possible to say which deficiency was responsible for the altered susceptibility of the nervous tissue.

REFERENCES

The Response of the Central Nervous System of the Rat to Methylcholanthrene. II. The Effect of a Diet Deficient in Thiamine and Riboflavin on the Induction of Tumors Derived from Nervous Tissue

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