Table 2. Effect of ages of donors and of recipients on tumor development in transplanted and host mammary gland. The mammary glands removed from the untreated donors were transplanted into recipient hosts. Two weeks after transplantation, a single dose of 7,12-dimethylbenz(*a*)anthracene was given intravenously into the tail vein of the recipient host. Recipient hosts were killed 6 months later. Grafts and hosts' own mammary glands were removed for whole-mount preparations. The difference in tumor incidences in grafts from 56-day-old donors to 56- and 120-day-old recipients and from 120-day-old donors to 56- and 120-day-old recipients is statistically of borderline significance (Yates-corrected chi-square test, $\chi^2 = 3.64$). The tumor incidence in mammary glands of both 56-day-old recipient hosts is significantly higher than that in 120-day-old recipient hosts. HAN, hyperplastic alveolar nodules. The number of rats indicated in column two is the original number of rats in each experimental group.

Age (days)		Rats (No.)	Surviving grafts (No.)	Rats with "preneoplastic" lesions or tumors or both (No.)			
				In surviving grafts		In host glands	
Donor	Recipient			HAN	Tumor	HAN	Tumor
56	56	14	13	9	3 (23%)	5	6 (46%)
56	120	16	15	3	4 (26%)	2	4 (25%)
120	56	16	14	1	1 (7%)	6	8 (57%)
120	120	14	14	3	0 (0%)	1	2 (14%)

120-day-old recipients were 14 and 25 percent. The tumor incidences in the control groups of 56- and 120-day-old rats were 80 and 20 percent, respectively (Fig. 1). It thus appears that the mammary glands *in situ* of the recipient hosts bearing mammary grafts have a lower tumor incidence than do those of control rats that receive no mammary grafts.

The most important factor in the development of tumors in transplants seems to be the age of the transplant. The mammary glands from 120-day-old rats are clearly less susceptible to carcinogenic stimuli than their 56-day-old sisters. Why the age of the target tissue is so critical to carcinogenesis in the mammary gland is a question that cannot be answered here. The exact biological mechanism that determines susceptibility to cancer induction on the basis of age has yet to be elucidated. Our data nevertheless demonstrate that the critical factor that influences the susceptibility of mammary cancer induction by 7,12-dimethylbenz(*a*)anthracene in rats resides in the target tissue. In every instance, the tumor incidence is significantly higher in younger grafts, regardless of the age of the hosts. Could there be a difference in the state of differentiation of the mammary gland of the 56-day-old and 120-day-old rats? Examination of the mammary glands of these two age groups by means of whole-mount preparations revealed no significant morphological difference. However, in most instances, the lobulo-alveolar growth in the 56-day-old mammary glands is more extensive than that seen in the 120-day-old rats.

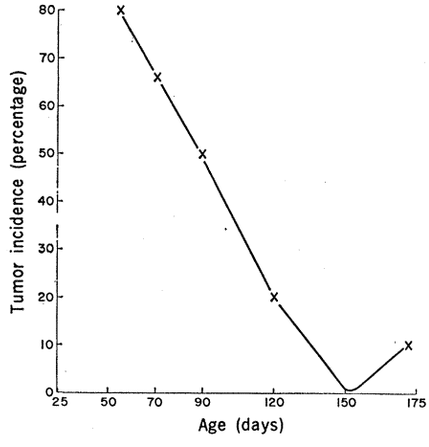

Fig. 1. Mammary tumor incidence related to age in Wistar-Furth female rats. The experiment consisted of six groups of ten rats each. The ages of the rats in the six groups are: 56, 70, 90, 120, 150, and 175 days old, respectively. They were given a single dose of 3 mg per 100 grams of body weight of 7,12-dimethylbenz(*a*)anthracene intravenously into the tail vein and were killed at the end of 6 months.

DeOme *et al.* (*4*) used the fat-pad transplantation technique to show that outgrowth of hyperplastic alveolar nodules from mice infected with mammary tumor virus gave rise to tumors more frequently than did normal tissue from the same animals. Recently Beuving (*5*) demonstrated that when hyperplastic alveolar nodules and ducts from mammary glands of rats treated with a carcinogen were transplanted into gland-free fat pads of isologous animals, 8 of the 37 resulting nodular outgrowths gave rise to mammary tumors. Our data (Table 2) seem to suggest that mammary transplants from 120-day-old donors are less apt to develop hyperplastic alveolar nodules, but that once produced the hyperplastic alveolar nodules are as apt to develop into tumors as are the hyperplastic alveolar nodules from 56-day-old donors. It suggests that the age factor influences normal mammary glands to form "preneoplastic" lesions rather than the subsequent development into tumors. This explanation, however, is not in agreement with the results from the first experiment (Table 1) demonstrating that the development of hyperplastic alveolar nodules in the grafts does not exhibit a significant difference in relation to the age of the grafts. It appears that not all hyperplastic alveolar nodules will ultimately become neoplastic. The study suggests that neoplastic cell variants are lacking in cell population of the hyperplastic alveolar nodules that develop in the mammary glands of 120-day-old female rats. The susceptibility of the mammary gland to neoplastic transformation may be an "intrinsic factor" residing in the mammary tissue, and that the latency of tumor appearance and the progression of tumor growth are dependent on the host factor or factors (*6*).

THOMAS L. DAO
Department of Breast Surgery, Roswell Park Memorial Institute, New York State Department of Health, Buffalo 14203

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Rats Enriched with Odd-Carbon Fatty Acids: Maintenance of Liver Glycogen during Starvation

Abstract. *In young rats a diet containing triundecanoin as the major source of fat produces substantial enrichment of adipose tissue triglycerides with undecanoate and higher fatty acids with odd-numbered carbons. The terminal three-carbon residues arising from beta-oxidation of these acids are glucogenic and help to counteract the decreases in liver glycogen and serum glucose ordinarily induced by prolonged fasting.*

The propionate residue arising from β-oxidation of odd-carbon fatty acids is known to be glucogenic (*1*). Studies have been described in which triglyc-

erides containing odd-carbon fatty acids have been administered over a short term to experimental animals (*1, 2*) and human subjects (*3*). However little attention has been given to the possible metabolic effects of the odd-numbered fatty acids after their incorporation into adipose tissue triglyceride. It was our purpose to ascertain whether, in rats in which adipose tissue was enriched with undecanoate (C_{11}), the terminal C_3 units would provide enough available carbohydrate to animals in the fasted state to counteract both the depletion of glycogen in liver and muscle and the drop in glucose in the plasma which are characteristic of starvation.

We used male Sprague-Dawley rats approximately 4 weeks of age (mean weight, 70.2 g). The animals were housed in individual, wire cages and permitted free access to water and one of two nutritionally complete diets. Thirty percent of calories in the experimental diet was supplied by fat, with 90 percent of that being triundecanoin (Drew Chemical) and 10 percent corn oil. The control diet was identical to the experimental diet except that all of the fat was corn oil. The diets were fed for 4 to 6 weeks before the starvation phase of the experiment.

The concentrations of glycogen in liver and muscle and of glucose in serum were measured in the control and experimental animals in the nonfasted state and after 24, 48, 96, and 144 hours of fasting. During starvation, all animals continued to have access to water. In 12 experimental and 12 control rats measurements were made of the individual fatty acids of the epididymal fat body when the animals were either in the nonfasted state or had been subjected to 2 to 6 days of starvation. In four experimental and four control animals that were fasted for 48 hours individual fatty acids of the free fatty acid and triglyceride fractions in plasma were also determined.

The animals were killed by decapitation, and liver and blood samples were obtained within 1 minute. Muscle samples (quadriceps) and epididymal fat bodies were removed immediately. Muscle and liver were directly crushed in 30 percent KOH. Glycogen in these tissues was measured by the indirect anthrone method (*4*). Serum glucose was determined by the Somogyi-Nelson procedure (*5*). Fatty acid composition of free fatty acids and triglycerides in serum was determined by gas-liquid chromatography after separation of the lipid classes by thin-layer chromatography. The fatty acid composition of lipid extracts of epididymal fat was measured by gas-liquid chromatography.

The gain in weight (mean ± standard deviation) of the control rats was 4.34 ± 0.86 g/day (or 6.83 percent per day, based on initial weight) as compared to 3.45 ± 0.82 g/day (or 5.80 percent per day, based on initial weight) in the experimental group ($P \lesssim .01$). Although daily food intakes were not measured, the rats given the experimental diet generally ate less than the controls throughout the feeding period. In all other respects the animals appeared normal. The average weight loss during starvation was 10.7 g/day in the control animals and 10.6 g/day in the experimental group.

Concentrations of undecanoate in epididymal fat ranged from 26 to 40 percent in rats fed the experimental diet. In addition, the epididymal fat also contained 3.3 to 6.1 percent of longer odd-numbered fatty acids ranging from C_{13} to C_{17}. Starvation up to 6 days did not change the proportions of odd-chain fatty acids in adipose tissue. Undecanoate in free fatty acids in the serums of rats fasted for 48 hours ranged from 7.5 to 13.5 percent. Campbell *et al.* (*6*) have also observed incorporation of odd-carbon fatty acids longer than C_{11} in the depot fat of dogs fed triundecanoin for 6 to 10 months.

The effect of starvation on concentration of glycogen in the liver and muscle and on concentration of glucose in the serum in the control rats and rats enriched with undecanoate is summarized in Table 1. In the control animals liver glycogen concentrations decreased dramatically during the course of starvation. In striking contrast, the experimental animals exhibited far less depletion of liver glycogen. During starvation there was a decrease in glycogen in muscle in the control group that was significant at 24 and 48 hours. Although glycogen in muscle remained unchanged during fasting in the experimental group, the differences (means) between the experimental and control groups were statistically not significant at the several intervals studied.

In the control animals concentrations of serum glucose (means) were significantly lowered throughout the fasting

Table 1. Effect of starvation on glycogen concentrations in liver and muscle and glucose concentrations in serum in control and experimental rats (rats enriched with undecanoate). Data presented are means ± standard deviations. Size of groups and significance (P) of differences between means are shown in parentheses; N.S., not significant.

Groups	Duration of fast (hours)				
	0	24	48	96	144
			Liver glycogen (grams per 100 g)		
Control	6.12 ± 0.85 (5)	0.12 ± 0.06 (5)	0.16 ± 0.07 (6)	0.58 ± 0.19 (4)	0.88 ± 0.68 (4)
Experimental	4.98 ± .62 (5)	1.30 ± .29 (5)	2.22 ± .70 (6)	2.22 ± .63 (4)	1.93 ± .42 (4)
	N.S.	($P < .01$)	($P < .01$)	($P < .01$)	($P < .05$)
			Muscle glycogen (grams per 100 g)		
Control	0.31 ± 0.06 (10)	0.21 ± 0.08*(5)	0.21 ± 0.05†(7)	0.23 (2)	0.25 ± 0.10 (4)
Experimental	0.27 ± .06 (5)	0.27 ± .05 (5)	0.26 ± .05 (7)	0.33 (2)	0.31 ± .12 (4)
	N.S.	N.S.	N.S.		N.S.
			Serum glucose (milligrams per 100 ml)		
Control	153 ± 20 (5)	89 ± 14†(5)	83 ± 31†(7)	91 ± 13†(4)	103 ± 16†(4)
Experimental	126 ± 17 (5)	101 ± 11 (5)	135 ± 20 (7)	129 ± 6 (3)	121 ± 16 (4)
	N.S.	N.S.	($P < .01$)	($P < .01$)	N.S.

* When compared to nonfasted control group, $P < .05$. † When compared to nonfasted control group, $P < .01$.

period. By contrast, in the experimental group there was a decrease only after 24 hours of starvation, and this change was not significant. The differences between the means for the two groups were statistically significant after 48 and 96 hours of fasting.

Previous studies (*7*, *8*) of rats subjected to prolonged starvation have shown that rapid depletion of liver glycogen takes place, reaching its lowest concentrations during the first 24 to 48 hours. This phase is followed by a gradual increase, but the liver glycogen remains far below concentrations in nonfasting animals. Muscle glycogen and plasma glucose concentrations also diminish during starvation, but the changes are less dramatic.

The liver glycogen changes in our control animals during starvation are similar to those described (*7*); however, the relative maintenance of the stores of liver glycogen in our experimental animals during starvation appears to be unique. Animals studied in the past have never exhibited comparable concentrations of liver glycogen during prolonged fasting, regardless of the sex or strain of the animals or the nature of the diet before starvation.

The amount of incorporation of undecanoate into adipose tissue triglyceride in our animals is in agreement with the mean of 31.4 percent reported by Campbell and Hashim (*9*) in weanling rats fed a diet rich in triundecanoin for 4 weeks. Calculations show that when undecanoate constitutes approximately one-third of the fatty acids of depot fat in the rat, the stores of carbohydrate precursors other than protein are increased more than threefold.

During fasting, free fatty acids, including those with odd-numbered carbon chains, are mobilized in increasing quantities from adipose tissue stores and are utilized by liver and extrahepatic tissues. The odd-carbon fatty acids are broken down in the same sequence as even-chain fatty acids, except that the final thiolytic cleavage of the C_5 fatty acid residue yields propionyl coenzyme A (CoA) rather than acetyl CoA. As shown by Ochoa and co-workers (*10*), propionyl CoA can be carboxylated, forming methylmalonyl CoA which isomerizes in two steps to form succinyl CoA. The succinyl CoA in turn is convertible to glucose and glycogen.

Since animals enriched with undecanoate appear to differ from normal animals only in respect to the substantial expansion of nonprotein glucogenic units stored in depot fat, they should prove valuable in studies of the influence of the carbohydrate stores on the physiologic response to starvation.

THEODORE B. VANITALLIE*
AVEDIS K. KHACHADURIAN
Departments of Biochemistry and *Medicine, American University of Beirut, Beirut, Lebanon*

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* Present address: Department of Medicine, St. Luke's Hospital Center, New York 10025.

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Human Liposarcomas: Tissue Cultures Containing Foci of Transformed Cells with Viral Particles

Abstract. *Viral particles occurring in foci of human liposarcoma cells in tissue culture are morphologically similar to the sarcoma viruses of the avian and murine species. Antibodies in the serum of the patient from whom the culture was originated reacted with cytoplasmic antigens in the original liposarcoma and in the cultured liposarcoma cells.*

Many avian sarcoma virus strains have been isolated since Rous in 1911 (*1*) described a filterable agent etiologically associated with a naturally occurring sarcoma of chickens. However, no comparable agents associated with sarcomas of any mammalian species were known until the discoveries of the murine sarcoma viruses (*2*) which are similar in many respects to their avian counterparts. Both contain RNA, are about the same size, have similar morphology, and are formed by a process of budding from the plasma membrane (*3*). Virus particles of this characteristic morphologic type (type C of Bernhard) can usually be demonstrated by electron microscopy in the sarcomas induced by these agents.

Under certain circumstances, both the avian and murine sarcoma viruses can induce sarcomas in which the intact virus particle cannot be found (*4*). Yet, the defective viral genome is present in the avian neoplastic cells because a group specific antigen of the sarcoma virus can be demonstrated in these tumor cells (*5*). Furthermore, intact infectious virus particles can be recovered upon cultivation in vitro of the sarcoma cells with normal embryo cells to which leukemia virus has been added as a helper. The leukemia virus supports the necessary synthesis of coat components of the sarcoma virus which are required for infectivity (*6*). Both the murine and avian sarcoma viruses induce foci of morphologically altered cells on infection of normal susceptible cells in tissue culture (*7*).

Since the sarcoma viruses of the avian and murine species are so similar, it might be expected that these viral characteristics would also be typical of human sarcoma viruses, if they exist. There is no direct evidence for the role of viruses in the etiology of human neoplasia. However, immunologic evidence suggests the association of an infectious agent with human osteosarcomas. Antibodies to osteosarcomas were demonstrated by immunofluorescence in the serums of patients with this disease and their close associates (*8*). Also, hamsters develop a low incidence of osteosarcomas after inoculation with extracts of human osteosarcomas (*9*).

We now describe the appearance of foci of cells in a tissue culture derived from a human liposarcoma. These foci contain abundant viral particles quite similar to the avian and murine sarcoma viruses. Antibodies in the serum of the patient from whom the culture was originated reacted with cytoplasmic