The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents

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The present study was undertaken to investigate the carcinogenic reaction of rats treated with two different and differently acting carcinogens. This seemed to be of interest for various reasons. First, it would be possible to study the influence of one carcinogenic agent on the action of another. Although there are numerous publications on the action of carcinogenic substances on established tumors, transplanted and spontaneous, the interaction of two differently acting chemical carcinogens has, as far as we know, not yet been studied. The second reason is that it may be possible to decide whether in the animals susceptible to the action of one carcinogen there exists a predisposition for developing also the type of tumors characteristic for the second agent applied at the same time, e.g., whether a rat that will develop a hepatic tumor by the action of \( p \)-dimethylaminoazobenzene is more susceptible to the effect of methylcholanthrene than one that resists the carcinogenic diet.

The experiments were made with albino rats of our own unselected breed, which has a relatively high incidence of spontaneous tumors. Such a strain seemed more suited for this study than a highly selected one, in which response to carcinogenic stimuli and localization of induced tumors are influenced by artificially selected genetical factors. It may be assumed certain differences in reaction to carcinogenic stimulation exist in these unselected animals, which should be revealed by the experiments. Moreover, it should be possible to observe the effect of specific carcinogenic agents on the incidence of spontaneous tumors.

MATERIALS AND METHODS

The strain of albino rats used has a spontaneous tumor incidence of 5 per cent at the age of 1½ years, chiefly of malignant retroperitoneal sarcomas, squamous epitheliomas and benign mammary fibroadenomas.

The animals were fed \textit{ad libitum} the stock diet consisting of peanut meal, ground corn, milk powder, salt, calcium carbonate, sesame oil and a concentrate of vitamins A and D. A 0.66 per cent solution of \( p \)-dimethylaminoazobenzene (Merck) in sesame oil was added to this diet in the proportion of 2.25 per cent, resulting in a food mixture containing 0.015 per cent of the dye. The rats of each series were kept in a common cage. Each animal that died or that was sacrificed, was autopsied and examined for the presence of tumors. Tissue from liver, lungs, heart, kidney, spleen, and tumor was fixed in Bouin’s solution and microscopic examination was performed after the material had been cut and stained in the usual manner. We are indebted to Dr. R. Jaffé for the microscopic examination of this material.

The rats were 2 to 3 months old at the beginning of the experiments and gained weight during the experimental period. The carcinogenic diets were fed continuously throughout the experiments. Those animals which survived 15 months after the beginning of the experiments were killed with gas and examined as already described.

The diet given series 70 was prepared by using a solution of \( p \)-dimethylaminoazobenzene in cod liver oil, whereas all the other series received diets prepared with a solution in sesame oil. This modification in the first diet was made because it had been found that cod liver oil reduced the number of tumors produced by methylcholanthrene in rats (4). Series 73 was fed a diet which contained the same amount of the dye, but with the addition 0.15 per cent of ethyl urethane. This substance has been found to produce pulmonary adenomas and hepatomas in rats (6). Series 67 and 70 received one intraperitoneal injection of 0.1 ml. of a 2 per cent solution of methylcholanthrene in olive oil 1 week after the beginning of the carcinogenic diet.

RESULTS

A summary is presented in Table I showing the different types of tumors observed in the various series and at various time intervals after the beginning of the experiments. It can be seen that no hepatic tumors occurred earlier than 12 months after the beginning of
the diet. Therefore, the final percentage of liver
tumors was calculated on the basis of the survivors at
9 months. The incidence of hepatomas was 75, 78,
61, and 77 per cent respectively. Series 70 showed
only a slight decrease but the difference is not suffi-
ciently great to be significant. Obviously the simul-
taneous application of methylcholanthrene or urethane
with the \( p \)-dimethylaminoazobenzene diet did not alter
the number of hepatic tumors elicited by the latter to
a significant degree.

Series 67 had a tumor incidence of 46 per cent when
hepatomas are not included in the calculation. These
tumors must be regarded as produced by the injection
of methylcholanthrene. Of 54 rats maintained under
identical conditions and injected with the same amount
of methylcholanthrene, but not receiving the carcino-
genic diet, 22 (42 per cent) developed tumors. There
was apparently no difference in the number of tumors
induced by methylcholanthrene in the rats fed
\( p \)-dimethylaminoazobenzene in addition, and in the
controls. There was a certain difference in the
distribution of the observed tumors between carcino-
mas and epitheliomas in the two groups. It seems
difficult to decide whether this was due to the treat-
ment of the one group with the carcinogenic dye or not.

The rats of series 73 received \( p \)-dimethylaminoazob-
enze and urethane simultaneously with the diet. Eighteen per cent of the animals from this group sur-

Viving 9 months developed pulmonary adenomas. Of
38 controls that received the same amount of urethane
without the dye, 26 per cent showed pulmonary adeno-
mas. It is sometimes difficult to determine whether
the microscopic lesions observed in the lungs of these
animals are adenomas or not. Therefore, the numbers
cannot be considered very accurate. But it can be said
that the results obtained in the present experiments do
not warrant the statement that the treatment with
\( p \)-dimethylaminoazobenzene had any influence on the

<table>
<thead>
<tr>
<th>Table I: Tumors Observed in Rats Treated Separately and Simultaneously with Various Carcinogens</th>
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<tbody>
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<td><strong>No.</strong></td>
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The animals of series 70 received the same treatment
of \( p \)-dimethylaminoazobenzene and methylcholanthrene
as series 67 with the only difference that the carcino-
genic diet had been prepared with cod liver oil instead
of sesame oil. This modification was made because a
previous study had shown that the application of cod
liver oil reduces the number of tumors produced by
methylcholanthrene in rats (4). It seemed of interest
to produce a smaller number of local tumors in one
group without reducing the dose of the carcinogen in
order to check the correlation of the occurrence of
hepatic and methylcholanthrene-induced tumors in the
same animal as presented in Table II; a very high
incidence of both types of tumor gives less accurate
results in this calculation. The reduction of local tumors
observed in this series compared with that of series 67
is similar to that previously described, namely 50 per cent. The number of liver tumors observed in this series was somewhat lower than that of the other series, but the difference cannot be considered to be significant.

TABLE II: COMPARISON OF EXPECTED AND FOUND PERCENTAGE OF RATS THAT DEVELOPED HEPATIC TUMORS AND OTHER TUMORS AFTER TREATMENT WITH p-DIMETHYLAMINOAZOBENZENE AND ANOTHER CARCINOGEN

<table>
<thead>
<tr>
<th>Series</th>
<th>Animals with hepatic tumors,</th>
<th>Animals with other tumors,</th>
<th>Animals with hepatic and other tumors, found,</th>
<th>Animals with hepatic and other tumors, expected,</th>
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In series 68, which received p-dimethylaminoazobenzene only, 1 retroperitoneal polymorph cellular sarcoma was found; in series 73, which received the carcinogenic dye and urethane, 1 retroperitoneal sarcoma and 1 adenoma of the kidney cortex were observed. The sarcomas must be considered spontaneous. As the kidney adenoma was the first case observed among our animals, treated and untreated, it is impossible to decide whether it was spontaneous or elicited by the treatment. The 2 sarcomas found in 41 animals which survived 1½ years should be expected to develop without any treatment as the spontaneous tumor rate is about 5 per cent. Apparently there was no influence of the treatment on this spontaneous tumor rate.

In Table II the tumors observed in the series treated with two different carcinogens are summarized in respect to their simultaneous occurrence in one animal. Metastases have not been included. Only those rats which survived 9 months have been included. The mathematical probability for the simultaneous development of 2 types of tumor produced by 2 independent carcinogens in 1 animal has been calculated according to the formula:

\[ P = \frac{a}{q} \times \frac{a}{v} \]

where \( a \) is the total number of animals, \( q \) the number of animals bearing one type of tumor and \( v \) the number of animals bearing the other type of tumors. These calculated values are compared in the table with the values found in each series. It can be seen from this comparison that the calculated and found values do not vary considerably. The number of animals included in the calculation is not great enough to reveal slight differences between the two values.

DISCUSSION

The experiments presented in this paper were performed in order to investigate the influence that may be exerted by one carcinogen on the action of another, when both carcinogens were applied simultaneously. The results obtained with our strain of albino rats do not justify the conclusion that interaction occurred. The incidence of liver tumors produced by feeding a diet containing p-dimethylaminoazobenzene did not vary significantly when the animals were treated at the same time with methylcholanthrene by injection, or when urethane was given simultaneously with the diet. This negative finding indicates that the carcinogens, methylcholanthrene and urethane, did not influence the production of hepatomas by the carcinogenic dye. Urethane alone produced a few hepatomas in rats when fed in the same amount without p-dimethylaminoazobenzene, but its action in producing liver tumors was very weak compared with that of the dye. The dose of the carcinogenic dye used in the present experiments was 25 per cent of that usually applied, but the application was continued for the whole experimental period of 15 months because it was hoped that by prolonging the experimental period it would be easier to observe a possible influence on the action of the second carcinogen applied.

It has been stated that a diet containing p-dimethylaminoazobenzene renders rats more susceptible to carcinogenic stimulation (7). It should be expected in this case, that tumor development with methylcholanthrene would be enhanced in rats receiving such a diet. No such stimulation was observed in the present experiments. The same was true for the animals receiving p-dimethylaminoazobenzene and urethane at the same time. In this case the number of pulmonary adenomas, which must be regarded as caused by urethane, did not differ significantly from that obtained in the controls that were treated with urethane only.

Similar results have been obtained by Rusch and his collaborators in experiments on the additive effect of ultraviolet light and carcinogenic hydrocarbons (8). Although mice rendered procarcinogenic with one hydrocarbon developed tumors with another, ultraviolet light did not have the same effect nor did it increase the carcinogenicity of Shope papilloma virus. A stimulation of the development of spontaneous pulmonary adenomas in mice injected subcutaneously with carcinogenic hydrocarbons has been observed by Andervont (1), while various authors found such substances to have an action on established spontaneous or implanted tumors, slowing their growth and even causing regression (9). Our results are no evidence of the existence of a similar influence of p-dimethylaminoazobenzene on tumors produced by methylcholanthrene or urethane and vice versa.

The comparison of the expected and found percentages of animals bearing two types of tumors elicited
by two differently acting carcinogens does not prove the existence of a linkage between the two actions. The values do not differ significantly. This result is the opposite of that obtained by Blum with inbred mice in which cutaneous tumors were induced by ultraviolet radiation and which have a high spontaneous rate for pulmonary tumors (2). A certain linkage between the appearance of both types of tumors in the animals has been found by means of an exact statistical analysis of the development time of pulmonary adenomas in animals bearing cutaneous tumors. The number of animals used in the present experiments is not sufficient for a similar analysis of the results. Dunlap and Warren observed a percentage of lung tumors in pure strain mice injected with carcinogenic hydrocarbons which was several times higher in the animals developing local tumors than in those which did not (3).

The failure to observe a correlation between the occurrence of two types of tumor gives rise to a number of theoretical questions. It has been assumed that the development of cancer in a given individual depends on the special genetical and environmental conditions. The genetical conditions may be such that they favor development of neoplastic growth or that they inhibit it. By selecting animals with such different genetical characteristics, the inbred strains with varying tumor incidence have been obtained. But, these strains do not show an equally high susceptibility or resistance toward all types of tumor. Susceptibility therefore cannot be dependent on a single genetical factor, but special factors must be assumed for the different kinds of tumors. Nevertheless, it has been proposed that one factor may exist in certain inbred strains which produces higher susceptibility for all types of tumors and that each special type depends moreover on other genetical factors. This should be reflected by a certain linkage of susceptibility toward different types of tumors. While the authors cited above observed such a linkage in their inbred strains of mice, it was not detectable in our experiments performed with an non-inbred strain of rats. Similar negative findings were obtained with non-inbred mice in which no linkage between methylcholanthrene-induced sarcomas and pulmonary adenomas could be observed (5).

It therefore seems likely that a factor or factors can exist in inbred strains which render the animals more susceptible to any kind of tumor, but that such a factor is not necessarily present in any given strain.

SUMMARY

Three groups of rats were fed a diet containing 0.015 per cent of p-dimethylaminoazobenzene and simultaneously were treated with methylcholanthrene by intraperitoneal injection or received in addition 0.15 per cent ethyl urethane in the diet. The incidence of hepatomas observed after 9 to 15 months were 61 to 78 per cent. The controls fed only the carcinogenic diet developed hepatomas in 75 per cent.

The incidence of sarcomas and epitheliomas in the groups injected with methylcholanthrene and fed the carcinogenic diet did not vary significantly from that observed in the controls, which were injected with the carcinogenic hydrocarbon only. The number of pulmonary adenomas observed in rats fed p-dimethylaminobenzene and urethane simultaneously did not vary to a significant degree from that obtained in the control group which was fed a diet containing urethane but no carcinogenic dye.

A comparison of the calculated and observed number of animals that had hepatic liver tumors and other tumors at the same time showed no significant difference.

There was apparently no mutual influence of two carcinogenic agents applied at the same time under the experimental conditions employed.

REFERENCES

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