DOES CHOLESTEROL STIMULATE TUMOR DEVELOPMENT? 1

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Certain tumors are known to be relatively rich in cholesterol (1-4) as well as in other lipoids (5-8). The cholesterol content of human cancers is said to increase with the degree of malignancy (3, 9), with such regularity that the life expectancy of cancer patients is inversely proportional to the cholesterol content of the neoplasm (10). v. Christiani and co-workers have pointed out the abnormal behavior of cancerous serum toward cholesterol butyrate (11-13). Borst found that the addition of cholesterol to the diet increased the rate of growth of rabbit tumors (14). Rondoni (15), Tesauro (16), and Maisin and Pourbaix (17) all regard cholesterol as a growth stimulator. Roffo considers cholesterol of prime importance in the development of tumors due to ultraviolet light and has stressed the fact that cholesterol accumulates in certain precancerous lesions in rats and in man (1, 18-20). The close chemical relationship between the sterols and certain carcinogenic hydrocarbons might favor this hypothesis (21). In fact, tumor formation has been attributed to the application of irradiated ergosterol (22), to the feeding of irradiated cholesterol (23), and to the injection of cholesterol treated with X-rays (24). Auler (25) concludes that doubts as to the growth-stimulating action of cholesterol are no longer justified.

Certain of our own experiments, however, failed to support such a conclusion. Cholesterol in amounts sufficient to produce fatty livers did not affect the development of mouse tumors induced by ultraviolet light (26), nor did a high cholesterol intake alter the development of tumors induced by painting with benzpyrene, or by injecting benzpyrene, methylcholanthrene, or dibenzanthracene (27). In agreement with Lang and Rosenbohm (28), cholesterol in the diet was found not to alter the rate of growth of transplanted tumors. The ineffectiveness of the cholesterol was particularly striking in view of the fact that certain of these tumor types are sensitive to other dietary changes. Since, however, these results were all obtained with tumors arising in tissues which do not normally accumulate cholesterol, the possibility remained that a stimulating effect might manifest itself in tumors originating from tissues known to be cholesterol-"sensitive." The effect of cholesterol on tumors arising in "sensitive" tissues has therefore been determined. In addition, cholesterol has been applied locally to skin in which tumors were developing. The "sensitive" tissues studied were rabbit tissue and mouse liver: the former because dietary cholesterol may increase the cholesterol content of rabbit blood as much as ten-fold, thus effectively increasing the available cholesterol in all tissues with an adequate blood supply; the latter because it accumulates cholesterol when this is added to the diet.

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**Ultraviolet Irradiation of Rabbit Ears:** The first carcinogenic agent employed was ultraviolet light. Abrikosoff (29) reported precancerous changes in rabbit ears after three months of irradiation. Furthermore, tumors can be produced readily by this means in the rat (20, 30) and mouse (31).

Fourteen young adult rabbits, approximately 2 kg. in weight, were divided into three groups. Group 1 contained 6 rabbits, which were irradiated with a quartz mercury vapor lamp (Burdick sun ray) for one hour daily at a distance of 50 cm. Their food consisted of Purina rabbit chow. Group 2 also numbered 6 animals, which were irradiated like those of Group 1, but received, in addition to Purina rabbit chow, hardened cottonseed oil (Primex) and cholesterol. The cholesterol was dissolved in the molten fat, which was then poured over the pellets and mixed as thoroughly as possible. The final composition of the diet was rabbit chow 96.78, cholesterol 0.22, hardened cottonseed oil 3.0. The 2 animals constituting Group 3 received the cholesterol diet but were not irradiated. All groups received food and water *ad libitum* plus limited amounts of dry alfalfa twice weekly. The ears of the irradiated animals were epilated with Na$_2$S at biweekly intervals.

A marked hypercholesterolemia developed in all animals receiving cholesterol. After one month, those on the Purina diet alone contained 1.16 to 1.53 mg. of cholesterol per c.c. of whole blood, while those receiving cholesterol showed from 5.20 to 12.0 mg. per c.c. There was no significant difference between irradiated and non-irradiated animals. Xanthomatous nodules of the feet developed in rabbits receiving cholesterol (32), but the only symptom attributable to irradiation was a slight redness of the ears. The experiment was discontinued after eleven months; no tumors, or even precancerous changes of the ears, were evident in any of the groups. Cholesterol, therefore, did not appear to promote tumor formation in rabbit ears.

**Shope Rabbit Papilloma:** When it became evident that the rabbits were not developing tumors as a result of irradiation with ultraviolet light, some of them were treated with the Shope papilloma virus. Papilloma tissue was washed with three quick changes of sterile saline solution, minced with a pressure mincer, and suspended in twenty times its weight of saline solution. The mixture was shaken for forty-five minutes with glass beads and then centrifuged. The clear supernatant fluid was used for injection, 10 c.c. being injected into the ear veins of 4 rabbits on the cholesterol diet, and of 2 rabbits on the stock diet. In addition, the virus was tattooed into the skin of one rabbit in each group.

Virus warts appeared in two to three weeks, and increased in size and number. They were found in many parts of the body—ears, neck, back, legs, and buttocks—but in general were largest and most numerous in the ears receiving the injection. The number of warts found in other parts of the body roughly paralleled the degree of papilloma development in the injected ear. Many of the papillomata disappeared within eight to ten weeks. When the virus was tattooed, a large and very dense cluster of papillomata appeared over the treated area but nowhere else. These growths were persistent.

Individual differences in extent of papilloma development were observed between members of the same group, but no differences between groups were

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2 For this we are indebted to Dr. R. E. Shope.
evident, either in the rate of appearance, distribution, number, size, or persistence of the papillomata. In other words, cholesterol feeding and hypercholesterolemia did not affect the development of rabbit papilloma.

**Aminoazotoluene Tumors in Mice:** Another carcinogenic procedure employed was the feeding of 2:5 aminoazotoluene (4-amino-3:2'-dimethylazo-benzol). The production of hepatomata with this agent is well known (33–34). The dye was incorporated in a standard stock ration (35), in a cholesterol ration, and in a cholesterol ration containing a brain concentrate. The composition of the diets was as follows: (a) Steenbock stock ration 94.9, hydrogenated cottonseed oil (Primex) 5.0, aminoazotoluene 0.05; (b) Steenbock stock ration 92.9, hydrogenated cottonseed oil 5.0, cholesterol 2.0, aminoazotoluene 0.05; (c) Steenbock stock ration 89.9, hydrogenated cottonseed oil 5.0, cholesterol 2.0, aminoazotoluene 0.05, brain concentrate 3.0. The brain concentrate was prepared according to the method of Maisin and Pouibaix (17) and was the same preparation which retarded the development of tumors induced by ultraviolet light (26).

Fifty male mice of the A strain 8 were placed on each of the diets and allowed to consume as much as they would. Originally the diets contained 0.1 per cent aminoazotoluene, but after two months the amount of dye was reduced to 0.05 per cent of the ration because deaths occurred with the larger amounts. Animals were killed for examination at intervals of two months, and the fat, water, and cholesterol content of the livers was determined. Partially fatty livers were noted in all animals receiving cholesterol plus aminoazotoluene, the average liver fat being 7.47 per cent after six months. Mice receiving the cholesterol diet but no aminoazotoluene averaged 14.8 per cent of liver fat. The mice on the stock ration plus aminoazotoluene contained 4.7 per cent liver fat as compared to 4.9 per cent in our stock animals.

Gross changes in the liver became apparent after four months, the livers of mice receiving the dye being definitely more granular than those of normal animals. This appearance grew progressively more pronounced, until after seven months occasional small nodules were observed. All animals killed after eleven months had hepatomata regardless of diet. There were no clear-cut differences in the degree of hepatoma formation on the three diets. The nodules in 4 animals on the cholesterol diet killed at eleven months appeared somewhat larger than those on the other diets, but even this gross difference was not observed consistently. We had no mathematical means of expressing differences in degree of development of these tumors, since they were too numerous and indefinite in outline to permit dissection and weighing. The dry weights of the livers were determined, since the water content of tumor tissue is appreciably higher than that of liver tissue. Both the actual dry weight and its percentage of the total were higher in the livers of animals fed cholesterol than in the others, due to the higher fat content of these livers. When a correction was applied for the altered fat content, however, no significant difference between groups appeared. The histologic appearance of the nodules obtained on the various diets was identical. The number of metastases and the period of survival were essentially the same. After eleven months, survivors numbered 17 on the stock ration, 18 on the cholesterol

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Table I: Effect of Cholesterol on Tumors Induced by Benzpyrene Painting

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<thead>
<tr>
<th></th>
<th>Effective Total</th>
<th>3½ mo.</th>
<th>4 mo.</th>
<th>4½ mo.</th>
<th>5 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four control groups</td>
<td>62</td>
<td>1.6</td>
<td>16</td>
<td>34</td>
<td>56</td>
</tr>
<tr>
<td>Cholesterol applied in benzene</td>
<td>17</td>
<td>12</td>
<td>24</td>
<td>35</td>
<td>53</td>
</tr>
<tr>
<td>Cholesterol applied in cottonseed oil</td>
<td>9</td>
<td>22</td>
<td>66</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>9</td>
<td>33</td>
<td>55</td>
<td>77</td>
<td>89</td>
</tr>
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Cholesterol, therefore, did not significantly affect hepatoma development.

Local Application of Cholesterol: In contrast to the ineffectiveness of cholesterol in the diet in promoting tumor development, its local application appeared to stimulate tumor production under certain circumstances. Mice were painted twice a week with 0.3 per cent benzpyrene in benzol, one drop being applied to each ear. On days when no benzpyrene was given, cholesterol was applied to the ears, either as a 1 per cent solution in benzene or as a saturated solution in cottonseed oil. The oil was saturated at 100°C; at room temperature some of the cholesterol precipitated out. The mixture was shaken thoroughly before application.

Control animals were painted only with benzpyrene and received either the stock diet or one of three other diets which did not alter tumor development. In addition, one group received a diet high in fat, which is known to accelerate the development of this type of tumor (27).

There were 20 animals in each group. The mice were examined twice monthly for tumor formation. The results were expressed as the number with tumors divided by the effective total—the number alive when the first tumor appeared. This was necessary because in some groups the mortality during the precancerous phase was high.

Cholesterol markedly stimulated the rate of tumor production when applied in cottonseed oil, but not in benzene (Table I). In fact, when cholesterol was applied in benzene solution, the rate of tumor production was almost exactly the same as that of the control groups. Cholesterol in cottonseed oil was as effective as a high-fat diet in accelerating tumor development. Since we have repeatedly failed to observe a marked effect with cottonseed oil alone, it is evident that both cholesterol and oil must be present to produce acceleration.

Identical results have been obtained in the induction of tumors by ultraviolet light (36). Cholesterol applied in oil accelerated the development of this type of tumor; cholesterol in benzene was without effect, and the oil itself was only slightly active. The mechanism by which cholesterol in oil hastens tumor development is unknown, but in view of the inactivity of cholesterol when applied in the absence of fat, or when taken internally, it would appear that cholesterol is not the significant factor. Fat, on the contrary, has been shown to hasten the development of epithelial tumors (26–27), and Twort and Twort (37) have stressed the stimulating effect of oleic acid. Perhaps the function of cholesterol in oil is to facilitate the penetration of the latter to the proliferating basal layer of the skin. Lanolin, which is rich in cholesterol, has long been used to aid the absorption of substances by the skin.
SUMMARY

(1) Cholesterol in the diet failed to stimulate the production of tumors in the rabbit by ultraviolet light.

(2) Shope virus papillomata developed at essentially the same rate in rabbits in which a cholesterolemia had been produced as in other rabbits.

(3) The addition of 2 per cent of cholesterol to the diet of mice failed to alter the rate of formation, the number, or the histological character of hepatoma induced by aminoazotoluene.

(4) The production of epitheliomata due to the painting of benzpyrene was accelerated when cholesterol was applied locally in cottonseed oil, but not when applied in benzene. It is therefore doubtful that cholesterol is a tumor-promoting agent.

NOTE: The authors are indebted to Prof. H. Steenbock for his valuable criticism.

BIBLIOGRAPHY