The Relationship of the Endocrine System to Carcinogenesis*

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Since several members of the endocrine system are known to influence metabolism, and other hormones are concerned in both genital and somatic growth, the abnormalities of metabolism and growth which are characteristic of tumor development might conceivably be due to, or at least influenced by, derangements in the supply of hormones normally governing such processes. A number of studies concerning one or another of the endocrine glands in relation to spontaneous, transplanted, or chemically induced tumors have been published. However, conclusions based on a comparison of results when tumors of different origin are under consideration are probably not valid. The wide variations in susceptibility between strains of animals used by different investigators must be taken into account. In the present study, the influence of each of the endocrines upon tumors having the same origin was determined in one strain of rats. As this study concerns only chemically induced tumors, no attempt will be made to review the literature covering endocrine influences upon spontaneous and transplanted tumors. References dealing with such relationships to chemically induced tumors will be cited under the appropriate sections.

Materials and Methods

The plan adopted was to investigate separately each member of the endocrine system, both as regards under-supply and over-supply of its secretion. As this work was primarily to serve as a base for further study, no combination of hormones was used. The state of under-supply was produced by extirpation of the gland involved; the condition of over-supply by administration of the appropriate hormone, usually in the form of a commercial preparation, whose biologic activity was checked by us.

Carcinogen used.—Methylcholanthrene was employed throughout, with the exception of one group in which 1,2,5,6-dibenzanthracene was used. The primary reason for the choice of methylcholanthrene was the fact that in our strain of rats we obtain 100 per cent tumor production with a 1 per cent solution of this agent in paraffin. This is an advantage when compared with the uncontrollable variability of occurrence of spontaneous tumors and the uncertainty of transplants’ taking. The comparatively short latent period with methylcholanthrene is also advantageous. Dibenzanthracene has a much longer latent period; it was used in one of the hyper-estrin groups because it was felt that the suspected stimulating power of estrin on tumor growth might be more apparent in the case of a more slowly acting carcinogen.

Administration of the carcinogenic agent.—The carcinogens were administered by the subcutaneous route. A 1 per cent solution was prepared in paraffin of low melting point and a total of 1 cc. injected into each animal at 5 different sites, 2 mgm. thus being placed at each point. One injection was made over the shoulders, one over each hip, and one in the mammary line at either side of the abdomen. Exact duplication of these points was attempted in each animal injected.

Animals used.—Young rats 6 weeks old at the time of injection were used. The animals were of the Denver strain, developed through 10 years of in-breeding and having nearly a zero incidence of spontaneous tumors.

Measurement of tumors.—The presence of growing tumors was determined by simple palpation. The size adopted as showing termination of the latent period; that is, the time elapsing between injection of the carcinogen and the formation of a growing tumor, was a mass of 1 cc. Some difficulty was experienced in the accurate measurement of certain tumor masses, as occasionally they have a tendency to grow as flat discs, even after active growth is in process. In these cases, allowance was made for thinness by greater surface area, so that a total of 1 cc. mass was present before it was counted as an active tumor. In all cases, several individuals frequently checked the tumors independently and agreement was

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reached before the tumor was considered to have the required volume.

**Experimental groups.**—1. Gonadal hormones.

a. Hyper-estrin. The estrogen used was estradiol benzoate (progynon-β, Schering Corporation). A noncancerous group was injected as controls with the same doses of estrogen, no tumors resulting. The work of many investigators has led to the belief that estrogens may be concerned in some types of tumors. This literature has recently been reviewed by Gardner (4).

b. Hyper-progestin. This group was injected with progestin (Parke, Davis & Co., Lakeside Laboratories, Inc.) in oil, from crystalline preparations. A noncancerous group was injected with the same amount of progestin, with no tumors resulting.

c. Hypo-estrin. The animals were ovariec-tomized 20 days previous to the administration of the carcinogen. Boyland and Warren (11) have obtained with what they believe to be a significant difference in the percentage of tumors induced with methylcholanthrene in ovariectomized mice.

d. Hyper-testosterone. Testosterone propionate (per- andren, Ciba Pharmaceutical Products, Inc., and orenone, Schering Corporation) was administered at two dosage levels. Noncancerous groups were given equivalent amounts, with no tumors resulting. Lacassagne and Raynaud (7) found testosterone to inhibit mammary tumor development. Flaks and Ber (3) found that testosterone acts to delay formation of methylcholanthrene-induced tumors in mice.

e. Hypo-testosterone. Castration was performed 10 days before injection with the carcinogen. Stewart (9) has been unable to reach a definite conclusion on the effects of castration in the development of 1,2,5,6-dibenzanthracene tumors.

2. Gonadotropic hormones. a. Pregnant mare serum. Two groups of rats were used, one on a high, the other on a low dose. The material used was gonadin (Cutter Laboratories).

b. Pregnancy urine. Again two dosages were given; the preparation was korotrin (Winthrop Chemical Co., Inc.).

3. Adrenal hormones. a. Hyper-cortin. The material used was: adrenal cortex extract (Wilson Laboratories), eschatin (Parke, Davis & Co.), cortalex (The Upjohn Co.), and cortisorbate (Schieffelin & Co.). Because of the large amounts necessary in an experiment of such long duration, only 5 rats were used. The hormone was given partly orally, in the form of a charcoal adsorbate mixed with the diet, and partly subcutaneously.

b. Hyper-desoxy corticosterone. The material used was percorten (Ciba Pharmaceutical Products, Inc.) Again only 5 rats were used.

c. Hypo-cortin. The adrenals were removed one week after the injection of the carcinogen. A maintaining dose of cortin was supplied in minimal amounts, one-half r.u. daily in the diet, and supplemented, when necessary to maintain life, by injection of cortical extract.

d. Hyper-adrenalin. The hyper-state was obtained by subcutaneous injection of adrenalin in oil (Parke, Davis & Co.), twice daily, in an effort to maintain a nearly constant action of the hormone. Goetsch (5) has found that adrenalin at this concentration will act for about 12 hours.

e. Hypo-adrenalin. The medulla of each adrenal was removed as completely as possible through a slit in the adrenal cortex.

4. Thyroid and pancreas. a. Hyper-thyroid. An extreme degree of hyperthyroidism was maintained by the injection of thyroxyl (E. R. Squibb & Sons). When the toxicosis threatened the survival of the animals, injections were stopped and smaller amounts administered in the diet for a few days.

b. Hypo-thyroid. Thyroidectomy was performed a week after injection of the carcinogen. Every effort was made to remove the entire gland, weights were recorded daily, and any fair gains in weight disqualified the animal from further consideration. No further injections were given, although calcium lactate was given in the water for a few days after the operation.

c. Hyper-insulin. Protamine zinc insulin (E. R. Squibb & Sons) was employed because of its prolonged action. Much higher doses than of ordinary insulin were tolerated. Because of the long duration of the experiment, the animals could not be starved. Blood sugar determinations were made at frequent intervals, and a fluctuating, but continuous, condition of hypoglycemia maintained.

d. Hypo-insulin. The pancreas was removed as completely as possible, using the method of Richter and Schmidt (8), one week following administration of the carcinogen. Determinations of glucose in the urine were made frequently during the development of the tumors.

5. Pituitary hormones. a. Hyper-pituitary. A crude pituitary extract was prepared from fresh sheep pituitaries, using the method of Van Dyke and Lawrence (11). Five mgm. of the precipitate was equivalent to 1 gm. of fresh tissue. The preparation was given in saline solution made up daily.

b. Hyper-growth hormone. The preparation used was phyon (Armour and Co.) of which 1 cc. contains the activity of 0.133 gm. of fresh anterior lobe tissue. Weight records were kept of each rat.

c. Hyper-prolactin. International standard prolactin was used in this group, the preparation being made up daily.
d. Hypo-pituitary. The pituitaries were removed by a modification of the method of Thompson (10). No serial sections were made at autopsy because, if cessation in weight gain and failure of gonadal development became apparent, it was felt that the hypopituitary state had been reached. Any animals showing normal development were disqualified. Korteweg and Thomas (6) have shown, as have others, that removal of the pituitary results in some inhibition of tumor formation in mice.

6. Controls.—Several lots of methylcholanthrene were used during the course of this experiment and a number of control rats injected from each lot. No significant difference in the length of the latent period in these groups was observed. Special care was exercised in the checking and measuring of these groups to insure reliable control data. A second group of controls consisted of animals injected with paraffin only. These were followed for a period of one year; no tumors of any sort developed.

**RESULTS**

The results are summarized in Tables I and II.

**DISCUSSION**

This work represents an attempt to influence the rate of development of chemically induced tumors by

**Table I: Methylcholanthrene**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Number and sex of rats</th>
<th>Dose (r.u.)</th>
<th>Injection schedule</th>
<th>Mean latent period, in days</th>
<th>Critical ratio (Dma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-estrin</td>
<td>5 M</td>
<td>30</td>
<td>3 x weekly</td>
<td>111.0</td>
<td>15.36</td>
</tr>
<tr>
<td>Hyper-estrin</td>
<td>5 F</td>
<td>30</td>
<td>3 x weekly</td>
<td>105.1</td>
<td>14.96</td>
</tr>
<tr>
<td>Hyper-estrin</td>
<td>5 M</td>
<td>750</td>
<td>3 x weekly</td>
<td>119.0</td>
<td>34.40</td>
</tr>
<tr>
<td>Hyper-estrin</td>
<td>5 F</td>
<td>750</td>
<td>3 x weekly</td>
<td>106.6</td>
<td>20.61</td>
</tr>
<tr>
<td>Hyper-progestin</td>
<td>10 M</td>
<td>3 mgm.</td>
<td>3 x weekly</td>
<td>107.5</td>
<td>14.53</td>
</tr>
<tr>
<td>Pregnant mare serum</td>
<td>10 M</td>
<td>15.0 r.u.</td>
<td>3 x weekly</td>
<td>80.2</td>
<td>18.02</td>
</tr>
<tr>
<td>Pregnant mare serum</td>
<td>10 M</td>
<td>15.0 r.u.</td>
<td>3 x weekly</td>
<td>70.1</td>
<td>9.04</td>
</tr>
<tr>
<td>Pregnancy urine</td>
<td>10 M</td>
<td>1.5 I.U.</td>
<td>3 x weekly</td>
<td>87.8</td>
<td>19.41</td>
</tr>
<tr>
<td>Pregnancy urine</td>
<td>10 M</td>
<td>15.0 I.U.</td>
<td>3 x weekly</td>
<td>87.4</td>
<td>19.81</td>
</tr>
<tr>
<td>Hyper-testosterone</td>
<td>10 M</td>
<td>60 gamma</td>
<td>3 x weekly</td>
<td>100.9</td>
<td>15.74</td>
</tr>
<tr>
<td>Hyper-testosterone</td>
<td>10 M</td>
<td>600 gamma</td>
<td>3 x weekly</td>
<td>105.1</td>
<td>19.17</td>
</tr>
<tr>
<td>Hyper-testosterone</td>
<td>10 M</td>
<td>23 r.u.</td>
<td>Daily</td>
<td>123.3</td>
<td>15.30</td>
</tr>
<tr>
<td>Hyper-desoxycorticosterone</td>
<td>5 M</td>
<td>3 mgm.</td>
<td>3 x weekly</td>
<td>97.8</td>
<td>11.70</td>
</tr>
<tr>
<td>Hypo-cortin</td>
<td>5 M</td>
<td>5</td>
<td>§</td>
<td>Daily</td>
<td>106.6</td>
</tr>
<tr>
<td>Hyper-adrenalin</td>
<td>10 M</td>
<td>3.5 cc. 1:10,000</td>
<td>2 x daily</td>
<td>97.3</td>
<td>17.77</td>
</tr>
<tr>
<td>Hypo-adrenalin</td>
<td>8 M</td>
<td>25 mgm./kg.</td>
<td>Varied</td>
<td>106.5</td>
<td>19.68</td>
</tr>
<tr>
<td>Hyper-thyroid</td>
<td>31 M</td>
<td>48 U./kg.</td>
<td>Varied</td>
<td>105.4</td>
<td>17.12</td>
</tr>
<tr>
<td>Hypo-thyroid</td>
<td>14 M</td>
<td>48 U./kg.</td>
<td>Varied</td>
<td>109.7</td>
<td>16.69</td>
</tr>
<tr>
<td>Hyper-insulin</td>
<td>12 M</td>
<td>10 I.U.,</td>
<td>Daily</td>
<td>114.0</td>
<td>19.5</td>
</tr>
<tr>
<td>Hypo-insulin</td>
<td>10 M</td>
<td>0.2 gm. fresh tissue</td>
<td>Daily</td>
<td>97.9</td>
<td>15.9</td>
</tr>
<tr>
<td>Hyper-pituitary</td>
<td>10 M</td>
<td>2 gm. fresh tissue</td>
<td>Daily</td>
<td>111.8</td>
<td>10.69</td>
</tr>
<tr>
<td>Hypo-pituitary</td>
<td>8 M</td>
<td>48 U./kg.</td>
<td>Daily</td>
<td>112.0</td>
<td>11.13</td>
</tr>
<tr>
<td>Controls</td>
<td>50 M</td>
<td>3 mgm.</td>
<td>3 x weekly</td>
<td>266</td>
<td>58.14</td>
</tr>
<tr>
<td>Controls</td>
<td>50 M</td>
<td>3 mgm.</td>
<td>3 x weekly</td>
<td>287</td>
<td>25.43</td>
</tr>
<tr>
<td>Controls, paraffin only</td>
<td>20 M</td>
<td>23 r.u.</td>
<td>Daily</td>
<td>106.8</td>
<td>16.34</td>
</tr>
</tbody>
</table>

* Coward-Burn rat unit.
† Cole-Saunders rat unit.
§ Daily amount necessary to maintain completely adrenalectomized rat, as defined by D'Amour and Funk (2).
|| Injection schedule varied, depending upon the condition of the individual animal.
" Injection schedule varied, depending upon the condition of the animal.
* Injection schedule varied, depending upon blood sugar.

**Table II: 1,2,5,6-Dibenzanthracene**

<table>
<thead>
<tr>
<th>Number and sex of rats</th>
<th>Hormone dose per week</th>
<th>Injection schedule</th>
<th>Mean latent period, in days</th>
<th>Critical ratio (Dma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-estrin</td>
<td>5 M</td>
<td>30 r.u.</td>
<td>3 x weekly</td>
<td>266</td>
</tr>
<tr>
<td>Hyper-estrin</td>
<td>5 F</td>
<td>30 r.u.</td>
<td>3 x weekly</td>
<td>287</td>
</tr>
<tr>
<td>Controls</td>
<td>5 F</td>
<td>23 r.u.</td>
<td>Daily</td>
<td>238</td>
</tr>
<tr>
<td>Controls</td>
<td>5 M</td>
<td>23 r.u.</td>
<td>Daily</td>
<td>246</td>
</tr>
</tbody>
</table>

* In the hyper-estrin groups all rats developed tumors; in the controls, only 4 out of each 5.

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means of endocrine derangement. It was felt that if
certain endocrine influences resulted in a deviation
from the usual rate of tumor growth, new avenues
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from the usual rate of tumor growth, new avenues
of research would be opened up. This is, therefore,
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of research would be opened up. It is felt that if
endocrine influences resulted in a deviation
can be measured with relative accuracy. The
The carcinogen used, methylcholanthrene, produces 100 per
cent tumor formation in the Denver strain of rats,
with considerable uniformity of growth. The mean
latent period of tumor production in normal animals
was 166.8 days. Consequently, any deviation from
the usual development, as compared with an adequate
number of control animals, could be traced to a
known hormone which had been administered or
withheld under controlled conditions.

The choice of the endocrine glands as a possible
influence on rapidly growing tissue is based on a num-
ber of points, both proved and theoretical. Although
their functions, in broad outline, are known, the
mechanism of hormonal action remains obscure.
Thus, while the growth-stimulating effect of the gona-
dal hormones upon the accessory sex structures is recog-
nized, their mode of action is unknown. If the re-
sponsiveness of cells forming the accessory structures
is an inherent property of these cells, it is possible
that other (tumor) cells, peculiarly susceptible to some
unknown growth-stimulating factor might also be
responsive. The high incidence of cancer of the
prostate, uterus, and mammary glands must not be
overlooked in this connection. A similar argument
can be advanced for the chorionic gonadotropins,
present in some species during the time of rapid growth
of the fetus, and for the more generally acting growth-
promoting factor of the pituitary. The influence of the
thyroid, pancreas, and adrenal medulla on general
and carbohydrate metabolism indicates their possible
importance. Finally, the anterior pituitary and adrenal
cortex present two structures whose functions are so
complex, as measured by the number of hormones
secreted and their known and suspected inter-relations
with other glands, that speculation as to the possible
effects of deprivation or excess of their secretions has
wide limits.

Examination of the data reveals, however, that few
of these plausible expectations were realized. We felt,
in view of the drastic difference between opposing
groups (complete deprivation compared with great
excess) that deviations from the normal, to be sig-
ificant, should be of considerable magnitude. The
data were submitted to statistical analysis. On the side
of decreasing the latent period; i.e., of stimulating the
rate of tumor development, the results with the four
groups treated with gonadotropins are statistically sig-
nificant, $D_{sw}$ (critical ratio) being, in sequence, 2.7, 7.1,
2.5, and 2.5. Influence exerted in the opposite direc-
tion, toward retarding tumor growth, is significant for
the hyper-cortin groups, $D_{sw} = 2.1$. It should be noted
that these figures are significant in the statistical
sense; that is, the differences observed are not due to
errors in random sampling. It must be noted also,
however, that it is admittedly difficult to say with
certainty on just what day a tumor reached the stan-
ard mass of 1 cc, and therefore the experimental
data on which the statistical analysis is based may be
somewhat inaccurate. We do believe, in the case of
the gonadotropins which are entirely consistent in all
four groups, that these findings are suggestive enough
to warrant further study with other dosages and
other preparations of the same type. As for the re-
main ing groups, the lack of influence on the devel-
oment of tumors displayed by complete absence, or
presence in excessive amount, of various hormones is
interesting. When one contrasts the two thyroid
groups, for instance, (thyroid secretion being a hor-
mone having a long carryover effect from one injection
to the next) and notes the close agreement between
the latent periods, the conclusion is inescapable that
this hormone is of no great significance in influencing
the development of a growing tumor from a focus
of chemical irritation. The same argument applies,
with a little more variation in the results, to other
hormones.

Records were kept of the time required from the
beginning of growth; that is, the development of a
tumor of 1 cc, volume, through progressive stages,
until death of the animal. This period of maturation
was subject to almost infinite variation within each
group and seemed to depend upon which particular
locus gained the ascendency. This seemed to be
simply a matter of chance. Usually only one, some-
times two, rarely more, of the injected sites developed
into full-blown tumors, and again there was no con-
sistency in any groups as regards this result. Usually,
if the tumors tended to grow inward, especially from
a focus on the abdominal wall, growth was rapid
death resulted early, although many animals lived
for months with tumors which, upon dissection, nearly equalled the mass of the animal itself.

All animals eventually died and were autopsied, records being taken of metastases. The percentage of grossly observable metastases in all groups was low, 10.5 per cent, with no significant variations between groups. The most frequent site of secondary invasion was the lungs.

A great deal of histologic material has been accumulated and is being studied. Sections of the endocrine system of tumor-bearing control animals were prepared to determine whether the tumor process itself had any influence upon the endocrine system. This is the reverse of the relationship to which the main study was devoted. Histologic sections were also prepared of the endocrine glands from representatives of each of the hormone-treated, tumor-bearing groups, in the hope that, should the tumor process produce a change in a given gland, the effect might appear to be more pronounced, or less pronounced, in the group to which that hormone had been supplied or from which it had been withheld. Studies of many tumors and metastases from different groups are in progress. This material will form the basis of another report.

**Summary and Conclusions**

An attempt was made to determine whether deficiency or excess of hormones would alter the rate of development of chemically induced tumors. The carcinogen used was methylcholanthrene in a 1 per cent paraffin solution, the total dose being 10 mgm., injected into 5 sites. Deficiency of each hormone was produced by extirpation of the gland involved; excess, by injection of the particular hormone. The rate of development was measured in terms of the elapsed time between injection of the carcinogen and the appearance of a tumor having a mass of 1 cc.

Neither deficiency nor excess of any hormone tested produced great deviation from the normal, although statistically significant differences, in the direction of increased rate of development, were shown by all groups of rats treated with gonadotropins. In the direction of decreased rate of development, differences were shown by the hypercortin group. Because of weaknesses in experimental technic, no stress is placed upon these statistical findings.

Considering the extreme physiologic contrast between animals completely deprived of a given hormone, as compared with animals receiving that hormone in excess, deviations from the normal were not large, under the conditions of the experiment. Since the dose of carcinogen used was capable of producing tumors in 100 per cent of the control animals, it may have been too large to be affected by the endocrine changes, whereas a more nearly border-line dose might have given the endocrine changes a better opportunity of exerting an influence.

**References**

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