Biosynthesis of Cholesterol and Fatty Acids in Tumor-bearing Animals*

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Much information has been published on the relationship of lipides to tumorigenesis (17), but most of the work has been concerned primarily with the more quantitative aspects of lipides in tumors and in the tissues of tumor-bearing animals. Investigations on the course of lipide biosynthesis have been few. In a continuation of earlier work in this laboratory with normal animals (23), it seemed advisable to study the biosynthesis of cholesterol and fatty acids in tumor-bearing animals. In this report, as before, the incorporation of C\textsuperscript{14} from acetate-1-C\textsuperscript{14} into these substances is used to demonstrate their synthesis.

**MATERIALS AND METHODS**

Carcinogen-induced and transplantable tumors were employed. The former consisted of a hepatoma (28) obtained by feeding \( p,p'N,N'-\text{dimethylaminoazobenzene (DMAB) to Sherman rats (Exp. I) and a spindle-cell sarcoma caused by implantation of 20-methylcholanthrene (MC) into Sprague-Dawley rats (Exp. II). The transplantable neoplasms included a fast-growing spindle-cell sarcoma maintained in the thigh of golden hamsters (\textit{Mesocricetus auratus}) and which was received from Boston University,\textsuperscript{1} where it was induced several years ago by the implantation of MC (Exp. III). Another fast-growing sarcoma was obtained by the injection of Yoshida ascites tumor into the thigh of Sprague-Dawley rats (Exp. IV). Wistar rats bearing Yoshida ascites tumor were employed in Exps. V and VI, and ascites tumor from the same strain of rats was used for Experiments VII and VIII.

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In each experiment tumor-bearing and control animals were given intraperitoneal injections, at certain time intervals of tumor growth, of an aqueous solution of sodium acetate-1-C\textsuperscript{14} (0.5 mc/kg). Groups of two animals each were killed at 10, 20, 40, 80, and 240 minutes after the injection. In certain experiments some of these intervals were omitted. The animals were anesthetized with ether and bled to death. Liver, gastrointestinal tract, leg containing the tumor, nontumor leg, and, in those instances where no visible tumor developed, the remaining carcass, were removed. The tissues were hydrolyzed for 15 hours in 30 per cent KOH on a steam bath. The hydrolysates were chilled by adding ice, acidified with concentrated hydrochloric acid, and the hydrolysates of the liver and legs were extracted directly 3 times with pentane. The hydrolysates of gastrointestinal tracts and carcasses had to be filtered with celite, and the filtrate was extracted 3 times with pentane which had first been used to extract the filtrate. The pentane solutions from each tissue were extracted twice with 10 per cent KOH to eliminate the fatty acids. The acid-free pentane extracts were washed twice with water and evaporated to dryness under reduced pressure. Precipitation of cholesterol with digitonin in these dried residues and subsequent bromination were carried out as previously described (24). The combined alkaline wash solutions from each hydrolysate were acidified and extracted with pentane and the fatty acids isolated by evaporation of this extract.

To obtain information on the changes of the cholesterol contents in tissues during tumorigenesis, the freshly removed tissues were weighed before hydrolysis, and the cholesterol isolated from the digitonides was also weighed. These figures were used to calculate the mg of cholesterol/gm of wet tissue.

The method of Kingsley et al. (19) was used for the determination of total cholesterol in blood serum at different times during the growth of the tumor. Blood for this procedure was obtained by heart puncture of a separate series of animals. Radioactivity count was made on all cholesterol samples as obtained from the digitonides, which were then purified by bromination and subsequently recounted. The differences of the two counts, representing what has been called "Higher Counting Companions, HCC" (23), are shown in all charts as the white part of the bars. All counts were made on a thin end-window counter. All these operations have been described before (30).

Samples of the livers and tumors were fixed in 10 per cent formalin, prepared on slides, and stained with hematoxylin and eosin.

**EXPERIMENTS AND RESULTS**

\textit{Experiment I.}—Female Sherman rats (140–160 gm. each) were fed, ad libitum, a diet consisting of 20 ml. of a 3 per cent solution of \( p,N'N'-\text{dimethylaminoazobenzene (DMAB) in olive oil mixed thoroughly with 1 kg. of unpollished rice. Each}
animal received 1 gm. of fresh carrot daily and unlimited tap water and consumed between 5 and 10 gm. of the rice mixture per day; this contained approximately 3–6 mg. of DMAB. Control animals received the same diet as the experimental animals except that it lacked DMAB, and one control series was kept on a normal diet. In the first 2 weeks the animals were slow to accept the special diet, but despite this fact the weights of the experimental and control animals increased between 5 and 20 gm. for each animal during the entire experiment. The experimental animals were divided into three groups of ten animals each, which were kept for 30, 90, and 145 days, respectively, after the feeding of the special diet began. Because of the long duration of the experiment the control animals consisted of three groups of ten animals which were left for 0 (normal diet control), 90, and 165 days after the feeding began. All animals were given injections intraperitoneally of 0.5 mc sodium acetate-1-C14/kg body weight. Each group of animals was subdivided into five pairs which were killed, respectively, at 10, 20, 40, 80, and 240 minutes after injection. A separate series of eight rice-carrot diet control and eight tumor animals, which were injected 15 days after the start of feeding, was also studied. These were killed in pairs at 10, 20, 40, and 80 minutes after injection.

Mortality was minimal in both the experimental and control groups, although some animals on the DMAB diet died during the 1st month of the experiment. The animals on the rice-carrot diet gained little weight but appeared healthy and active. With the development of advanced hepatoma, however, they became thin and inactive.

Chart 1a shows that, in the liver, incorporation of C14 from acetate-C14 into cholesterol decreased and was definitely lower 30 days after the start of the experiment, while after only 15 days no significant change compared with the controls could be detected. At the 90-day interval the decrease in cholesterol synthesis had become considerable; after 145 days, however, cholesterol biosynthesis increased again. The values for the corresponding rice-carrot-fed controls were higher than those for the tumor animals, but lower than those for the control animals fed a normal diet. Decreases of the biogenesis of cholesterol were manifested also in the gastrointestinal tracts and the carcasses; they corresponded to the results in the livers. At all times the isolated cholesterol contained high counting companions (HCC).
Both experimental animals and the rice-carrot-fed controls manifested a definite increase in the incorporation of C\(^{14}\) into the fatty acids of the livers as the animals grew older (Chart 1b). The increase was more marked after 90 days than after 15 and was more pronounced in the rice-carrot-fed controls than in the experimental animals, both surpassing the normal control. No such effects were seen in the gastrointestinal tracts and the carcasses.

The blood cholesterol determinations in the tumor-bearing animals at the 30-day interval and later were higher in value than those in the rice-carrot-fed controls and they were also higher than in the controls on a normal diet (Chart 7).

There were no significant changes in the amounts of cholesterol (mg.) per gram wet tissue compared with the controls even at the 90- and 145-day intervals, although the livers at the latter stage were much heavier than the livers of the controls and contained large tumors (Table 1).

The livers of the tumorous animals even at 30 days were normal in size and macroscopically normal in appearance. Microscopically, a swelling of the parenchymal cells was observed, and the sinusoids were narrowed, but there was no evidence of hepatoma. After 90 days extensive nodular cirrhosis of all lobes was found, and under the microscope beginning malignant transformation of the bile ducts and infiltration of lymphocytes in the bile duct areas were discernible. Malignant transformation in the liver could be seen at this time in only one animal. At the 145-day interval the livers were from 2 to 4 times their normal size and had large nodules. Microscopically, they now showed at least 90 per cent investment with hepatoma, and large areas of necrosis had appeared.

Experiment II.—Male Sprague-Dawley rats (120–140 gm. each) were given one subcutaneous injection in the right thigh of 8 mg. of 20-methyl-cholanthrene (MC) dissolved in 0.5 cc. of oil of lard. Control animals were given injections similarly of only 0.5 cc. of oil of lard. All animals were fed a diet of Purina Laboratory Chow (consisting of 23.0 per cent protein and 5.0 per cent fat) and tap water, ad libitum, during the experiment. Each animal consumed approximately 10 gm. of food per day. The experimental animals were divided into four groups of ten animals and kept, respectively, for 28, 90, 166, and 205 days after the MC injection. Because of the slow induction of the tumors, three groups of controls consisting of ten animals in each group were left for 0, 28, and 223 days after the injection of oil of lard (controls A, C, and D of Charts 2a and 2b). Another series of eight control and eight tumor-bearing animals was
made separately and killed 15 days after the implantation (Control B). As a check, the 90-day series was repeated, together with a 90-day control series (Control E). The weight of the animals increased normally during the experimental period, reaching a peak of about 500 gm. in the final series. After the indicated time intervals the rats were given injections of sodium acetate-C\textsuperscript{14}, dissected, and treated as described above.

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HCC were encountered in all tissues of the different series. No significant differences in the radioactivity of the fatty acids isolated from the livers or the gastrointestinal tracts were found, but there was a considerably higher specific count in the fatty acids from the tumorous leg than from the control legs at the 166- and 205-day intervals (Chart 2b).

Blood cholesterol determinations were lower in the tumor series than in the control animals (Chart 7), with the exception of the 90-day figures.

The amount of cholesterol (mg.) per gram wet tissue, for livers and gastrointestinal tracts, did not differ significantly between tumor and control series, but the carcasses of the tumor-bearing animals contained more cholesterol than did those of the controls. Tumorous legs also showed more cholesterol than the legs of the normal series, but all these differences were not statistically significant (Table 1).

Nothing abnormal was found in the animals macroscopically at the 15-, 28-, and 90-day intervals; the implanted animals showed a nodule at the site of implantation. Well developed tumors were present in the 166- and 205-day tumor series which microscopically were recognized as spindle-cell sarcoma. No abnormality was found microscopically at any time in the livers of any of the animals whether they had tumors or not. The parenchymal cells showed indistinct cords separated by normal-width sinusoids. Bile ducts and vascular elements also remained normal.

Experiment III.—Male golden hamsters (about 100 gm. each) were inoculated subcutaneously in
the right thigh with a 1-mm. cube of a methylcholanthrene-induced sarcoma, with a 17-gauge needle used as a trocar. Tumor-bearing animals and controls received a diet of Purina Laboratory Chow, containing 23 per cent protein and 5 per cent fat, and tap water ad libitum. Each animal consumed 5–10 gm of the food/day. In addition, each hamster received approximately 2 gm of fresh carrot 3 times a week. The tumor-bearing animals were divided into four groups of ten animals and left for 6, 16, 21, and 35 days, respectively, after inoculation with the tumor. Only one set of ten control animals was used because of the short duration of the experiment and because there is little change in the animals' weight during this time. All animals were given injections at the indicated times intraperitoneally of sodium acetate-1-C¹⁴, dissected, and treated as described above.

Chart 3a shows that the biosynthesis of cholesterol in the livers of the tumor-bearing hamsters was considerably increased over the controls in the 1st week and then decreased continuously as the tumors grow. At the 35-day interval, when the tumors had attained nearly one-seventh of the weight of the whole animal and became necrotic, the cholesterol counts rose again. However, even at the lowest level of biosynthesis in the 21-day series these counts were higher than those in the control series. These results were also noted in studies of cholesterol in the gastrointestinal tracts. Here the tumor-bearing animals also had higher counts than did the controls. At the 16- to 35-day intervals the counts of cholesterol isolated from the tumor samples were higher than those from the controls. A considerable amount of HCC was found in all cholesterol samples. The 6-day series was repeated, together with a separate control series. As in the main experiment there was an increase of cholesterol synthesis in the livers and gastrointestinal tracts over that in the controls.

The radioactivity of the fatty acids of the liver was higher in all tumor-bearing animals than in the controls and reached a peak at the 16-day interval. The fatty acids of the gastrointestinal tract showed such a peak after 35 days. In the tumorous legs the increase in counts was small (Chart 3b).

Seven days after the start of this experiment, blood cholesterol determinations in the tumorous animals were all considerably higher than those in the controls. However, at 2 days the blood cholesterol in the tumor-bearing animals was lower than in the controls (Chart 7).

The results obtained from the amounts of cholesterol (mg.) per gram wet tissue were similar to those in the foregoing experiments. There were no appreciable differences between control and tumor-bearing animals regarding cholesterol in the livers, gastrointestinal tracts, and carcasses. However, the tumorous legs contained considerably more cholesterol than did the control legs. This increase was statistically significant (Table 1).

Microscopically, the livers of control and tumorous animals appeared normal at 35 days. In the livers of the 35-day tumor-bearing hamsters there were large numbers of leukocytes in the
sinusoids and also an apparent increase in von Kupffer cells, with many transitional forms of these cells present. They evidently were caused by the necrosis in the tumors at this late stage of growth.

**Experiment IV.**—Male Sprague-Dawley rats weighing 180–250 gm. each were used in this experiment and were fed as in Exp. II. They received an inoculation of 1 ml. of Yoshida ascites fluid containing about 1.6 million cells into the right thigh. The ascites fluid was obtained from a Yoshida ascites tumor, maintained intraperitoneally in young Wistar rats. After a cell count was taken, the fluid was injected into the thigh of the recipient rats. Series of ten animals were used 3, 6, 16, and 20 days after the implantation of tumor material. The transplants rapidly developed into solid tumors, whose average weight after 20 days was almost 100 gm. in some of the animals and seriously hindered their movements. At the indicated times each series was given injections intraperitoneally of 0.5 me sodium acetate-1-C14/kg, and two animals each were killed, respectively, 10, 20, 40, 80, and 240 minutes after the injection. They were dissected and treated as before.

From Chart 4a it can be seen that, 3 days after the implantation of the tumor cells, the biosynthesis of cholesterol in the livers of the tumor-bearing animals was considerably higher than that in the controls. It then rapidly decreased, and at the 14- and 20-day interval was much lower than in the controls. In the gastrointestinal tracts the values for the tumor-bearing animals were lower than those for the controls throughout the experiment. The 8-day series, because of its importance, was repeated with a separate control, and an even greater incorporation of C14 into the liver cholesterol was observed than in the original experiment. No significant difference in the cholesterol samples from the tumorous legs and from the controls was found at any time.

The radioactivity counts of the fatty acids of livers and gastrointestinal tracts showed some decrease in the tumor-bearing animals as compared with the controls, and the tumorous legs showed no difference from their controls (Chart 4b).

The blood cholesterol of the tumorous animals...
was lower in this experiment than in the controls (Chart 7).

The amounts of cholesterol (mg.) per gram of wet tissue did not differ between tumor-bearing animals and controls for any tissues and were practically the same as in the other experiments with rats. A slightly higher though statistically not significant value was found for the cholesterol content of the tumor legs compared with the controls (Table 1).

Microscopically, the livers of all animals including those bearing tumors were normal at 21 days, except for an apparent increase in the number of von Kupffer cells. Some transitional forms of these cells were visible in the sections.

**Experiment V.**—Male Wistar rats weighing 110–120 gm. were fed Purina Laboratory Chow and water ad libitum as in the other experiments above. They were given injections intraperitoneally of Yoshida ascites fluid containing about 1.6 million cells per animal. One series of eight animals was retained for 3 days, and another series of eight for 6 days after tumor inoculation. A control series of eight normal rats was also run. At the indicated times, all animals received injections of 0.5 mc. of sodium acetate-1-C\(^14\) intraperitoneally, and they were killed in pairs 10, 20, 40, and 80 minutes after the injection. They were dissected and treated as before.

Chart 5a shows that, 2 days after the start of the experiment, biosynthesis of cholesterol in the livers of the tumor-bearing animals was much lower than in the controls, although at this time no ascitic fluid had formed, and the only indication of tumor development was an increase of dampness of the organs in the peritoneal cavity. Six days after the implantation, however, there was a considerable increase of the biosynthesis in the livers of the tumor-bearing animals. At the same time, the animals showed well developed ascites tumors with volumes ranging from 5 to 15 cc. The tumor had formed nodules on the mesentery, along the intestines. Two other experiments which are not reported here gave the same result. Neither the gastrointestinal tracts nor the carcasses of the tumor-bearing animals showed differences from the normal organs. The tumor itself did not show any change in the cholesterol compared with the carcass.

There were no appreciable differences in the fatty acid radioactivity counts, shown in Chart 5b, between tumor-bearing animals and controls for livers, gastrointestinal tracts, and carcasses; but the radioactivity in the tumor itself was definitely higher than that in the carcasses.

There was no difference in the blood-cholesterol determinations in the tumorous compared with those in the controls in the 2-day series, but at 6 days a very considerable increase was found (Chart 7).

The amounts of cholesterol (mg.) per gram of wet tissue were nearly the same for tumorous
animals and controls and did not vary from the figures for the other rat experiments, although Wistar rats were used for this experiment (Table 1).

Microscopically, the livers appeared normal at 6 days. However, there was an apparent increase in the number of von Kupffer cells, and some transitional forms of these cells were visible in the section.

Experiment VI.—Three Wistar rats weighing about 120 gm. were injected intraperitoneally with Yoshida tumor ascites containing about 1.6 million cells per animal as in Exp. V. After 6 days they were anesthetized with ether, and a total of 300 ml. of ascites fluid with a count of 67,600 cells/cu mm was withdrawn. It was mixed with an equal volume of White's fluid, 0.2 mc. sodium acetate-1-C\textsuperscript{14} was added, and the mixture was incubated at 37° C. Samples of 150 ml. each were removed from the mixture 30, 60, 150, and 300 minutes after the start of incubation. They were hydrolyzed by the addition of 50 gm KOH/flask, heated overnight on the steam bath, and then processed for cholesterol and fatty acids.

Chart 6 reveals that there was no appreciable synthesis of cholesterol in the isolated tumor cells. However, these cells were not completely devoid of synthetic ability, because there was a certain amount of C\textsuperscript{14} incorporated in digitonin-cholesterol. There was also some incorporation of C\textsuperscript{14} into the fatty acids.

Experiment VII.—Twenty-four male Wistar rats weighing 80–100 gm. were given injections intraperitoneally of Yoshida tumor ascites containing about 1.6 million cells per animal. After 6 days they were anesthetized with ether, and a total of 300 ml. of ascites fluid with a count of 67,600 cells/cu mm was withdrawn. It was mixed with an equal volume of White's fluid, 0.2 mc. sodium acetate-1-C\textsuperscript{14} was added, and the mixture was incubated at 37° C. Samples of 150 ml. each were removed from the mixture 30, 60, 150, and 300 minutes after the start of incubation. They were hydrolyzed by the addition of 50 gm KOH/flask, heated overnight on the steam bath, and then processed for cholesterol and fatty acids.

Table 2 demonstrates that more cholesterol-C\textsuperscript{14} was retained in the tumor than in the carcass.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>CHOLESTEROL-C\textsuperscript{14} Counts/min/mg</th>
<th>Total Counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>150</td>
<td>11,544</td>
</tr>
<tr>
<td>G.I. tract</td>
<td>58.9</td>
<td>572</td>
</tr>
<tr>
<td>Carcass</td>
<td>65.1</td>
<td>586</td>
</tr>
<tr>
<td>Tumor</td>
<td>200.0</td>
<td>268.0</td>
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Chart 6.—Experiment 7.—Time course of cholesterol-C\textsuperscript{14} and fatty acid synthesis after incubation of Yoshida ascites tumor cells with acetate-1-C\textsuperscript{14}.

with Yoshida tumor ascites containing about 1.6 million cells per animal as in Exp. V. After 6 days, when the tumor was fully developed, they weighed 130–140 gm. They then received 66 mg. cholesterol-C\textsuperscript{14} of 2296 counts/min/mg\textsuperscript{4} dissolved in 6 ml. Tween-20. Each animal was given, via stomach tube, 0.5 ml. of this solution twice daily for 2 days. Between the stomach tube feedings, the animals were given Purina Laboratory Chow and water ad libitum. They were killed 4 hours after the last cholesterol-C\textsuperscript{14} feeding, dissected, and treated as before. The tumor nodules found in the peritoneal cavity were combined with the ascites fluid for hydrolysis.

As in the previous experiment the cholesterol from the digitonide had a low count, while the cholesterol after purification contained not more than traces of radioactivity. The fatty acids had a higher count than the digitonin cholesterol.

The fact that the implantation of the reaction mixture after 5 hours of incubation caused ascites tumor growth in the three injected rats shows that the tumor cells were viable throughout the experiment. As in Exp. VII the lack of incorporation of C\textsuperscript{14} into the pure cholesterol signifies the inability of the tumor cells to synthesize this substance, although the incorporation of C\textsuperscript{14} into digitonin-precipitable compounds and in fatty acids proves that the tumor cells still have synthetic potential.
DISCUSSION

The work, reported here, shows that, during the tumorigenic process, biosynthesis of cholesterol is changed even before a neoplasm becomes histologically defined. At this time cholesterol is synthesized in the liver at a lower level than in the normal animal, but synthesis increases when solid tumors become evident. Data for the gastrointestinal tracts and the carcasses follow a similar pattern. These changes of biosynthesis vary considerably with the development of the neoplasm in the five types of tumors studied. There are—as the best example—two distinctly separate stages in the development of the Yoshida ascites tumor (Chart 5a). The first (“initial phase”) is defined by Berenblum (4) and Butenandt et al. (7) as that phase when the tumor grows in the peritoneal cavity and during which biosynthesis in the liver is depressed; the second (“development phase” [4, 7]), at about the 6th day, occurs when the tumor invades the mesentery and intestines, and biosynthesis is stimulated considerably.

The two carcinogen-induced tumors in Exps. I and II behave similarly: as long as there is no visible tumor, cholesterol biosynthesis first decreases gradually but increases after 3 months, when solid tumors appear. When fully developed solid tumors are implanted, as in Exps. III and IV, an immediate stimulation of biosynthesis takes place. Evidently the first stage, as seen in Exps. I, II, and V, is obliterated, and only the second part of liver involvement is observed. This stimulation, however, is soon followed by another period of decrease. These findings suggest the possibility that the tumor has an effect on the liver. That the liver, as the main organ which synthesizes cholesterol (9), should be influenced by the tumor, is not without analogy. Biosynthesis of cholesterol is an enzymatic process, and effects of the tumor on enzymes, especially liver catalase, were observed by Nakahara et al. (“toxohormone”) (21), Greenstein (15), and Adams (1). (Compare also Boyd et al. “metabolism directing substance” [5] and Haven et al. “hormone given off by the tumor” [17, p. 304].)

The stimulation of cholesterol biosynthesis during the developmental phase could be a stress reaction of the body cells against the invading tumor. Adrenals are enlarged in tumor-bearing animals (22, 26), and cholesterol increases during stress (10). This second stage, as seen in Exps. III, IV, and the latter part of V, is, however, very often interfered with by necrosis which may increase cholesterol synthesis. A higher cholesterol content in the necrotic part of a tumor has been observed by Haven (16) and others, but no effect on the liver was established.

It has been reported (compare Haven and Bloor, p. 287 [17] and also Bauer [2]) that tumors contain and possibly produce more cholesterol than do normal tissues. From Table 1 it is obvious that in this work the quantity of cholesterol/gm wet tissue is remarkably constant. Only the tumor-bearing parts of the body show at certain stages statistically significant increases over the controls. From in vitro studies Emmelot et al. (12), Medes et al. (20), and others have concluded that tumor slices synthesize cholesterol from acetate, and Smith et al. (27) found the same by perfusing a human adrenal tumor. Contrary to these findings, Exps. VII and VIII prove that isolated Yoshida ascites cells do not synthesize any appreciable amount of cholesterol from acetate. This agrees, however, with Busch et al. (6), who claim that tumors are “virtually unable to metabolize acetate” and with Fish et al. (13), who believe that tumor cholesterol is actually synthesized in the liver. The discrepancy of the results of this work and the earlier in vitro work is very probably caused by the great difficulty in obtaining tumors free from adherent normal tissue. That the tumor acts to trap cholesterol from the circulation is also assumed by Emmelot et al. (12). This view is supported here by Exp. V and the data of Table 2, in which cholesterol-C14 fed to tumor rats is shown to be deposited in the tumor-bearing leg to a greater degree than in the carcass.

As in normal animals (25), differences between the specific counts of cholesterol from digitonin and after bromination (HCC) are found in all experiments, suggesting that biosynthesis in tumorous animals is not taking a course different from that in normal animals.

Determinations of blood cholesterol (Chart 7) show agreement with the biosynthetic patterns in Exps. II–V. Surprisingly, however, in Experiment
I (DMAB-hepatoma), values for the blood cholesterol are higher in the tumor-bearing animals, possibly because of the derangement of lipide metabolism caused by the rice-carrot diet.

The data presented show that, during tumorigenesis in animals, liver biosynthesis of cholesterol and its amount in the blood may be lower or higher than normal according to the stage of development of the tumor. In cancer patients, where only the latter can be determined, Hellman et al. (18) found no alteration of cholesterol, while Gould et al. (14) observed an increase of 4 percent. However, the phase of tumor development was not determined in these investigations.

Fatty acid synthesis from acetate in tumor-bearing animals is somewhat depressed, and in a general way shows a pattern similar to that of cholesterol. Only in the tumorous legs are the specific counts occasionally higher than those in the controls. The two peaks of specific counts, as also observed by Beekmanns et al. (9), were seen in most of the experiments and may account for the variations found. Little fatty acid synthesis takes place in the isolated tumor cell (Chart 6), in agreement with Medes et al. (20), who find that the tumor cannot synthesize all fatty acids which it requires and takes them from the circulation.

It is important to emphasize that the changes in cholesterol biosynthesis are not accompanied by corresponding changes in the histology of the liver cells even at the time of the greatest development of the neoplasms. Similarly, normal histology of the liver was reported in experiments on liver enzymes of tumor animals (15). Even in the hepatoma Experiment I, the drop in cholesterol biosynthesis is considerable at a time when the liver, histologically, is still in a preneoplastic stage (compare Sugiura [28]).

SUMMARY

Biosynthesis of cholesterol and fatty acids, as judged by the incorporation of C14 from acetate into these compounds, is affected soon after initiation of the tumorigenic process and is dependent upon the stage of development of the tumor.

In the livers of rats with carcinogen-induced tumors, biosynthesis of cholesterol dropped below normal, while the tumor was in its latent form, but increased again when the tumor became visible. In rats and hamsters with transplantable tumors, there was an immediate rise and later a decrease in biosynthesis.

Cholesterol biosynthesis in the gastrointestinal tracts and carcasses followed in general the liver patterns.

Fatty acid synthesis was only slightly affected in tumor-bearing animals.

Cholesterol determinations in the blood of tumor-bearing animals were in agreement with the biosynthesis patterns with the exception of the hepatoma experiment.

No significant changes have been observed in the cholesterol contents of tissues of tumor-bearing animals as compared with the normal.

Isolated Yoshida ascites tumor cells incubated with acetate-1-C14 did not synthesize more than traces of cholesterol. Tumors might, however, trap cholesterol.

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SCHWENK AND STEVENS—Cholesterol and Fatty Acid Synthesis


Biosynthesis of Cholesterol and Fatty Acids in Tumor-bearing Animals

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