Abnormal Fat Absorption and Utilization in Rats Bearing Walker Carcinoma 256*

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SUMMARY

A method was modified for the assay of 113I-labeled lipides in microquantities of plasma or in lymph collected from cannulated rats.

The changes in plasma lipide activity in response to triolein-113I test diets fed to fasted rats were studied. Higher mean tolerance curve peaks were observed in tumor-bearing than in normal or pair-fed control animals. The elevated peaks of alimentary hyperlipemia increased with tumor growth.

Fat absorption studies in rats with thoracic duct cannulas showed a marked decrease in amount and rate of fat absorption in the tumorous animals.

In vivo clearance studies indicated that the hyperlipemia is due to an impairment of the clearance mechanism in the tumor-bearing rats. The results of these investigations were confirmed by in vitro studies of lipoprotein-lipase activities in post-heparinized plasma of tumor-bearing and their pair-fed control mates.

The above findings led to the conclusion that the cachexia associated with tumor growth is due to (a) a decrease in absorption of dietary fat and (b) debilitated utilization with a consequent mobilization of fat depots.

It has been established that tumor growth in rats leads to anorexia, loss of body weight, depletion of carcass fat depots, and eventually to cachexia (2, 14, 15, 19). The concomitant hyperlipemia may be highly exaggerated in force-feeding experiments in which attempts are made to prevent body weight loss (19, 24). The causes of neither the cachexia nor the hyperlipemia had satisfactorily been elucidated.

Tumor growth probably increases the caloric requirements of the host (20, 21) owing to increased energy expenditure (19). Although the tumor may synthesize fatty acids, this process is too slow, and the tumor has to secure preformed fat from the host (18).

Haven and Bloor attributed the hyperlipemia to fat mobilization (15). Begg et al. (1, 2), on the other hand, maintained that the hyperlipemia is due to defective clearance mechanisms, in which lipoprotein lipase was implicated (1).

The methods available for the use of 113I-labeled lipides were adapted, modified, and applied to the investigation of the above problems. Three types of studies were conducted: (a) fat tolerance studies, (b) in vivo and in vitro clearance studies, and (c) fat absorption studies. A preliminary report of the absorption studies was published (22).

MATERIALS AND METHODS

Experimental animals.—Sprague-Dawley male rats were kept in large individual cages and weighed daily. The amount of diet consumed by each rat in a 24-hour period was determined by differential daily weighing of the diet. For a period of 7–10 days from their arrival in the laboratory, the rats were allowed to feed ad libitum on a normal synthetic diet complete with vitamins and a salt mixture. Next, the rats were divided into groups of three on the basis of growth rate and food consumption, i.e., in each group one rat was designated “normal,” one “pair-fed control,” and the third rat “tumor-bearing.” The normal rats were not treated in any way prior to experiments; they were fed ad libitum at all times. The tumor-bearing rats were given transplants of Walker carcinoma 256 and were also permitted food ad libitum. On the day following the trans-
plantation of the tumor, the pair-fed control rats were fed only as much of the diet as was consumed on the previous day by their tumor-bearing mates. Consequently, with progress of tumor growth and with the development of anorexia in the tumor-bearing group, the diet of the pair-fed control rats was comparably restricted. The rats of all three groups had free access to water.

Tumors were transplanted by the standard piece technic (23). Tumor size was estimated by the Schrek method (23) and was expressed in percent of total body weight. Whenever possible, tumors were removed and weighed on a balance. Tumor growth, when expressed in time units (days or weeks), designates the time elapsed from the moment when growth of a transplanted tumor could be detected.

**Triolein-^111 test diet.**—The chemical addition of iodine (I^111)-monochloride to the double bond between carbons 9 and 10 of the oleic acid moiety in the triolein molecule results in a radioactively iodinated product. The I^111 binding in this compound is stable to gastric juice, bile, pancreatic juice, 25 percent hydrochloric acid (3), 10 percent potassium iodide (KI), and 40 percent trichloroacetic acid (TCA) (23). The triolein-I^111 used in these experiments was procured from Abbott Laboratories, Chicago, Illinois, and contained about 0.2 percent I^111 in the nonlipide form.

The test diet was prepared by diluting 0.2 to 1.0 ml. of the stock triolein-I^111 with pure olive oil to a total volume of 10.0 ml. Since stock triolein-I^111 activities varied greatly owing to the short half-life of I^111 (8 days), the concentrations of labeled triolein in the test diet were not constant. However, attempts were made to keep the concentrations within narrow limits and to use identical test diets, with activities of 20 μc/ml, in studying pair-fed mates. The activity of the test diet was 20 μc/ml. In the experiments in which this test diet was used, doses of 0.5 ml. (10 μc), fed to each rat, constituted the "total dose" (TD).

**Fat tolerance and method of assay of plasma lipide activity.**—The administration of test diet to the rats by the use of a metal stomach tube is referred to as "force-feeding." After an overnight fasting period, the rats were force-fed 0.5 ml. triolein-I^111 test diet followed by 5 ml. of water. The administration of water following the test diet was indispensable, since its omission caused the rats to eliminate the diet in about 1 hour after the feeding in the form of a severe diarrhea. At 1, 2, 3, 4, and 6 hours from the time of the feeding, tail vein blood was collected into 10-cm., heparinized, melting-point capillary tubes open at both ends. The tubes were then flame-sealed and spun in the centrifuge for 10 minutes at 2,000 r.p.m.

The method of Turner (25) was modified and adapted for microdeterminations of plasma lipide activity. The essential modification was the dilution of the plasma to be analyzed with human plasma devoid of any radioactivity. The dilution of the plasma samples with unlabeled plasma provided bulky precipitates which effected recoveries of 90–95 per cent, and agreement of 5–10 per cent was found between duplicate runs. Plasma samples analyzed by the Turner method and by the present modification of that method were identical in plasma lipide activity.

For the fat tolerance studies, one capillary tube was collected for each blood sampling; 0.02 ml. of the rat's tail vein blood was transferred with micropipettes into test tubes containing 0.3 ml. of the stock plasma. Five ml. of a 10 per cent KI solution was then added, and the whole mixture was thoroughly stirred. Next, 3 ml. of a 40 per cent TCA solution was added slowly with constant stirring of the mixture.

The tubes, after standing for 15 minutes for complete precipitation of the plasma chylomicrons and plasma lipoproteins, were centrifuged for 15 minutes at 2,000 r.p.m. The precipitates were washed with 4-ml. lots of 3:1 10 per cent KI and 40 per cent TCA and the tubes spun again for 10 minutes. The washing was repeated two more times or until the supernatants contained no more countable radioactivity. In this way, all the free I^111 resulting from metabolic breakdown of triolein-I^111 was removed (25). The radioactivity of the tubes was measured in the deep-well scintillation counter, allowing for a counting error of not more than 10 per cent.

The results of the fat tolerance and of the absorption studies were expressed in terms of "per cent total dose" (% TD). The values for %TD were derived by multiplying the net counts/min in any assay sample by 100 and dividing by the net counts/min in the test diet dose (TD). In the fat tolerance studies, the values for %TD were multiplied by a correction factor of 5,000, so that the final results were in terms of %TD/100 ml plasma. No correction was made for differences in plasma volume, since these were not found to be large enough to affect the results. The plasma lipide activity was plotted as %TD vs. time in hours. The resulting curve was referred to as "fat tolerance curve."

**Thoracic duct cannulation, collection, and assay of lymph lipide activity.**—Rats in the various groups were force-fed with 0.1 ml. olive oil. From
2 to 4 hours later thoracic ducts of these animals were cannulated according to the technic of Bollman et al. (8). The animals were secured to the restraining cages immediately after surgery (7). The animals had access to 1 per cent sodium chloride contained in the drinking bottles, but had no access to food from the time they were placed in the restraining cages until the completion of the experiments. The lymph was collected into 3.8 per cent sodium citrate (12) or into heparin. In all the experiments where rates of absorption were studied, the lymph was collected at half-hourly intervals.

Twenty-four hours after the cannulation, triolein-I\(^{131}\) test diet was administered in 0.5-ml. portions per rat by means of a metal stomach tube was followed by 5 ml. of water. Collection of lymph for lipide activity assay was started immediately after the administration of the test diet. The lymph was assayed in a manner similar to that employed for plasma analysis. In all these experiments, the addition of stock plasma to the lymph samples was mandatory, since otherwise no precipitate was formed upon the addition of TCA. The TCA precipitated the plasma proteins with which the chylomicrons and lipoproteins present in the lymph coprecipitated.

To each tube, containing from 1.5 to 2.5 ml. of thoracic duct lymph, 1 ml. of stock plasma and 5 ml. of 10 per cent potassium iodide were added, the mixture was stirred, and then 3 ml. of 40 per cent trichloroacetic acid was added with constant stirring. The tubes were then centrifuged for 20 min. at 2,000 r.p.m. The precipitates were washed three times with 5-ml. fractions of 3:2 10 per cent KI and 40 per cent TCA and counted in a deep-well scintillation counter.

**Intravenous injection of lymph for clearance studies.**—Lymph was collected from thoracic duct cannulas of rats fed with the triolein-I\(^{131}\) test diet either into 8.8 per cent sodium citrate solutions or into heparin solutions. The fractions of lymph which gave the highest radioactive counts were used for the clearance studies. In all the experiments, the lymph had activities of about 200,000 counts/min/ml. Heparin concentration in injected lymph was 0.85 mg/kg body weight. In one experiment, the citrated lymph was diluted 1:1 with isotonic saline.

**In vivo rate of clearance of the lymph by the rats** was studied by injecting 5 ml. of the radioactive lymph into the exposed femoral vein of fasted rats under mild ether anesthesia. Tail blood was collected at time intervals up to 60 min. and in some cases 120 min. from the time when the lymph injection was completed. Three capillary tubes were collected in each case, and the plasma was analyzed as described above.

As soon after the injection as possible, the needle was withdrawn, and pressure was applied to the femoral vein until the bleeding stopped. The incision in the skin was stitched, and the rats, after recovering from the anesthesia, were returned to their cages for the rest of the experimental period. The above manipulations usually took about 10 min. from the time of the injection, so that the rats were conscious throughout most of the blood-sampling period.

The lipemia found at any time after the injection of the radioactive lymph was expressed in terms of per cent of initial plasma activity. The initial plasma activity was the activity in net counts/min of the first sample taken after the injection. Per cent clearance was arrived at by dividing the activity in the plasma test sample, in net counts/min, by the initial plasma activity and multiplying by 100. Per cent of initial plasma activity (per cent clearance) in the various samples was plotted against time in minutes.

**In Vitro clearance studies.**—A 0.2-ml. solution containing 200 units/ml heparin was injected into the exposed femoral vein of the rat under mild ether anesthesia. Three min. later, the peritoneal cavity of the rat was exposed by a longitudinal incision along the linea alba. A polyethylene tube was inserted into the dorsal aorta and the rat’s blood collected in a test tube. From 8 to 12 ml. of blood could be collected in this fashion. The blood was centrifuged, and this “post-heparinized” plasma—constituting the enzyme system—was removed into a separate test tube.

The substrate for these studies was provided by mixing absorptive lymph, collected through thoracic duct cannulas, with a saline-phosphate buffer at pH 7.4. The buffer was added slowly to the lymph with constant mixing until the resulting mixture gave a reading of 0.3–0.4 optical density units (O.D. units) when read in a Coleman Junior Spectrophotometer at 450 m\(\mu\).

The substrate and each of the post-heparinized plasma samples were incubated in a constant temperature bath at 37° C. prior to and during the entire test period, when except readings were made.

Two-tenths ml. of post-heparinized plasma was transferred into a test tube to which 1.4 ml. of the buffered lymph substrate was introduced rapidly through a long #31 gauge needle. After the whole mixture was stirred thoroughly, the tube was placed in the Coleman spectrophotometer...
without delay. Readings were taken at time intervals for a period of 10 min. The optical density changes in the system were determined at 450 mμ. The results were expressed in terms of decreased absorbance, i.e., initial O.D. reading minus the reading of any time interval.

RESULTS

FAT TOLERANCE STUDIES

To test the methodology, duplicate runs were made on the same rats at time intervals of 1 week. In about 75 per cent of the animals studied (a total of twelve rats), the curves either overlapped or were very close in shape and levels of plasma activity. In the other 25 per cent of the rats, the curves were very dissimilar. However, in all the rats studied, the plasma lipide activity levels were always within a close range of values (Chart 1).

a) Normal rats.—Chart 1 (A) shows the fat tolerance curves of fourteen normal rats weighing 151 ± 19 gm. Chart 1 (B) shows the fat tolerance curves of twelve normal rats weighing 263 ± 38 gm. Peak values of 24 per cent TD/100 ml. plasma in the small rats and only 14 per cent TD/100 ml. plasma in the large rats were observed. The minimal values for both groups were similar; the maximal values, however, were considerably larger in the small than in the large rats, and, as a result, the values in the small animals were scattered over a wider range than in the large ones.

b) Large tumor-bearing rats.—The Walker carcinoma 256 was transplanted into rats weighing between 175 and 200 gm. At different stages of tumor growth, the rats were subjected to fat tolerance studies. Of the rats studied, only those in which the tumors grew at the same rates were chosen.

Tolerance curves of eight tumor-bearing rats weighing 331 ± 42 gm. and bearing 9-day-old tumors are shown in Chart 2 (A).

In Chart 2 (B), the tolerance curve for eight tumor-bearing rats weighing 332 ± 29 gm. is seen. These rats were studied at the time when the tumors were 2 weeks old.

Chart 2 (C) shows the tolerance curve of eight rats bearing 3-week-old tumors and weighing 353 ± 37 gm. at the time of the experiments.

To express the results of the fat tolerance experiments listed above for the tumor-bearing rats in a different manner, peak values were treated statistically; mean values and the standard deviations from the mean were calculated for the vari-
ous groups and plotted in Chart 3 against time in weeks of tumor growth. The mean peaks represent in fact the highest degree of alimentary hyperlipemia attained in any one group of rats. As can be seen from Chart 5, tumor growth was associated with very high plasma lipide activities representing markedly elevated alimentary hyperlipemias.

c) Small tumor-bearing rats and their pair-fed controls.—Tumors were transplanted into rats weighing between 125 and 150 gm. Each tumor-bearing rat had a pair-fed control mate. In a group of eight tumor-bearing rats, weighing 178 ± 14 gm. and bearing 1-week-old tumors, Chart 4 (T1), a peak value for the mean tolerance curve was found to be 44 per cent TD/100 ml. plasma. Maximal values of 72 per cent were observed.

Chart 4 (C1) represents the tolerance curve of eight control rats weighing 180 ± 16 gm. and pair-fed with the tumor-bearing rats in (T1). The tolerance curve of this group was identical in all respects with the curve for the small normal rats.

The tolerance curves observed at 2 weeks of tumor growth are seen in Chart 4 (T2) and (C2) for the tumor-bearing and for the pair-fed controls, respectively. In the tumor-bearing group, weighing 205 ± 9 gm., the picture was much the same as in the first week of tumor growth. However, in the control group with weight of 189 ± 8 gm. a very pronounced change took place. The peak of the

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**Chart 2.**—Fat tolerance curves determined after feeding of triolein-14C test diets to large tumor-bearing rats at (A) 9 days, (B) 14 days, and (C) 21 days of tumor growth, after an overnight fasting period. (●) mean values; (○) minimal and maximal values; and the range of values (shaded area).

**Chart 4.**—Peaks (± S.D.) of the mean fat tolerance curves determined in large tumor-bearing rats after feeding of triolein-14C test diets at different stages of tumor growth. N is the peak of the mean fat tolerance curve of the large normal rats.
mean tolerance curve dropped to half its value of the previous week.

In the 3d week of tumor growth, the eight tumor-bearing rats weighed 231 ± 19 gm., Chart 4 (T₃), and the eight pair-fed control rats, Chart 4 (C₃), weighed 199 ± 17 gm. In neither group were any significant changes from the previous week noticed.

In the 4th week of tumor growth, only six tumor-bearing rats survived. The tumor-bearing animals, Chart 4 (T₄), weighed 255 ± 18 gm. and the pair-fed control rats, Chart 4 (C₄), weighed 207 ± 21 gm. The tolerance curves of the pair-fed controls were as in the previous 2 weeks. In the tumor-bearing rats, the plasma lipide activities were extremely elevated. The peak of the mean tolerance curve reached a value as high as 80 per cent TD/100 ml. plasma, more than 3 times higher than the peak of the large normal mean tolerance curve and about 8 times as high as the peak of the mean control group curve. Maximal values as high as 121 per cent TD/100 ml plasma were found. At the same time, all the minimal values were above the maximal values of the pair-fed control rats' tolerance curve.

As in the case of the large tumor-bearing rats, the peaks of the tolerance curves were treated statistically; mean peak values and their appropriate standard deviations were calculated. These were plotted for the various groups against time in weeks of tumor growth, as shown in Chart 5. The mean plasma lipide activity peaks of the tumor-bearing rats were higher than both the normal and the pair-fed control peaks. Beyond the 1st week, these differences were all statistically significant. From the 3d to the 4th week, the plasma lipide activity peak doubled.

d) Turbidity changes after feeding the triolein-I¹¹ test diet.—The physical appearance of the plasma from the two groups also differed. Where identical radioactive measurements were found in two tubes—one containing tumor-bearing rat's plasma and one from a normal or a pair-fed control rat's blood plasma—the plasma of the tumor-bearing rat was always much more turbid than the plasma of the control or normal rat. Even when the radioactivity was so low that the tube containing the plasma of the normal or the pair-fed control rat was clear, the plasma of tumor-bearing rats was distinctly turbid. The fasting plasma of tumor-bearing rats was also turbid, whereas the plasma of fasted normal or control rats was not.
LYMPHATIC ABSORPTION OF TRIOLEIN-1\(^{131}\) TEST DIET

Difficulties were encountered in cannulating the thoracic ducts of the tumor-bearing rats. The tumor interfered physically with the surgical manipulations involved. In certain cases, the cannulation was practically impossible, and, because of the generally low resistance of the tumor-bearing animals, the mortality rate in the absorption studies was high. Only those animals which appeared to be in a satisfactory condition in the post-operative period were studied. No results were used unless the flow of lymph in the animal under consideration was continuous and was not interrupted for any length of time throughout the collection period.

The method for lymph analysis employed in these experiments was satisfactory as judged from recovery experiments. Essentially quantitative recoveries were effected. In analyzing the absorptive lymph, a constant difference of 5–8 per cent was found between the activity of the non-treated lymph and the activity in the lymph precipitates. The decrease was assumed to be due to the removal of inorganic \(^{131}\)I present in the lymph.

Lymphatic absorption of triolein-\(^{131}\) test diet in groups of small rats in a period of 6 hours following the feeding was studied. As can be seen in Table 1, the control rats absorbed the same amounts of lipide activity as did the normal rats of equal age. The tumor-bearing rats absorbed much less fat into their thoracic duct lymph than did the normal and control rats \((P < .001)\).

In studying normal, tumor-bearing, or pair-fed controls, large rats absorbed more fat than did small rats of the same group (Table 1). All of these differences were statistically significant \((P\) values were less than 0.02). But, as in the small rats, the controls absorbed more fat than did the tumor-bearing rats. The large tumor-bearing rats absorbed about 3.5 times the amount of fat absorbed by

![Chart 5. Peaks (±S.D.) of the mean fat tolerance curves determined in small tumor-bearing (○) and in pair-fed control (□) rats after feeding of triolein-\(^{131}\) test diets at different stages of tumor growth. N₈ and N₉ are the peaks of the mean fat tolerance curves of the small and the large normal rats, respectively.](chart-5.png)

**TABLE 1**

THE LYMPHATIC ABSORPTION OF TRIOLEIN-\(^{131}\) TEST DIETS BY SMALL AND LARGE NORMAL (N), TUMOR-BEARING (T), AND PAIR-FED CONTROL (C) RATS

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**TABLE 1**

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† Per cent TD/carcass wt.
the small tumor-bearing rats, but only $\frac{1}{2}$ of the amount of fat absorbed by their pair-fed control animals ($P < .001$).

**PLASMA ANALYSIS IN CANNULATED RATS DURING FAT ABSORPTION**

Blood plasma samples were taken from the tails of the cannulated rats during the period of lymph collection. Some counts were found in the plasma, but after treatment with 10 per cent KI and 40 per cent TCA these counts were "washed out" of the precipitate. This was an indication that none of the plasma activity was due to the presence of lipide-bound $^{131}$I.

**RATE OF ABSORPTION OF TRIOLEIN-$^{131}$ TEST DIET IN NORMAL AND TUMOR-BEARING RATS**

The rate at which triolein-$^{131}$ test diet was absorbed from the intestine into the thoracic duct lymph was determined from cumulative curves like the ones shown in Chart 6 for a large tumor-bearing rat and its pair-fed control. These curves represent absorption in those rats which had the maximum amount of labeled fat recovered from their lymph. In both cases, absorption was almost complete by 6 hours after the feeding.

**CALCULATED RATE OF PLASMA CLEARANCE**

The data from the studies on fat tolerance and rate of fat absorption were combined and treated mathematically to suggest the possible rates at which the absorbed fat was cleared from the blood plasma (Chart 7).

The fat tolerance curve of the control rat showed that, at 3 hours after feeding of the test diet, 12.5 per cent TD/100 ml plasma were accounted for in the blood. The total plasma volume of the rat was assumed to be 7 ml. As a consequence, the total amount of fat in the rat at 3 hours after feeding time was 0.88 per cent TD. The fat absorption curve for the control rat indicated that in the 3-hour period following the administration of the test diet 15 per cent TD was absorbed by the rat through the thoracic duct lymph. Therefore, the percentage of the absorbed

**CLEARANCE OF INTRAVENOUSLY ADMINISTERED FAT**

Studies of rate of clearance of absorptive lymph were approached in three ways. Lymph, collected in citrate and diluted 1:1 with isotonic saline, was injected intravenously into fasted rats, and the
rate at which this lymph was removed from the plasma was determined (Chart 8). The rate at which three tumor-bearing and two pair-fed control rats cleared the undiluted citrated lymph are shown in Chart 9 (A). Practically no lipide activity was found in the plasma of the control rats 2 hours after injection, whereas in the tumor-bearing rats as much as 20 per cent of the initial dose could be recovered from the plasma.

The injection of lymph collected in heparin was followed by an accelerated rate of plasma clearance, Chart 9 (B), in both tumor-bearing and pair-fed control rats.

The results of the in vivo experiments can be expressed in two ways as summarized in Table 2. On the one hand, the half-life of the lymph lipides in the plasma of the tumor-bearing rats was twice that in the control rats when the diluted lymph was injected; 3.5 times longer when the lymph was not diluted; and 2.5 times longer when the nondiluted, heparinized lymph was injected. On the other hand, in terms of lipide activity at 40 min. after the injection, the concentration of the uncleared lipide in the plasma of the tumor-bearing rats was more than 3 times higher than it was in the plasma of the pair-fed control rats when citrated, diluted lymph was injected. It was 3 times higher when nondiluted, citrated lymph was injected, and 10 times higher when the heparinized lymph was injected.

**In Vitro Clearance Studies**

Lipoprotein lipase activity in plasma from each group was assayed spectrophotometrically. The results for the two groups of animals as seen in Chart 10 indicate that the rate of the in vitro clearance in the pair-fed control rats was considerably faster than in the tumor-bearing rats. For all intents and purposes, then, practically no lipoprotein-lipase activity was present in the post-heparinized plasma of the tumor-bearing rats.
DISCUSSION

In attempting to discuss the hyperlipemia of cancer, a dichotomy should be made between post-absorptive (essential) and absorptive (alimentary) hyperlipemia. The two lipemias, although related, are vastly different phenomena. In the post-absorptive state, "in which neither absorption from the guts nor mobilization from the fat depots is occurring" (13), the plasma lipides are maintained in a steady state. The hyperlipemia reflected in the tolerance studies is clearly defined as an alimentary hyperlipemia, since the tolerance curve represents a state of flux within the plasma during the process of absorption. The peak of the tolerance curve is a measure of the highest level of absorptive hyperlipemia.

![Chart 10](chart.png)

**Chart 10.**—*In vitro* clearance of absorptive chyle buffered with saline-phosphate buffer at pH 7.4, by postheparinized plasma of six rats bearing tumors 15–18 per cent of body weight (●) and by five pair-fed control rats (○).

The tolerance curve could be considered, for all practical purposes, as a function of absorption and utilization, or clearance. The tolerance curve is not a measure of absorption alone, and fat tolerance studies would, therefore, be meaningless unless they were supported by both absorption and clearance studies.

In the fat tolerance studies, the height of the mean tolerance curve peaks was markedly elevated in the tumor-bearing rats of both age groups. The extent of this effect of the tumor on plasma lipides seemed to be directly related to tumor growth. Almost all of the fat absorbed by the tumor-bearing rats in the last week of tumor growth could be accounted for in the plasma at the time the tolerance curve peak occurred. The behavior of the pair-fed control rats was in the opposite direction. The decline in the tolerance curve peak in these animals beyond the 9d week to below normal values was significant.

Only the amount of fat absorbed in rats of the three groups was studied, since the exact mechanism of fat absorption has not been worked out in complete detail. The assumption that the I- labeled lipide recovered from the lymph of the cannulated rats is a true measure of fat absorption is based on sound and acceptable experimental evidence. Through the contributions of Chaikoff et al. (10) and of Borgström et al. (4), it has been shown that "long chain (≥C16) acids whether saturated or unsaturated and whether fed as free acids or as esters are predominantly absorbed via the lymphatics" (4). Frazer states that "the bulk of absorbed fat passes in the chyle into the systemic blood" (11). Furthermore, it was shown that long-chain fatty acids do not undergo changes in chain length during absorption (9). In the present work it was found that the amount of free I- in the lymph was a minimal level, 5–8 per cent of the total activity in the collected chyle. No difference was found between thoracic duct and intestinal lymph (5).

The total amounts of fat absorbed by the large rats of all three groups (Table 1) were significantly greater than those absorbed by the smaller rats of each respective group. However, in terms of per cent TD/gm body weight, all normal and control rats absorbed equal amounts. Here, the apparent difference between the normals and controls can be accounted for on the basis of body weight discrepancy in a similar age group due to the semi-starvation of the control rats. The small tumor-bearing rats absorbed less fat per gm. body weight than did the large tumor-bearing rats; but in the small ones all other manifestations characteristic of cancerous animals were more pronounced, i.e., anorexia, loss of body weight, hyperlipemia, rate of tumor growth, and cachexia.

The tumor-bearing rats of both age groups absorbed considerably less fat than did their respective pair-fed controls; also the fat was absorbed at a much reduced rate (Chart 6). This was true whether the amount of fat absorbed was expressed as total per cent TD, or as per cent TD/gm total body weight. Even in terms of per cent TD/gm carcass weight, the tumor-bearing rats absorbed markedly less fat than did their pair-fed mates. These findings amplify the statement by Bloor and Haven (6, 15) that the amount of the intestinal tissue is inadequate for maintenance of life and growth in competition with the tumor. The extent of the decrease of fat absorption is such that the intestine seems to be insufficient for
maintenance not only of the whole body of the host, but even of the carcass alone.

A marked delay in plasma clearance by the tumor-bearing rat was found in the in vivo clearance studies confirming the findings of Begg and Lotz (2). The initial concentration of the injected fat in the plasma of both experimental groups was lower than at the time of the peak of the normal mean tolerance curve, which was only a fraction of the mean peak of the tumor-bearing rats' tolerance curve. Higher concentrations of fat were cleared at even slower rates. Also, when chyle was injected a second time before the complete clearance of fat injected previously, the resulting new "initial" concentration of fat in the plasma was much higher, the rate of clearance much slower, and the fat was found in the plasma of the tumor-bearing rats much longer than was found with a single injection. However, in the pair-fed control rats the differences between the first and second injection were not very pronounced.1

The in vitro studies indicated that the tumor-bearing rats' plasma was lacking in heparin-clearing factor—Korn's lipoprotein-lipase (16, 17). Whereas the normal rats' post-heparinized plasma, withdrawn from either pair-fed control or normal rats, showed a rapid rate of clearance, post-heparinized plasma withdrawn from tumor-bearing rats had practically no clearing ability. The data available from this work do not permit any statements as to whether lipoprotein-lipase was inadequately synthesized in the tumor-bearing rats, whether it was not released from the tissues by heparin, or whether its action was inhibited. Nevertheless, lipoprotein-lipase apparently plays an important role in the production of the alimentary hyperlipemia in the cancerous animals.

The tumor-bearing rat, with its defective clearance mechanisms, was unable to handle the influx of absorbed fat as efficiently as the normal or the pair-fed control rat and, as a consequence, this fat piled up in the plasma of the tumor-bearing rat, whereas in the normal rat it was rapidly removed immediately following absorption. This situation persisted throughout the course of tumor growth leading to extremely high peaks of alimentary hyperlipemia.

That the elevated absorptive hyperlipemia was caused by exogenous fat entering the plasma during absorption is ascertained, since originally only exogenous fat carried the radioactive label. The plasma lipide activity in the tumor-bearing rats was elevated to levels higher than those found in the normal and in the pair-fed control rats despite a decrease in absorption. This could be due, then, to a debility on the part of the tumor-bearing rats to clear their plasma at rates commensurate with rates of fat absorption.

In contradistinction to the findings of the tolerance studies, the lipemia observed in fasted rats is post-absorptive. The plasma total lipides of tumor-bearing rats fed a normal diet was found to be quite elevated despite an overnight fasting period.1 In the post-absorptive state, lipemia is due, at least in part, to endogenous fat mobilized from the fat depots, possibly coupled with an inability to utilize the mobilized fat. The post-absorptive lipemia thus constitutes a disruption of the mechanisms by means of which the plasma lipides are otherwise maintained in a steady state.

Possibly, the extreme hyperlipemia observed in rats force-fed a high-fat diet might also be explained in view of the present findings. As described by Stewart and Begg (24), their rats were fed twice daily until the tumor reached a size 20 per cent of total body weight (16 days). Since, as was shown in the present work, the tumor-bearing rats are unable to clear even small amounts of fat, overloading the plasma of the tumor-bearing rats with large amounts of fat by constant force-feeding of a high-fat diet leads to an over-all increase in the plasma lipides. This might be due to the exhaustion of an already defective clearance system, or simply as a piling up of lipides in the plasma because of the inability of the tumor-bearing rats to clear the lipides completely before an onrush of newly absorbed fat. The fat mobilized from the fat depots aggravates the condition. The extent of this piling up is such that even after an overnight fasting period, i.e. in the post-absorptive stage, an extremely high hyperlipemia is observed.

It has, therefore, been concluded that in addition to the anorexia, with its resultant decrease in food intake, the decrease in fat absorption may be a major factor contributing to the cachexia associated with cancer. To meet the needs for high energy requirements of the tumor, fat is mobilized from the fat depots. The fat which is absorbed from the intestine, once it enters the blood stream, is inefficiently removed from the circulation for purposes of deposition and utilization and, as a consequence, a hyperlipemia ensues.

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Abnormal Fat Absorption and Utilization in Rats Bearing Walker Carcinoma 256

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