

Effects of Krebs-2 Carcinoma on the Lipide Metabolism of Male Swiss Mice*

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SUMMARY

Total body composition of mice bearing Krebs-2 carcinoma transplanted subcutaneously was studied at several times during the course of tumor growth. Adult male Swiss mice, averaging 38 gm. in weight, sustained a profound fat loss during growth of Krebs-2 tumor. Fat depletion occurred in three stages. The first stage, involving loss of approximately 50 per cent of the lipide of the animal, occurred by 7 days after transplantation, before the tumor had reached appreciable size. The second phase was one of steady state in which no further fat loss occurred despite active tumor growth. This stage lasted approximately from day 7 to day 28. Premortally, the tumor-bearing animal lost another substantial quantity of fat.

The first stage fat loss has been reproduced with nonviable preparations from tumor. The possible nature of the lipolytic factor is discussed.

Profound alterations of the lipide metabolism of tumor-bearing rats are well known (1, 2, 4, 6). Rats bearing implants of Walker carcinosarcoma 256 undergo a progressive depletion of host fat which bears a linear relation to the increasing tumor mass. Little information is available from other laboratory models, and it has not been known how faithfully the data from rats reflect the pattern seen in other animals bearing other tumors.

The effects of Krebs-2 carcinoma on total body composition of Swiss mice differ considerably from the pattern induced in rats by the Walker tumor (3). Certain aspects of the disordered fat metabolism are the subject of the present report.

MATERIALS AND METHODS

Adult, nongrowing Swiss Ha/ICR male mice, averaging 38 gm. in weight, were inoculated subcutaneously on the back with a bacteriologically sterile suspension of Krebs-2 carcinoma cells. Tumor "sizes" were estimated as the product of maximum length and width of tumors, measured with calipers. At appropriate times, mice were deprived of food and water for 5 hours and then dropped into a large Waring Blendor containing 300-400 ml. of distilled water, homogenized in the

cold, and brought to constant volume. This procedure produces instantaneous death and homogeneous suspension suitable for analysis.

Total fat was determined after filtration of an aliquot of the suspension equal to one-fourth the total volume. The filtered liquid phase was extracted exhaustively with chloroform in a separatory funnel. The filter paper containing the solid phase was cut in small pieces, placed in an extraction thimble, and refluxed in a soxhlet extractor for 16 hours with a 3:1 mixture of chloroform and ethanol. Total fat was determined gravimetrically on the combined extracts, after evaporation of the solvents at 60° C. under reduced pressure to constant weight.

Nitrogen was determined as described previously (5). Total water was calculated as the difference between wet and dry weight, the latter obtained by evaporating an aliquot of the homogenate to constant weight at 95° C. under reduced pressure. An aliquot of the homogenate was ashed in a muffle type furnace for 8 hours at 630° C. using silica crucibles. Total ash was then measured gravimetrically.

RESULTS AND DISCUSSION

In preliminary trials with groups of twelve male animals each, it had been found that the body composition (in grams of component per mouse)

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of adult Swiss mice remained constant over a time-span of at least 7 weeks. In Experiment 1, 200 mice were given injections of 0.1 ml. of Krebs-2 carcinoma suspension. Tumor "sizes" were measured weekly and a histogram plotted. Groups of nine to twelve animals were selected randomly from among those mice having a tumor "size" equal to the mode for that week, with the exception of the 5th week, where animals were chosen from among those surviving with the largest tumors. Total body weight, total water, total nitro-

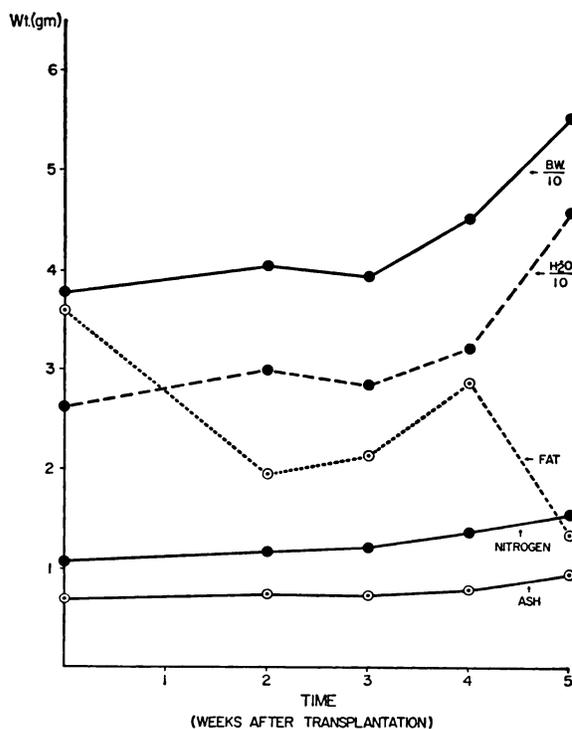


CHART 1.—Composition of Swiss mice (♂) + Krebs-2 tumors (S.C.) as function of tumor growth. Two hundred mice given injections. Tumor mode chosen for points 0-4; only largest tumors chosen for point 5. Statistical data in the text.

gen, and total ash of the tumor-bearing animals increased as the tumor grew (Chart 1). These data will be presented in detail elsewhere (3). Suffice it to note (from these and other experiments where tumor and host were analyzed separately) that Krebs-2 carcinoma does not produce depletion of host nitrogen and that the increase in ash weight can be accounted for largely by accumulation of sodium.

During tumor growth, a massive loss of fat occurred. By the 5th week, when most of the animals were dying of the consequences of the tumor, the surviving mice contained only about one-fourth of the fat present in the normal uninjected controls.

Of particular interest is the triphasic shape of

the lipide curve. The first acute fat loss occurred during the period when tumors had just reached a measurable size ($P < 0.01$ when compared with fat content at time 0). The second phase is a period of essentially steady state, despite continuous tumor growth (no significant difference between fat content at 2, 3, and 4 weeks). The third phase began about the 4th week and comprised renewed fat loss in the premortal phase ($P < 0.01$ when fat content at 5 weeks was compared with that at 4 weeks). The behavior of the fat curve has been reconfirmed in three additional experiments, which have also shown that the first phase (acute initial fat loss) occurs within the 1st week after tumor implantation, at a time when the tumor is barely palpable.

Although anorexia might explain the fat loss in the third phase, in part or *in toto*, this is untenable as an explanation for the first phase. In other experiments, controls and tumor-bearing mice ate the same amount of food during the 1st week (mean food intake of ten normal animals = 36.90 gm/mouse; mean food intake of thirteen animals bearing Krebs-2 carcinoma = 36.86 gm/mouse). The possibility of a different mechanism of lipolysis was thus sought for the early acute fat loss of the first phase.

In Experiment 2, groups of adult male mice were selected from cage mates. Each group was of narrow weight range, and the group averages were 38 ± 1 gm. The groups were then randomly allocated to treatments. Five groups of animals were used.

Group I served as a control.

Group II received 0.1 ml. of Krebs-2 tumor.

Group III received 0.1 ml. of an aliquot of the same tumor frozen (3 minutes in a dry-ice alcohol mixture) and thawed (5 minutes in a 37° C. bath) ten times to rupture all cells.

Group IV received 1.0 ml. of the frozen and thawed (F&T) tumor. (Groups I-IV contained six animals each.)

Group V consisted of 50 animals given 0.1 ml. of the F&T tumor and observed for tumor growth. Only one in 50 developed a palpable tumor by 6 weeks, in contrast to the essentially 100 per cent take with palpable tumors by the 10th day in our usual transplantation of the tumor.

Groups I-IV were sacrificed on the 7th day after tumor implantation; 90 minutes before sacrifice, each animal received 5 μ c. of acetate-1-C¹⁴ (Nuclear-Chicago 8.12 mc/mole) dissolved in 0.5 ml. of saline by intraperitoneal injection. The total fat was determined as usual. The fat was then redissolved in CHCl₃, plated (samples of in-

finite thickness) and counted (Nuclear-Chicago Scaler-Model 183B, Automatic Sample Changer-Model 110, Printing Timer-Model C-111B).

The results, presented in Table 1, show: (a) The tumor was capable of producing fat loss (in 7 days). There was significant increase of the specific activity of *total* body fat when acetate-1-C¹⁴ was the precursor. Neither the precursor pool size nor the specific activities of fat components have yet

ever, the F&T tumor produced both fat loss and increased specific activity of fat.

In another experiment conducted with tumor which had grown for 21 days (the steady state period) to the average "size" of 2.2 sq. cm., the specific activity of the fat from the tumor-bearing animals (after injection of acetate-1-C¹⁴ as described above) was not significantly different from that of the controls, suggesting that the factor responsible for the enhanced acetate incorporation and perhaps the early fat loss was either no longer present or no longer active.

The metabolic changes induced in adult Swiss male mice by subcutaneous Krebs-2 carcinoma are different from those shown by the rat implanted with Walker carcinoma 256.

Early acute fat loss in Krebs-2-bearing mice is associated with increased acetate-C¹⁴ uptake into fat. Both phenomena can also be evoked by non-viable tumor preparations. Studies of the nature of the lipolytic factor (possibly an associated virus or a nonviral cell component) are in progress.

TABLE 1

(EXPERIMENT 2)

EFFECT OF KREBS-2 CARCINOMA ON THE CONTENT AND SPECIFIC ACTIVITY OF FAT OF SWISS MICE*

Group no.	No. of animals	Treatment	Total fat (gm/animal)	Specific activity† of fat (in arbitrary units)
I	6	None	3.6 ± 0.92	49 ± 25
II	6	0.1 ml. tumor suspension	2.6 ± 0.79	148 ± 34
III	6	0.1 ml. F&T‡ tumor	3.8 ± 0.99	118 ± 31
IV	5§	1.0 ml. F&T‡ tumor	2.7 ± 0.72	220 ± 45

* All animals sacrificed on the 7th day after tumor implantation.

† 5.0 μ c. of acetate-1-C¹⁴ injected 90 minutes before sacrifice.

‡ F&T Tumor Suspension was frozen (3 minutes in a dry-ice alcohol mixture) and thawed (5 minutes in a 37° C. bath) ten times.

§ One animal died from unknown cause the day following inoculation of F&T tumor.

been measured. The relationship between the fat loss and increased specific activity is thus unknown at present. (b) The frozen and thawed tumor, at the dose of 0.1 ml/animal, did not produce fat loss but significantly increased specific activity of fat from acetate. At the dose of 1.0 ml/animal, how-

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