Intestinal Absorption of Fat in Tumor-bearing Rats*

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SUMMARY

An attempt has been made to confirm the potentially important observation made by Posner that subcutaneous growth of Walker carcinoma 256 in rats results in defective fat absorption, as measured by the amount of I^{131}-labeled triolein in thoracic duct lymph. Posner's results could not be confirmed. It is concluded that, under the conditions of the present experiments, growth of the Walker tumor for 3 weeks has no effect on fat absorption. This result supports the findings of Colella, Reith, and Williams that humans with malignant neoplasms absorb fat normally.

Posner used a type of restraining cage which may have led to mechanical interference of intestinal function in his rats bearing tumors in the inguinal region, whereas in the present study a cage was used which allowed more clearance for inguinal tumors. The reason for the difference in results is not known, but this difference in method of restraint seems to be the most probable explanation.

Posner (9, 10) has reported data which demonstrate that rats bearing Walker carcinoma 256 have defective fat absorption. These data were obtained by measuring the radioactivity of the lipides found in thoracic duct lymph after feeding I^{131}-labeled triolein. Posner has suggested that this defect in fat absorption may be an important contributory factor to the development of cachexia. Thus, according to his report, malabsorption is compounded with the anorexia which occurs in animals bearing this tumor (7). The result is a further decrease in the supply of calories to the host, which is already in competition for available nutrients with a rapidly growing tumor.

On the other hand, Colella, Reith, and Williams (5) have recently reported that human patients with malignant disease showed normal fecal excretion of radioactivity after a test meal of I^{131}-labeled triolein, in spite of the fact that blood levels of radioactivity were below normal. They concluded that their tumor-bearing patients absorbed fat normally.

An attempt to confirm the results reported by Posner is reported here. This seemed a necessary step to take in view of the potential importance of Posner's observation in an understanding of tumor-host relationships and in view of the interesting studies which could be designed in an attempt to determine the mechanism of this effect. An effect of tumor growth on fat absorption could not be demonstrated by technics almost identical to those used by Posner.

MATERIALS AND METHODS

Male albino rats (Holtzman) were used. They were maintained on Purina Laboratory Chow and water, ad libitum. Seven of the animals bore tumors (Walker carcinoma 256)[1] which had been started from subcutaneous injections of tumor homogenates about 3 weeks before each experiment. The average size of the tumors at the end of the experiments was 17.9 ± 3.0 per cent[2] of the body weight. Animals in which the tumor had ulcerated through the skin were not used. Six normal animals were used as controls. Control rats were not pair-fed with tumor-bearing rats, since Posner reported (9, 10) that there was no difference in fat absorption between pair-fed controls and normal animals fed ad libitum. The mean body weights were 324 ± 29 gm. and 324 ± 20 gm.[2] for the normal and tumor-bearing animals, respectively.

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[1] Kindly supplied by Dr. J. M. Dabney, University of Oklahoma Medical Center.

[2] Mean ± standard error of mean.

The methods used for studying fat absorption were nearly identical to those used by Posner (10). Each rat was fed *ca.* 0.3 ml. of olive oil by stomach tube. About 1 hour later the thoracic duct was cannulated with polyethylene tubing (PE 10) just above the cisterna chyli by the method of Bollman, Cain, and Grindlay (4) with the following slight revision. A U-shaped bend was placed in the catheter so that after leaving the thoracic duct it was directed caudally. The distal (or delivery) end of the catheter was brought subcutaneously along the abdominal wall, and out the left inguinal region. The animal was then subjected to caudal restraint, in the restraining cage described in a previous publication (1). With this type of restraint only the tail is held stationary; the animal engages in a much more normal degree of activity than in the Bollman type of restraining cage (3) as was used by Posner. While in restraint, each rat was given access to Purina Laboratory Chow and 0.9 per cent NaCl.

Sixteen to 24 hours after thoracic duct cannulation, 0.5 ml. of triglyceride containing a trace of I^{131}-labeled triolein was administered by stomach tube. This test meal was immediately followed by 5.0 ml. of water by stomach tube, as suggested by Posner (10). During administration of the test meal the animal was not removed from the restraining cage but was lightly anesthetized with ether.

I^{131}-labeled triolein already diluted in peanut oil was obtained from the supplier.[3] This preparation was further diluted with olive oil (as was done by Posner) to give the desired concentration of radioactivity in the test meal which, at the time of administration, ranged from 1.2 to 23.2 μc/ml.

Lymph was collected in a heparinized flask for 6 hours after feeding of the test meal. Any animal from which lymph did not flow continuously during the entire postoperative period was discarded; this occurred only once. The proteins and lipides of lymph samples were co-precipitated with trichloroacetic acid after addition of human plasma as described by Posner (10). The precipitates were washed free of inorganic I^{131} and counted with a well-type scintillation detector. Lymph activity was compared with the activity in a duplicate sample of the test meal diluted in petroleum ether. Results are expressed as per cent of injected radioactivity found in the 6-hour collection of lymph lipides.

After each absorption test the rat was killed and weighed; if it bore a tumor the tumor was removed and weighed separately.

TABLE 1

PER CENT OF I^{131}-LABELED TRIOLEIN IN TEST MEAL ABSORBED INTO THORACIC DUCT LYMPH IN SIX HOURS IN NORMAL AND TUMOR-BEARING RATS

Rat*		Tumor†			Lymph flow (ml/6 hr.)	Absorbed (% of initial dose)
Weight (gm.)	Age (days)	Size (%)	Age (days)	Location of injection		
222	60	0	0		21.5	30.4
261	73	0	0		12.0	45.6
325	77	0	0		23.7	43.5
355	103	0	0		34.5	35.8
388	118	0	0		17.3	43.2
396	95	0	0		31.0	38.2
265	59	32.8	21	Rt. shoulder	23.5	42.9
290	83	12.8	26	Rt. shoulder	22.0	46.3
305	77	14.4	18	Rt. inguinal	36.5	39.7
311	76	12.5	25	Rt. shoulder	34.5	42.7
322	70	14.3	20	Rt. shoulder	37.2	37.8
339	82	25.1	22	Back of neck	36.5	29.6
434	105	13.6	25	Rt. inguinal	34.0	37.5

* Rat weight and age at end of experiment.

† Size of tumor is expressed as percentage of total body weight (carcass + tumor). Age of tumor is taken from the day of subcutaneous injection to day of absorption test.

RESULTS AND DISCUSSION

The data are presented in Table 1. No evidence was obtained for any effect of tumor growth on fat absorption. The two sets of data are, in fact, remarkably close to each other (normals: 39.4 ± 2.4 per cent; tumor-bearing: 39.5 ± 2.0 per cent)[2]. The effect described by Posner was in the order of a 50 per cent reduction of fat absorption in one set

[3] Abbott Laboratories.

of experiments and an 80 per cent reduction in another set. With the degree of variability obtained in the present experiments an effect of this magnitude should have been easily discerned.

Posner also presented data indicating that larger rats absorbed more fat in 6 hours than did smaller rats. The data in Table 1 are arranged in order of increasing body weight within the normal and tumor-bearing groups. There is no evidence from these data for any effect of body weight on fat absorption. However, it should be noted that the mean weight of one of Posner's normal groups of animals (156 gm.) was much less than the weight of the smallest animal studied here. It is possible that, if small enough animals had been used in the present study, an effect of body weight on the rate of fat absorption would have been seen.

The reason for the discrepancy between the present results and those of Posner is not apparent, but the following possibility should be mentioned. Posner's tumor-bearing rats all had tumors in the right inguinal region and were held in the Bollman type of restraining cage during the experiment. Tight restraint of a rat with a large tumor in its inguinal region could conceivably result in mechanical interference with blood or lymph flow in the intestine, or even with transit of chyme through the intestine. It seems possible, therefore, that in Posner's tumor-bearing rats mechanical effects of the tumor on the intestine accounted for the defective fat absorption observed. In the present study only two of the animals had tumors in the right inguinal region (see Table 1). However, these two animals had fat absorption values about equal to the mean; there was no evidence for any effect of tumor position on fat absorption. With the method of restraint used in this work the animals were held in such a way that, even with an inguinal tumor, there would probably be no more mechanical interference with the intestine than there would be in the unrestrained condition.

Table 1 shows a difference in the rate of lymph flow between the normal and tumor-bearing animals (means were 23.3 ml. and 32.0 ml. per 6 hours, respectively). Statistical analysis by "*t*" test revealed a P value between 0.05 and 0.10. Therefore, there was no convincing evidence obtained that thoracic duct lymph flow is greater in tumor-bearing animals than in normals. On the other hand, lymph flow was certainly not less in the tumor-bearing than in the normal animals; this fact indicates freedom from severe mechanical interference by the tumor as discussed above. Posner did not report rates of lymph flow.

Posner studied rats of the Sprague-Dawley strain. The present study utilized rats from the Holtzman Company; these were originally derived from the Sprague-Dawley strain. It seems doubtful that a strain difference could explain the discrepancy. Another difference between the two studies was that Posner did not allow his animals to eat during the entire postoperative period, whereas the animals in the present experiment were fed ad libitum; it is not probable that this factor could account for the difference in results.

The reason Posner obtained an effect of tumor growth on fat absorption, whereas the present study shows no such effect, remains obscure; however, the difference in method of restraint seems to be the most probable explanation. The present results support the findings of Colella, Reith, and Williams (5), who reported that patients with malignant disease absorbed fat normally.

Bloor and Haven (2) have demonstrated that the intestine of rats bearing the Walker tumor is smaller than the intestine of control rats of comparable age. This observation has been confirmed by Wiseman, Neame, and Ghadially (11) in rats with Sarcoma RD3. Bloor and Haven concluded that "the amount of intestinal tissue is insufficient to support life and growth in the face of the competition of the tumor." In spite of this decreased amount of intestinal tissue (not actually measured in the present study), the tumor-bearing rats reported in Table 1 absorbed fat normally. Thus, the present results do not support the contention that intestinal absorption is the step which is rate-limiting in the supply of calories to the tumor-bearing animal, at least not at the stage of tumor growth studied here. It seems more likely that food ingestion, decreased by anorexia in the tumor-bearing animal, is the step which limits the rate of calorie supply.

Wiseman, Neame, and Ghadially (11) studied D-glucose and L-histidine transport by everted sacs of small intestine from rats bearing Sarcoma RD3. They found that greater concentration ratios (serosal/mucosal) were established for both D-glucose and L-histidine by intestinal sacs from tumor-bearing than from control rats. Nunn, Bramante, and Sayeed (8) have confirmed this effect of tumor growth on glucose transport *in vitro* with Walker 256. In addition, the latter investigators demonstrated that this effect is much more apparent in the upper half of the small intestine, where glucose transport is normally the most vigorous, than in the lower half.

Thus, *in vitro* work not only fails to support the idea of intestinal malabsorption during tumor

growth but indicates that there may actually be an increased ability to absorb some nutrients. Similar findings of increased absorptive ability in rats during semistarvation have been reported (6, 11).

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