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Studies of the Regression of Spontaneous Adenocarcinomata in Mice

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Untersuchungen über die Regression spontaner Adenocarcinome bei Mäusen

Zusammenfassung. Die Beeinflussung der Wachstumsgeschwindigkeit und Regression von Tumoren durch Gewebesxtrakte von Mäusen, Rattus natalensis, Rindern, Ochsen und Stieren wurde an 673 Mäusen der Inzuchtstämme C3H/St und C3HB/St mit Adenocarcinomen der Brustdrüse untersucht. Die Tumoren können in zwei Gruppen unterteilt werden, je nachdem ob sie bei den Nachkommen von nicht mit Leberextrakten behandelten Tieren entstehen oder bei Nachkommen von mit Leberextrakten behandelten Muttertieren. Aus den Ergebnissen wird der Schluß gezogen, daß die Injektion eines säugenden Muttertieres mit einem speziell präparierten Leberextrakt 1. eine Hemmung der Wachstumsrate der Tumoren und 2. die Prozentzahl vollständiger Regression spontaner Milchdrüsentumoren beeinflußt.

Summary. 673 mice of the inbred C3H/St and C3HB/St strains bearing spontaneous adenocarcinomata have been used to study the influence on tumor growth rates and percentage of regressions of such tumors by tissue extracts derived from mice, mastomi, beef, bull and steer. The tumors can be divided into two classes depending upon whether they arose in the offspring of the non-treated series or in the offspring of mothers under treatment with liver extracts. The conclusions arrived at by this method are that the injection of a nursing mother with a specially prepared liver extract will affect: 1. the inhibition of growth rate of tumors and 2. the percentage of complete regressions of spontaneous tumors of mammary gland origin in mice.

Two of the primary sources of spontaneous tumors of mammary gland origin in mice are: 1. the well-known inbred strain C3H/St, and 2. one of C3H/St sublines, C3HB/St (STRONG, 1968). The latter subline is further characterized by a spontaneous mutation at the microphalmic anemia locus Mi, which occurred recently in a mouse of the C3H/St strain. In the original C3H/St as well as in the derived subline C3HB/St, virgin females, in addition to those that are used for breeders, are prone to develop mammary tumors (adenocarcinoma) in more than 90% of all individuals. These tumors grow progressively and at a relatively uniform rate. Under the normal laboratory conditions the mammary tumors never regress and eventually kill the host. These tumors are the resultant not only of a peculiar genetic constitution but also of the presence of Bittner's milk agent (BITTNER, 1934)

Another source of spontaneous tumors of similar histological appearance occurs in the offspring born to C3H/St and C3HB/St mothers who have been injected with a liver tissue extract. The possible effect of the extract on the growth rate and fate of spontaneous tumors is determined in the present study.

Two series of spontaneous tumors were obtained as follows: 1. those derived from normal or tumor-bearing mice maintained under controlled conditions, and 2. those derived from a mother being exposed to a liver extract.

Methods

The method of preparation of the liver extracts was, in essence, as follows (see also STRONG, 1968):

The livers of several species (mouse, mastomi South African rat, Rattus natalensis, beef, bull, steer, and horn shark) were first ground by a meat grinder. Then the ground material was reduced to fine particles by Virtis centrifugation in 80% grain alcohol for ten minutes. The mixture was then kept under refrigeration and shaken periodically. From time to time, the clear supernate was taken off and successive samples were pooled.

These successive 80% alcohol soluble solutions were then lyophilized (negative pressure, dry ice) to dryness. The dried material was then washed several times with 100% alcohol and the ensueing 100% alcohol soluble material was pooled and again lyophilized to dryness. Both the material soluble in 100% alcohol and that which was not (but soluble in 80% alcohol) was then taken up in distilled water separately. The 100% alcohol soluble material taken up in distilled water received the symbol A, whereas the 80% soluble remainder (but not soluble in 100% alcohol) received the symbol W. The final solutions in distilled water were obviously "emulsions" or "emulsoid" and not true solutions.

The mouse liver material was taken up in distilled water in the ratio of 2 cc of water per mouse liver for the W moiety, and 2 cc per mouse liver for the A moiety. For the other species with larger livers, the dilution factor was 40 cc distilled water per one gr of lyophilized dried material.

A pinch of thymol was added to the distilled water preparations as a preservative. The solutions were then heated at 56° C for 30 minutes in a water bath and sealed with rubber stopper in a vaccine bottle.

All solutions were kept in the ice box when not in use, but were warmed to room temperature before injection in order to avoid spasms following the injection of a cold solution. Subcutaneous injections were used at first at sites remote to the spontaneous tumor but this proceedure was discontinued since skin ulcerations were occasionally produced.

The dosage administered to the tumor bearing mouse was variable depending upon the physical appearance of the mouse or the surface examination of the tumor but varied only between 0.10 cc to 0.50 cc. Following a complete regression of a tumor the mouse was never injected with more than 0.10 cc of the material, and this procedure never exceeded once in two weeks (i. e. every sixth period).

The mice were examined three times weekly and their tumors measured by caliper in the two longest diameters. The size of the tumor was calculated by multiplying the two longest diameters together. This system of measurement has been used for many years by the author. It is realized that this method is not an absolute measure of a tumor but experience has shown that reproducible growth rates can be obtained by such means, and is, therefore, justified. The variables of internal necrotic material, hemorrhagic areas, and connective tissue cannot thus be determined.

The estimation of the effect of the treatment upon a spontaneous tumor is dependent therefore not exclusively upon an estimated growth rate of the tumor but upon the percentage of complete regressions of such growths and the survival time of the tumor-bearing mouse. Another estimate of effect can be determined by counting the number of mice developing multiple tumors but this again is not considered in this present investigation.

The treatment consisted of the injection of either A or W on alternate injection periods (three times a week) intraperitoneally.

Results and Discussion

673 mice bearing spontaneous tumors were used in this experiment as follows: 1. 59 of these mice belonged to the C series and were kept as controls. None of these mice regressed their spontaneous tumors. 2. 10 belonged to the E series and were also kept as untreated controls. Only 1 regressed its tumor. 3. 518 mice were included in the C^1 series used for experimental mice being injected with liver extracts; and 4. 86 were included in the E^1 (experimentally derived) series also

received liver extracts. Fig. 1 presents data on the growth rate of spontaneous tumors in mice (adenocarcinomata of mammary gland origin).

No.	Mice	Series	Cont'd as:	Some Growth	Regressions	Percent Regressions
1.	59	C	Controls	59	0	0.0
2.	10	E	Controls	9	1	10.0± ?
3.	518	C″	Exper.	430	88±5.8	17.0±1.1
4.	86	E′	Exper.	53	33±3.1	38.4±3.6
		P between 3. and 4. <0.01				

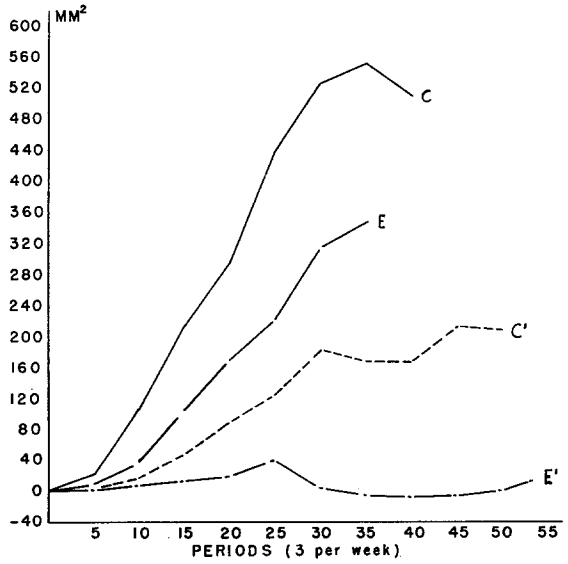

Fig. 1. Mice kept as controls (solid line C); mice kept as untreated controls (long dash line E); C mice with periodic injections of liver extracts 3 times per week (short dash line C′); and (4) E mice receiving periodic injections of liver extracts (long dash and dot line E′). Sizes of tumors are given on the vertical line (multiplication of the two longest diameters) and intervals of five successive periods of observation each are indicated on the base line. Measurements below the base line indicate complete regression of tumors

Thus it is obvious from the results shown in the Table 1 and Fig. 1, that the exposure of the offspring by injecting the nursing mother with a specially prepared liver extract has affected: 1. the growth rate, and 2. the percentage of complete regressions of spontaneous tumors of mammary gland origin that arises several months later in the offspring.

Another factor of the use of the injected material into tumor bearing mice was the observation that the "aging of the solutions" appears to be a variable in the effect upon the fate of the tumor. In some cases, this variable appears to be in the from of "rhythms" of effect. In order to resolve this problem completely,

considerably more research will be needed since the supply of mice bearing spontaneous tumors is always minimal. Some of this variable may be due to the use of small samples of tumor bearing mice. However, this factor should not be applicable to the present interpretation of the phenomenon of regressions of spontaneous tumors, but should be considered by investigators interested in verifying the present conclusions on the fate of spontaneous tumors.

The present experiment recalls the work of BITTNER (1934) on the discovery of the Bittner virus. The milk factor of Bittner is involved in the origin or initiation of spontaneous tumors in mice whereas the present study is involved not in the origin of spontaneous mouse mammary tumors but in the determination of their biological characteristics (growth rate) and fate (progressive or retrogressive changes).

References

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