Identification of Linoleic and Arachidonic Acids as the Factors in Hyperlipemic Blood That Increase \(^{3}\text{H}\)Thymidine Incorporation in Hepatoma 7288CTC Perfused in Situ

Leonard A. Sauer and Robert T. Dauchy

Laboratory for Cancer Research, Bassett Institute for Medical Research, The Mary Imogene Bassett Hospital, Cooperstown, New York 13326

ABSTRACT

Tumor growth and the incorporation of \(^{3}\text{H}\)thymidine into tumor DNA in vivo are increased about 3 times in adult rats (greater than 250 g) after 1 to 2 days of starvation or the induction of diabetes with streptozotocin. These tumor growth responses require hyperlipemia and are reversed by refedding or insulin treatment, respectively. They do not occur in young tumor-bearing rats (less than about 150 g) that lack appreciable fat stores. A direct relationship between the increased rates of both \(^{3}\text{H}\)thymidine incorporation and tumor growth and host hyperlipemia suggests that tumor cell renewal in vivo in fed rats is limited by substances that are present in hyperlipemic blood.

In this study we used a procedure for perfusion of solid tumors in situ to measure the sensitivity of tumor \(^{3}\text{H}\)thymidine incorporation to hyperlipemic blood and to identify the rate-limiting substances. Tissue-isolated Morris hepatomas (7288CTC) growing in young or adult Buffalo rats were perfused with blood from donor rats. Hyperlipemic blood for perfusion was obtained from 2-day starved tumor-bearing (Buffalo) or non-tumor-bearing (Buffalo or Lewis) rats. At the end of the perfusions the tumors were labeled with a pulse of \(^{3}\text{H}\)thymidine (2 \(\mu\)Ci/g estimated tumor wet weight). \(^{3}\text{H}\)Thymidine incorporation in tumors growing in fed adult rats was increased about 3 times in adult rats (greater than 250 g) after 1 to 2 days of starvation or the induction of diabetes with streptozotocin. A prominent feature of the tumor growth responses requires hyperlipemia and are reversed by refeeding or insulin treatment, respectively. They do not occur in young tumor-bearing rats (less than about 150 g) that lack appreciable fat stores. A direct relationship between the increased rates of both \(^{3}\text{H}\)thymidine incorporation and tumor growth and host hyperlipemia suggests that tumor cell renewal in vivo in fed rats is limited by substances that are present in hyperlipemic blood.

In these experiments we used a procedure for perfusion of tissue-isolated tumors in situ with donor blood. The primary goal of the study was to identify the active agent(s) in hyperlipemic blood that stimulate tumor \(^{3}\text{H}\)thymidine incorporation.

MATERIALS AND METHODS

Reagents. \([\text{methyl-}\text{}^{3}\text{H}]\text{Thymidine (6.7 Ci/mmole) and L-\text{[\text{1-14C]}Leucine (250 to 300 mCi/mmole) were purchased from Research Products International, Mt. Prospect, IL. Cycloheximide and palmitic, steatoc, oleic, linoleic, and arachidonic acids were obtained from Sigma Chemical Co., St. Louis, MO. The purity of these fatty acids was measured by gas chromatography and was greater than 98%, in agreement with the specifications of the supplier. Acetone, chloroform, and ethanol were purchased from Fisher Chemical Co. and were redistilled before use. Methyl esters of the standard fatty acids were obtained from Supelco, Bellefonte, PA. Affigel-blue is a product of Bio-Rad Laboratories. Animals and Tumors. Young and adult male Buffalo and adult male Lewis rats were obtained from colonies established here. The rats were fed a laboratory chow (Prolab mouse, rat, and hamster 1000 formula; Agway, Inc., Syracuse, NY), had water ad libitum, and were maintained at a constant temperature of 23°C in a room with lights on 6 a.m. to 6 p.m. At the time of tumor implantation the young Buffalo rats were 30 to 45 days old and weighed less than 200 g and the adult rats were 3 to 5 months old and weighed 250 to 350 g. All experiments were performed with the Morris hepatoma 7288CTC. Arterial blood for perfusion was obtained from adult tumor-bearing Buffalo rats or from adult non-tumor-bearing Buffalo or Lewis rats that were either fed ad libitum or were starved for 2 days prior to bleeding. After anesthesia (sodium pentobarbital, 25 mg/kg, i.p.) and administration of an anticoagulant (50 units sodium heparin/100 g body weight, i.v. in the exterior jugular vein) the rats were bled from a carotid arterial catheter. Tumors showed similar responses following perfusion with whole blood from Buffalo or Lewis rats. Preparation of Tumors for Perfusion. All experiments were performed using tissue isolated tumors grown s.c. in the left inguinal fossa (5). Briefly, a 3-mm cube of tumor was attached to the end of a vascular stalk composed of the truncated superficial inferior epigastric artery and vein. The implant and end of the stalk were enclosed in a Parafilm envelope, placed in the inguinal fossa, and the skin incision was closed. The tumor arterial blood supply and venous drainage were established through the epigastric vessels. Tumor attachment to other host tissues was blocked by the Parafilm envelope. The experiments were started...
when the growing tumors were estimated to weigh 6 to 7 g. In some experiments tumor growth in adult rats was stimulated by a 2-day period of starvation (1), which increased the tumor weight in these animals to about 12 to 13 g.

Tumors growing in either the fed or starved rats were prepared for perfusion in situ as described by Dauchy and Sauer (4); (a) a PE 50 catheter (Clay Adams, Parsippany, NJ), about 20 cm in length and containing a Y-tube connector about 7 cm from the distal end, was inserted into the left carotid artery of an anesthetized and heparinized host rat; (b) the femoral inferior epigastric venous trunk emerging from the tumor was exposed and catheterized with a butterfly catheter (ST Infusion Set; Abbott Hospital Products, North Chicago, IL); (c) the femoral artery proximal to the tumor was elevated and nicked and the free end of the carotid catheter was inserted (tip toward the tumor) and secured. The tumor received oxygenated blood by reversed flow through the distal femoral artery and its collateral branches during this procedure (4); (d) the clamp was released from the carotid catheter and host arterial blood flowed into the tumor. The femoral artery distal to the tumor was then ligated.

Perfusion with Arterial Blood from Individual Donor Rats. Arterial blood from the carotid catheter of a donor rat was introduced into the tumor through the side arm of the Y-tube connector; the flow of host blood was clamped at the same time. About 3 ml of blood were bled from each donor rat over a 30-min period. Thus, six donor rats were required for a 3-h perfusion. Donor rats recovered from this blood loss.

Perfusion with Pooled Donor Arterial Blood. Eighty to 90 ml of whole blood were collected from 8 to 10 adult donor rats, chilled to 4°C, and gently mixed on a mechanical rocking device. The blood was pumped from the chilled container into the tumor with a peristaltic pump (Model 1206; Harvard Apparatus, S. Natick, MA). Pressure was measured with a Condon's manometer (Harvard Apparatus) and adjusted to 60 to 80 mm Hg at a flow rate of 0.1 to 0.15 ml/min. The blood was warmed to 37°C before it entered the tumor. Except for a short section of Silastic tubing in the pump, all connections were made of vinyl tubing. Arterial oxygen saturation values were 97% or greater (AVL blood gas analyzer, Model 945; Graz, Austria).

Experimental Design for Measurement of \textsuperscript{3}HThymidine Incorporation into Tumor DNA. Tumors were labeled with [methyl-\textsuperscript{3}H]thymidine (2 \textmu Ci/g estimated tumor weight) injected into the carotid catheter. Unincorporated \textsuperscript{3}H-thymidine appeared in the tumor venous blood about 2 min after the injection, reached a peak after 3 to 4 min, and then decreased gradually and was nearly completely eliminated from the tumor after 20 min. Therefore, \textsuperscript{3}Hthymidine was administered 20 min before the end of the perfusion.

In some experiments tumor protein synthesis was inhibited during the perfusion by administration of cycloheximide (60 \mu g/g total body weight, injected i.v.) to host and donor rats. This dose inhibited tumor l-leucine incorporation about 90% \textsuperscript{-1} (1). Because the effect of cycloheximide \textsuperscript{in vivo} decreases with time after injection (6), individual cycloheximide-treated donor rats were used for these experiments. The procedure was as follows: the host rat was treated 30 min before the tumor was prepared for perfusion and donor rats were treated 30 min before connection to the tumor. Thus, the tumor was continuously perfused with blood from rats treated with cycloheximide for 1 h or less. Total perfusion time was 2 h and required four donor rats. The tumor was labeled with a solution that contained [\textsuperscript{3}H]thymidine (2 \textmu Ci/g estimated tumor weight), l-[\textsuperscript{U-14}C]leucine (0.5 \textmu Ci/g estimated tumor weight), and 100 \mu mol/ml unlabeled l-leucine (7).

After the perfusion the tumor was removed from the animal, weighed, and frozen until analysis. A 20% homogenate was made in 0.9% saline solution and the incorporation of \textsuperscript{3}Hthymidine into tumor DNA and the tumor DNA content were measured as previously described (2, 3). Incorporated \textsuperscript{14}C-leucine, when present, was contained in the pellet that remained after DNA extraction and was dissolved in Protosol (Beckman, Inc.) and counted by liquid scintillation.

Methods for Fractionation and Reconstitution of Donor Blood. Hyperlipemic arterial blood from starved adult donor rats was fractionated and the components were tested for their ability to increase \textsuperscript{3}H-thymidine incorporation when perfused into tumors growing in fed adult rats. The perfusates were prepared as follows.

Forty ml of pooled arterial blood from fed or starved adult donor rats were separated into plasma and cellular fractions by centrifugation. Two whole blood preparations were reconstituted by combining the plasma and cellular fractions from the blood of the starved rats with the cellular and plasma fractions, respectively, from the blood of the fed rats.

Twenty ml of plasma from the arterial blood of either fed or starved donor rats were extracted twice with 10 volumes of acetone:ethanol (1:1). The insoluble materials were separated by centrifugation and the supernatant solutions, which contained the plasma lipids, were combined and dried by rotary evaporation at 37°C. Two whole blood preparations were formed by dissolving the residue from the plasma of the fed rats and the residue from the plasma of the starved rats, respectively, with 20 ml of plasma and 20 ml of packed cells from the arterial blood of fed adult donor rats.

Plasma from the arterial blood of starved adult donor rats was separated into albumin and globulin fractions using a 1.6 - x 17-cm Affigel-blue column, as described by Travis and Pannel (8). Fifteen ml of plasma containing about 600 mg of albumin were dialyzed against 0.1 M sodium phosphate, pH 7.0, before loading on the column. The globulins and albumin were successively eluted with 50 ml of 0.1 M sodium phosphate and 50 ml of 2 M sodium chloride, respectively. Protein peaks were monitored at 280 nm (Photometer Model UA-5; ISCO, Lincoln, NE). Fractions comprising the peaks were pooled and concentrated in a stirred ultrafiltration cell (Model 52; Amicon Corp., Lexington, MA) equipped with a YM-30 filter. After three washes by resuspension in phosphate (5 mm)-buffered saline, pH 7.4, the albumin and globulin fractions were concentrated to 5 ml. Purity was measured by electrophoresis on cellulose acetate (Gelman Sciences, Ann Arbor, MI) at pH 8.6 in barbital buffer. The albumin fraction, which was estimated to be greater than 95% pure, contained a small amount of the globulins. However, albumin was absent from the globulin fraction. Two whole blood preparations were formed by adding either the globulin or albumin fraction to 15 ml of plasma and 15 ml of packed cells from the arterial blood of fed adult rats.

Individual fatty acids were dissolved in plasma from arterial blood of fed adult donor rats. An equivalent volume of oxygenated packed cells was added back to reconstitute a whole blood preparation for perfusion.

Analysis of Fatty Acids. The fatty acid content of plasma fractions was measured using a Perkin-Elmer Sigma 3 gas chromatograph with a 3.2-mm x 1.8-m 5% diethylene glycol succinate column at 200°C with a flame ionization detector (9). Plasma (0.25 ml) was added to 1.75 ml of 33 mm sodium phosphate buffer (pH 6.6); 10 \mu g of heptadecanoic acid were added as an internal standard and the mixture was extracted with 6 ml of chloroform:heptane:methanol (50:50:2). After centrifugation and removal of the aqueous layer, the organic layer was evaporated to dryness under a stream of N\textsubscript{2} at 37°C. The fatty acids in the residue were redissolved in isooctane, transferred to a clean tube, evaporated to dryness again, and converted to the methyl esters with boron trifluoride/methanol. Fatty acid methyl esters were identified by mass spectrometry (Model 3390A; Hewlett Packard, Sunnyvale, CA).

Statistical Analysis. Means are presented ± 1 SD and were compared by one-way analysis of variance (ANOVA) and the Duncan multiple range test. \textit{P} < 0.05 was considered significant.

RESULTS

Nutritional Status of Host and Blood Donor Rats and the Incorporation of \textsuperscript{3}HThymidine in Hepatoma 7288C TC Perfused \textit{in Situ}. Table 1, Experiment 1, lists the \textsuperscript{3}Hthymidine incorporation measured in tumors growing in fed adult rats. The mean rate of thymidine incorporation shown in Line A represents the zero time measurement made before perfusion with donor blood. Cells in these tumors were exposed to normolipemic blood from the time of implantation to the time of assay. Data listed on Lines B and C indicate that perfusion with
Table 1 Incorporation of [3H]thymidine in hepatoma 7288CTC perfused in situ

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nutritional status of host</th>
<th>Nutritional status of donor</th>
<th>Perfusion time (h)</th>
<th>[3H]Thymidine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adult host and donor rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Fed</td>
<td>Host</td>
<td>Zero time</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>B</td>
<td>Fed</td>
<td>Fed, TB</td>
<td>2</td>
<td>74 ± 4</td>
</tr>
<tr>
<td>C</td>
<td>Fed</td>
<td>Fed, NTB</td>
<td>2</td>
<td>89 ± 6</td>
</tr>
<tr>
<td>D</td>
<td>Fed</td>
<td>Starved, TB</td>
<td>2</td>
<td>194 ± 12</td>
</tr>
<tr>
<td>E</td>
<td>Fed</td>
<td>Starved, NTB</td>
<td>2</td>
<td>209 ± 9</td>
</tr>
<tr>
<td>2. Adult host and donor rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Starved</td>
<td>Host</td>
<td>Zero time</td>
<td>211 ± 13</td>
</tr>
<tr>
<td>G</td>
<td>Starved</td>
<td>Starved, NTB</td>
<td>2</td>
<td>201 ± 1</td>
</tr>
<tr>
<td>3. Young host and adult donor rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>Fed</td>
<td>Host</td>
<td>Zero time</td>
<td>74 ± 7</td>
</tr>
<tr>
<td>I</td>
<td>Fed</td>
<td>Fed, NTB</td>
<td>2</td>
<td>91 ± 8</td>
</tr>
<tr>
<td>J</td>
<td>Fed</td>
<td>Starved, NTB</td>
<td>2</td>
<td>225 ± 5</td>
</tr>
<tr>
<td>K</td>
<td>Starved</td>
<td>Host</td>
<td>Zero time</td>
<td>85 ± 5</td>
</tr>
<tr>
<td>L</td>
<td>Starved</td>
<td>Starved, NTB</td>
<td>2</td>
<td>214 ± 14</td>
</tr>
</tbody>
</table>

* TB, tumor bearing; NTB, non-tumor bearing.
* † [3H]Thymidine incorporation after perfusion with blood from starved TB donor rats is significantly greater than after perfusion with blood from fed TB donor rats, at P < 0.01.
† [3H]Thymidine incorporation after perfusion with blood from starved NTB donor rats is significantly greater than after perfusion with blood from fed NTB donor rats, at P < 0.01.
‡ [3H]Thymidine incorporation after perfusion with blood from NTB starved donor rats is significantly greater than after perfusion with blood from starved young host rats, at P < 0.01.

In Fig. 1, A and B, perfusion with hyperlipemic blood caused a gradual increase in [3H]thymidine incorporation in tumors growing in fed adult rats. The stimulation was preceded by a lag period of about 0.5 h. Incorporation levels measured after 3 h were equal to those observed at zero time in tumors growing in the starved adult rats (Table 1, Line F). Interestingly, perfusion of tumors growing in adult starved rats with normolipemic blood inhibited the high level of [3H]thymidine incorporation.
(Fig. 1, D and E). Again there was a short lag, before the gradual decrease in incorporation. The last values measured at 3 h were similar to the zero time values measured in tumors growing in fed adult rats (Table 1, Line A and Fig. 1, A and B). Tumor DNA contents were not changed.

Effect of Cycloheximide on the Incorporation of [3H]Thymidine into Hepatoma 7228 CTC Perfused in Situ. These experiments were performed to determine if the stimulations and inhibitions of tumor [3H]thymidine incorporation illustrated in Fig. 1, A and B and Fig. 1, D and E, respectively, required concurrent protein synthesis.

In Fig. 2, Columns 1 show the mean incorporations of [3H]-thymidine and [14C]leucine in tumors growing in fed adult rats after perfusion for 2 h with blood from other fed adult rats. The value for [3H]thymidine incorporation agrees with data from Table 1, Lines B and C. Treatment of the fed host and donor rats with cycloheximide prior to the start of the perfusion inhibited tumor leucine incorporation about 90% but did not affect tumor thymidine incorporation (Columns 2). Tumor [3H]thymidine incorporation was increased when these tumors were perfused for 2 h with hyperlipemic blood from starved adult rats (Fig. 2, Column 3, [14C]leucine incorporation was not measured). However, cycloheximide treatment of both the adult host and donor rats prior to the perfusion had no effect on the stimulation of [3H]thymidine incorporation even though [14C]leucine incorporation was reduced to a very low level (Columns 4).

The high [3H]thymidine and [14C]leucine incorporations observed in tumors growing in starved adult rats following perfusion with blood from other starved adult rats are shown in Fig. 2, Columns 5. Inhibition of tumor leucine incorporation by treatment of the starved host and donor rats with cycloheximide prior to perfusion did not influence the tumor [3H]thymidine incorporation (Columns 6). Tumor [3H]thymidine incorporation was inhibited when tumors growing in starved animals were perfused for 2 h with normolipemic blood (Columns 7, [14C]leucine incorporation was not measured). Prior treatment of the host and donor rats with cycloheximide did not alter this inhibition of tumor [3H]thymidine incorporation despite the inhibition of [14C]leucine incorporation (Columns 8). It seems reasonable to conclude, therefore, that new protein synthesis is not required for these acute increases and decreases in tumor [3H]thymidine incorporation.

Identification of Substances in Hyperlipemic Blood That Stimulate Tumor [3H]Thymidine Incorporation. The in situ tumor perfusion procedure was used as an assay for detection of these substances. Cells, plasma, and selected plasma extracts isolated from hyperlipemic arterial blood were reconstituted to whole blood mixtures for perfusion by combining with plasma, cells, and whole blood, respectively, from fed adult rats. Tumors growing in fed adult rats were perfused for 2 h with these blood mixtures and were labeled with [3H]thymidine at the end of the perfusion. The incorporations were compared to low and high control incorporation values obtained from tumors perfused with blood from fed or starved adult rats, respectively. Active blood preparations were identified as those that significantly increased tumor [3H]thymidine incorporation above the mean low control value.

The results of these experiments are listed in Table 2. Low and high control [3H]thymidine incorporation values are given under experiments 1 and 2, respectively. Reconstituted blood fractions that stimulated tumor [3H]thymidine incorporation contained plasma from hyperlipemic blood (experiment 4), the total lipid extracts of plasma from hyperlipemic blood (experiment 5), and albumin preparations isolated from plasma of hyperlipemic blood (experiment 7). Inactive reconstituted blood preparations contained cells (experiment 3) and globulin protein preparations from hyperlipemic blood (experiment 8). Total lipid extracts of plasma from normolipemic blood (experiment 6) were also inactive. Thus, the active substances in hyperlipemic blood are contained in the plasma, are soluble in lipid solvents, and are bound to albumin.

Palmitic, stearic, oleic, linoleic, and arachidonic acids, the major free fatty acids in normolipemic rat plasma, were each increased about four to five times in hyperlipemic plasma (Fig. 3). The effects of these fatty acids on tumor [3H]thymidine incorporation are shown in Table 3 and were tested as follows: either single fatty acids or groups of them were added to normolipemic plasma to give concentrations that equaled or exceeded the mean concentrations in hyperlipemic plasma; cells from the normolipemic blood were added back. The mixtures were perfused for 2 h through tumors growing in fed adult rats and [3H]thymidine incorporation was measured. The saturated fatty acids, palmitic and stearic, were inactive when added singly (data not shown) or together (Table 3, experiment 1). Oleic acid also had no effect (Table 3, experiment 2). However, blood enriched in linoleic and arachidonic acids stimulated tumor [3H]thymidine incorporation (Table 3, experiments 3 and 4); linoleate was more effective at the concentrations encountered in hyperlipemic plasma. Experiments 5 and 6, respectively, show that the stimulatory effects of linoleic and arachidonic acids were additive and that higher concentrations did not further increase the [3H]thymidine incorporation. Finally, perfusion of normolipemic blood enriched in the ketone bodies (10) had no effect on [3H]thymidine incorporation in tumors growing in fed adult rats (data not shown).

DISCUSSION

In this study we used a procedure for perfusion of intact solid rat tumors in situ to examine the stimulating effect of hyperli-
pemic blood on tumor [3H]thymidine incorporation and to identify the active substances. Linoleic and arachidonic acids, the two major polyunsaturated free fatty acids in the hyperlipemic blood of starved adult rats, were identified. Addition of these fatty acids to normolipemic blood at concentrations equaling those found in hyperlipemic blood reconstituted the normolipemic blood to full activity. Linoleic acid was the more stimulating and the effects of the two fatty acids were additive. Palmitic, stearic, and oleic acids, the ketone bodies, and the globulin protein fraction from hyperlipemic plasma, which contains the triglycerides, were all inactive. The ability of hyperlipemic blood to stimulate [3H]thymidine incorporation in tumors in situ was entirely accounted for by the elevated linoleic and arachidonic acid concentrations. Presumably, longer exposures to linoleic and arachidonic acids, as occurs after 2 to 7 days of acute starvation or diabetes in adult rats,

sized thymidine triphosphate pools. Experiments are now under way using autoradiographic and flow cytometric methods to determine how linoleic and arachidonic acids affect the cell cycle in tumors growing in fed rats. Since these polyunsaturated fatty acids could act by changing the rate of DNA synthesis in cells in active S phase and/or by stimulating active DNA synthesis in dormant S phase cells (11, 12) as well as by changing the specific activity of thymidine triphosphate pools, we considered these experiments to be beyond the scope of this report. A shift of tumor cells from G1 to S phase seems an unlikely explanation for the observed increase in [3H]thymidine incorporation due to hyperlipemia because protein synthesis is required for this transition (13, 14) and cycloheximide treatment had no effect on tumor [3H]thymidine incorporation (Fig. 2). An increase in the rate of DNA synthesis in S phase cells, however, may be cycloheximide insensitive (15).

Our results would appear to be in vivo counterpart to the studies of Kidwell et al. (16), who reported that rat serum stimulated the growth and incorporation of [3H]thymidine into WRK-1 rat mammary tumor cells in culture. The active substances in serum were localized to alipoprotein fractions containing albumin and were identified as free fatty acids. Both the saturated and unsaturated fatty acids stimulated growth and thymidine incorporation into WRK-1 cells but linoleic acid was the most effective. Subsequently, Wicha et al. (17) reported that unsaturated free fatty acids stimulated growth and [3H]thymidine incorporation into normal rat mammary epithelial and 7,12-dimethylbenz[a]anthracene-induced rat mammary tumor cells in culture. The stimulatory effect lasted for up to 72 h and required the presence of insulin, hydrocortisone, progesterone, estrogen, prolactin, and delipidized fetal calf serum. Linoleic and linolenic acids were the most effective stimulators for normal mammary epithelial cells, and oleic and linoleic acids were most effective for the tumor cells. Interestingly, stearic and palmitic acids inhibited growth of both cell types. In our experiments neither oleic nor stearic and palmitic acids affected [3H]thymidine incorporation in hepatoma 7288CTC perfused in situ (Table 3). Wicha et al. (17) also showed that linoleic acid stimulated and stearic acid inhibited [3H]thymidine incorporation into acid-precipitable material in normal mammary epithelial and tumor cells within 1 to 3 h after addition. The kinetics of these early stimulatory effects of linoleic acid described by Wicha et al. (17) is very similar to those shown in Fig. 1, A and B for the stimulation of [3H]thymidine incorporation in hepatoma 7288CTC following perfusion in situ with hyperlipemic blood.

It is now well known that diets high in vegetable oils containing linoleic acid stimulate the growth of transplanted and spontaneous tumors in rodents and increase the number of tumors arising from carcinogen treatments (see Refs. 18 and 19 for recent reviews). Epidemiological evidence suggests that high fat diets have similar effects in human tumor growth and carcinogenesis (20, 21). The linoleic acid content of human adipose tissue may be an important cancer risk factor (22). How diets rich in linoleic acid stimulate tumor growth and carcinogenesis is not known. Wicha et al. (17) demonstrated that oleic and linoleic acids had a direct effect on the growth of tumor cells in culture. Our experiments indicate that linoleic and arachidonic acids in the arterial blood stimulate [3H]thymidine incorporation in tumors perfused in situ and suggest that these fatty acids are rate limiting for tumor growth in vivo. Diets high in linoleic acid increase the concentration of this fatty acid in the blood (23). In view of our results, it seems possible that an elevated arterial blood linoleate concentration would stimulate growth of existing tumor cells and give selective growth advantages to recently transformed cells. Experiments are under way to test these proposals.

ACKNOWLEDGMENTS

Thanks are due Dr. Estelle Goodell for help with the statistical analyses and Dr. Roberta Reed and Chris Burrington for assistance with the plasma protein separations.

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