Effect of Hypocholesteremic Drugs on Tumor-Bearing Mice (36087)

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The structural and functional role of lipids in biological systems is extremely diverse. The mechanism by which fats reduce, induce, reenforce, or initiate biological reactions, however, are not known. While several approaches are available in elucidating this problem, the use of drugs which effect lipid levels has proven to be a useful tool. Since cellular cholesterol is associated with membrane material, it was of interest to study the effect of lipid-lowering drugs on actively dividing cells. The need of tumor cells for this material for organelle formation was thought to be critical. The present report is a summary of our biological findings; another report will deal with the effect of these compounds on tumor lipid metabolism.

Experimental Section. Methylphenidate (Ritalin) and aminoglutethimide phosphate (Elipten) were supplied by Ciba Pharmaceutical Co. Two other drugs, *trans*-1, 4-bis(2-chlorobenzylaminomethyl) cyclohexane dihydrochloride (AY9944) and ethyl 2-*p*-chlorophenoxy-2-methylisobutyrate (clofibrate) were obtained from Ayerst Laboratories.

G. D. Searle and Co. donated supplies of 20,25-diazacholesterol [*N*-methyl-*N*-(-3-dimethylamino)propyl-17β-amino-androst-5-en-3β-ol].

In these preliminary experiments, over 800 male Swiss mice (21–23 g) were used. Approximately 20% of these animals were used as controls. In each experiment with individual drugs, separate controls were used. Animals were distributed randomly and each group of 10 animals was injected with at least five concentrations of a drug. All mice were allowed food and water *ad libitum*. Twenty-four hours after the initial drug dose, 20,000 Ehrlich ascite cells were injected interperitoneally. Drugs were injected subcutaneously for 4 days more. After five consecutive daily doses of drug, the progress of tumor growth was followed by weighing of the individual animal groups, as well as recording their survival time.

Results. Effect of methylphenidate (Ritalin). Drug was tested at five concentrations. Animals receiving the highest dose (40 mg/kg) were highly agitated. At this dose level, mice were placed five in a cage rather than ten. Cumulative results for this drug are given in Fig. 1. The general effect of this drug is to shorten the life-span of the tumor-bearing animal. The length of survival (days), as well as the mean life-span is shortened by almost 3 days (2.8 days) at levels of 4.0 mg/kg. Higher doses of drug do not increase this adverse response.

Effect of clofibrate. The effect of this hypocholesteremic agent on tumor-bearing animals is erratic but more pronounced than Ritalin (Fig. 2). The most effective dose in lowering the life-span was observed in mice receiving 8 and 400 mg/kg. The latter dose is approximately one quarter the LD_{50} (1625 mg/kg). Again, as with Ritalin, a higher dose (800 mg/kg) of drug did not shorten the mean life-span of the animals. This dose did, however, cause the greatest loss in weight and some increase in survival time. Biological effects seen at such high doses are difficult to interpret because of the added effect of drug toxicity.

Effect of AY9944. This hypocholesteremic agent (LD_{50}, 155 mg/kg) has very little effect on the mean life-span of tumor-bearing animals, except at the toxic dose (100 mg/kg). While increased weight, as well as some increase in survival days, was noted at dose levels of 0.4 and 4.0 mg/kg, the changes

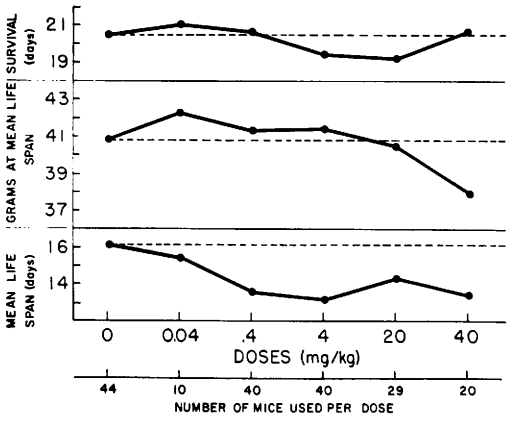

FIG. 1. Effect of Ritalin on Ehrlich ascites tumor: Five different doses of methylphenidate (Ritalin) were given ip for 5 days. At the effective dose levels (0.4 and 4.0 mg/kg) four separate experiments were carried out.

in these parameters are not as important as the lack of significant change in the mean life-span (Fig. 3).

Effect of 20, 25-diazacholesterol. The most consistent effect on tumor-bearing animals was recorded for this drug (LD_{50}, 90 mg/kg). All parameters measured showed a decrease level compared to control groups. Maximum effect was seen at 10 mg/kg, although similar changes were noted with drugs given over a tenfold range of concentrations, 4–40 mg/kg (Fig. 4).

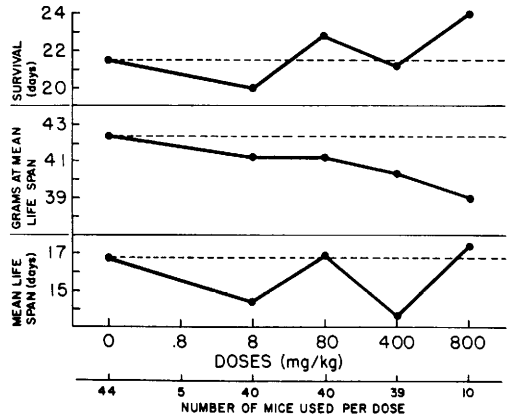

FIG. 2. Effect of clofibrate on Ehrlich ascites tumor: Clofibrate was administered ip for 5 days. Four separate experiments were conducted to test effective dose level. Nature of the biphasic response is not readily explainable.

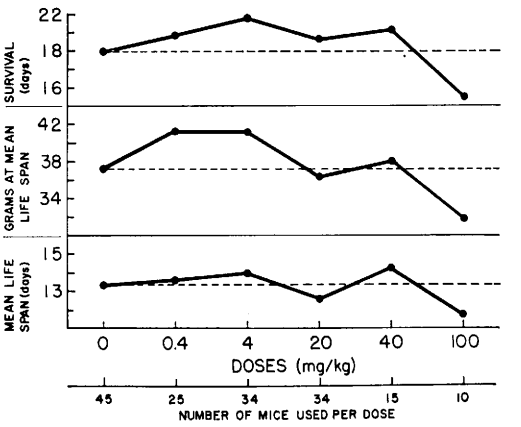

FIG. 3. Effect of AY9944 on Ehrlich ascites tumor: Results with AY9944 show little effect. The decrease of mean life-span and other changes at high drug levels is not considered important.

Effect of Elipten. This drug is not a hypocholesteremic drug. Elipten interferes with the conversion of cholesterol to pregenalone and, therefore, increases the level of cholesterol by decreasing sterol utilization.

The effect of this drug (40 mg/kg) was to *increase* the mean life-span of tumor-bearing animals (Fig. 5). While animals maintained a relationship between weight and drug dose, the survival time data was more erratic and more difficult to interpret.

Discussion. Hypothetically, the role of lipids in cancer is important because of the major role lipids play in biomembranes (1).

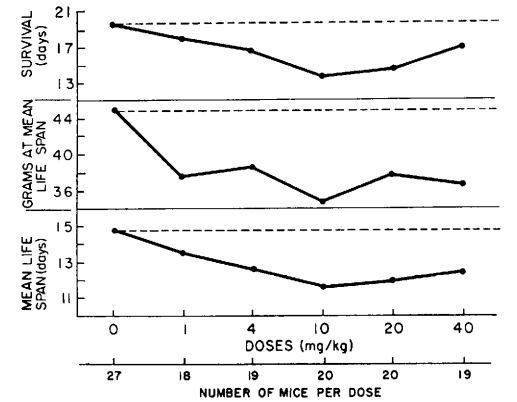

FIG. 4. Effect of 20,25-diazacholesterol on Ehrlich ascites tumor: This hypercholesteremic drug is most effective in decreasing the life-span of tumor-bearing animals. All injections were ip and continued for 5 days.

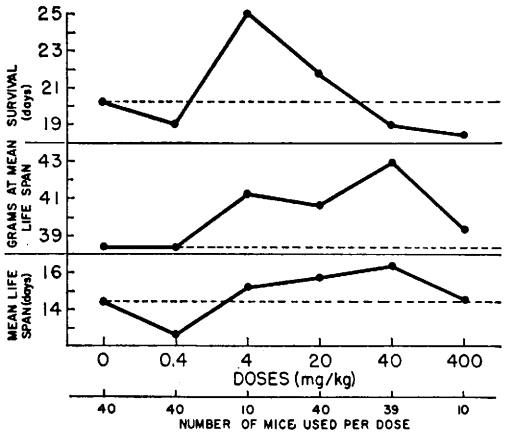

FIG. 5. Effect of Elipten phosphate on Ehrlich ascites tumor: Elipten inhibits the cholesterol desmolase system. Drug action raises the mean life-span and cholesterol level contrary to the action of other drugs tested.

While the precise role of cholesterol in biological membranes is not known, changes in the sterol component of cell membranes may alter events occurring at or in cell membranes (2). For this reason we began the investigation of drugs which would alter cholesterol metabolism. An early summary of studies on total lipid compositions and their significance was reviewed by Haven and Bloor (3). Most biochemical studies on tumor-bearing animals reenforce the idea of unidirectional flow of metabolites from host to tumor (2, 3). The significance of these early findings is difficult to fully understand since changes in whole tissue do not reflect more subtle changes as a cellular level (4).

The implication of some direct cause–effect relationship between cholesterol and tumor-growth has been suggested. Szepsenwol (5) fed cholesterol with lard and raw egg yolk to cause increased incidence of mammary carcinoma. Pharmacological drugs which are antimetabolites to cholesterol action have had a growth retarding action (6–9).

In this regard, a previous study on the use of hypercholesteremic drugs in tumor-bearing animals is of interest (10). Littman *et al.* (10) showed that all three tumors studied (Ca 755, Ehrlich carcinoma, and Novikoff hepatoma) maintained an ability to concentrate cholesterol. In these experiments, tumors developing in animals on a cholesterol-free, fat-free diet had a higher sterol and lipid content than those on a complete diet. In their experiment, the cholesterol-free diet restricted the size and weight of the developing tumors but not necessarily the survival rate. Since host weight loss has been shown to cause "false positive" activity in drug evaluation tests (11), the clear interpretation of restricted or special diets on tumor-growth is difficult (12, 13). Two drugs, AY9944 and triparanol (MER-29), were studied and relate directly to our present study, but with different interpretation of data. In their study, Littman *et al.* (10) found that in animals on a complete diet, AY9944 concentrated in diet (250 and 500 mg/kg per day) gave tumor inhibition with weight loss and increased the mortality of mice with sarcoma 180 from 3/10 to 6/10 in the high drug group.

Triparanol which inhibits the conversion of desmosterol to cholesterol (similar in action on lipid metabolism to 20,25-diazacholesterol) caused animals to lose weight as well as inhibit tumor growth.

Because of the high doses involved in their study, the specific effect of the drug is open to question. Our own results (Table I) do not agree with their conclusions; namely, reduction of serum cholesterol retards growth and development of transplantable animal tumors. Our findings summarized below indicate an opposite effect.

Compounds similar to Ritalin have been shown to inhibit Co-A derivative formation (14). In our experiments this compound decreased the life span of the tumor-bearing animals as drug concentration is increased. An optimum effect is reached in which a further increase in drug concentration has no adverse effect on the animal. Thus the decreasing life span brought about by Ritalin is not proportional to the amount of drug injected.

Clofibrate, in interfering with the conversion of the acetyl-CoA to mevalonic acid (15), gives us a similar effect. There is one difference. Note that the data give a biphasic curve. Whether this is real or not will need further clarification. That the effect is real

TABLE I. Summary of Drug Effects on Animals Bearing Ehrlich Ascites Tumor.

Drug	LD_{50} (mg/kg)	Effective dose (mg/kg)	Mean life-span (days) Control	Drug	Normal deviate (n)	Probability values
Ritalin	150	4	16.1 ± 2.7	13.3 ± 2.1	7.22 (42)	>.001
Clofibrate	1625	400	16.0 ± 3.1	13.6 ± 3.4	3.33 (42)	>.001
AY9944	155	40	13.4 ± 1.1	12.6 ± 2.6	1.16 (30)	.25
20,25-Diazacholesterol	90	10–20	14.7 ± 0.1	11.6 ± 1.0	14.0 (24)	>.001
Elipten	1800 (oral)	40	14.4 ± 3.8	16.4 ± 1.7	3.1 (40)	>.01

can be deduced from the fact that one would expect the life-span to decrease at higher doses, but this is not the case. It is apparent that there is more involved than just the toxicity of the drug. If toxicity alone were involved, a clearer relationship would be demonstrated between drug dose and observed effects.

The two drugs, AY9944 and 20, 25-diazacholesterol, affect different biosynthetic pathways but both just prior to cholesterol formation. Using AY9944, there is not a significant drop in the mean life-span at the effective dose level when compared to the control group. The high dose caused a marked decrease in life-span, but in our opinion, studies at such high drug levels are less meaningful and biologically less important because of possible toxic effects.

The effect of 20,25-diazocholesterol is on the formation of cholesterol from demosterol, which has an unsaturated side chain. Again, we have the characteristic decrease in life-span at low drug levels.

So far, the drugs we have discussed affect biosynthesis prior to cholesterol formation and all seem to decrease the life-span of the animals. Elipten, however, which increases the amount of cholesterol by blocking side-chain oxidation (16) has an effect opposite to that previously observed. By using this drug and increasing the available sterol pool, there was an increase in life-span of the tumor-bearing mice.

The total results of these experiments seem to suggest that drugs which interfere with cholesterol biosynthesis for one reason or another decrease the life-span of animals with Ehrlich ascites tumor. That this decrease in life-span was not due to toxicity is borne out by the fact that the effects were found to be greater at lower concentrations than that at higher concentrations of the drugs. This is contrary to Littman *et al.* (10) who used near toxic level of drugs.

Since the biosynthesis of cholesterol after squalene can proceed via two pathways, our data seems to indicate that the unsaturated pathway on which AY9944 is effective has little significance on formation of cholesterol or that 7-dehydrocholesterol is more readily substituted for cholesterol throughout the body tissue pool. On the other hand, the diazacholesterol which lowered the mean life-span significantly inhibits desmosterol conversions to cholesterol and may be a more physiologically important pathway or, since desmosterol is less like cholesterol structurally, one might expect a greater change in membrane function.

This interpretation is strengthened when we view the data on Elipten. This drug, which prevents the synthesis of cholesterol to other steroids, has an opposite effect. In other words, *there was an increase in mean life-span* averaging 2.2 days.

In summary, one may say that those drugs which are known hypocholesteremic agents at nontoxic dose levels decrease life-span of Ehrlich ascites tumor-bearing mice, while drugs such as Elipten, which prevent the side-chain oxidation of cholesterol into steroids, increase the life-span of tumor-bearing animals.

As a concluding remark, the report of Carroll and Khor is of interest (17). While their results provided evidence that a high-fat diet enhances tumorgenesis when fed *after* the administration of 7,12-dimethylbenz(a)anthracene, the incidence in rats on a low-fat diet was not statistically different from animals on a normal diet. This report indicates that the *effect of dietary fat is exerted at the promotional state* of carcinogenesis (18). However, a word of caution must be raised in evaluating data from nutritional experiments, where a number of variables are changed.

Summary. A large range of drug doses were used. Since effects were found at lower rather than higher drug levels, the results are significant. Hypocholesteremic drugs, such as methylphenidate, clofibrate and 20, 25-diazacholesterol lower the mean life-span of tumor-bearing animals. Another similar drug, AY9944, did not have a significant effect ($p = .25$). The drug, Elipten, which interferes with the side-chain oxidation of cholesterol produced an opposite effect: the mean life-span was significantly increased ($p > .01$).

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