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Obligatory Transformation of Fibroblasts by Bone Matrix in Rats Fed Sucrose Ration[1] (37834)

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Responding fibroblasts of mammals undergo a change in gene expression when they are placed in contact with demineralized residues of bone or tooth. Fibroblasts exposed to the transformants in this way are changed rapidly into chondroblasts and osteoblasts according to a timetable (1). In normal nutritional states hemopoietic bone marrow forms within 18 days and persists in the center of the ossicles created by transformation of the fibroblasts.

Certain tissues, few in number, have a profound nutritional advantage during serious dietary deficiencies insofar as they grow while the generality of cells are wasting. Rous (2) found that the growth of a transplanted carcinoma in the rat was unaffected by drastic underfeeding of the host. Growth of the prostate (3) was vigorous and selective in infantile dogs injected with testosterone while deprived of all food for 3 weeks. In the present study it was found that fibroblasts were transformed by the bone matrix in rats during severe dietary deprivation induced by feeding sucrose exclusively as their sole ration.

Material and Methods. Dehydrated acid-insoluble rat bone matrix (1) having particle size 74–420 μm was prepared. Control and experimental animals were 29-day-old male rats bred in this laboratory of Long–Evans strain; their weight was 51 ± 2 g. Control rats were fed a commercial ration of compressed pellets which has been the sole diet of our colony for 10+ years. Beginning 1 or 5 days prior to transplantation of the demineralized bone matrix, half of the rats were fed sucrose exclusively and this was continued until the termination of the experiment. Both control and sucrose-ration (S-R) rats were provided with a salt lick together with tap water to drink *ad lib.* The diet was nitrogen free. Under ether anesthesia, a subcutaneous pocket was prepared surgically; 10–15 mg of the powdered bone matrix was deposited therein and the incision was closed with a metallic clip. There were 6 transplantation sites of this sort in each rat; the day of operation is denoted Day 0. On the day of harvest, ^{35}S or ^{32}P was injected intravenously 4 hr prior to sacrifice to quantitate chondromucoprotein synthesis in cartilage and bone mineral formation, respectively (1); half of each transformation plaque was studied for concentration of alkaline phosphatase and radioactivity while the remainder was used for histological examination. One unit of alkaline phosphatase (EC 3.1.3.1) is defined as the enzyme activity which liberates 1 μmole of *p*-nitrophenol per 0.5 hr under stated conditions (4).

Results and Discussion. Rats fed sucrose ration had a progressive decline in body weight (Table I) which proved fatal. Permitted to live their life span, 12 S-R rats survived 18–30 days; the mean survival was 26 ± 3.6 days.

In one of our experiments, the sucrose ration was initiated on Day −1. On Day 7, cartilage was found in abundance in all of the transformation plaques both in S-R and control groups; moreover, there were no

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TABLE I. Transformation Plaques in Rats Fed Complete or Sucrose Rations.[a,b]

Harvest day	Ration	Change in body weight (g)	Transformation plaques: Weight (mg)	Alkaline phosphatase (U/g)	^{35}S (cpm $\times 10^{-3}$)	^{32}P (cpm $\times 10^{-3}$)	Ensiform ^{35}S cartilage (cpm $\times 10^{-3}$)	Bone ^{32}P (cpm $\times 10^{-3}$)
			(a) Sucrose ration started on Day -1					
+7	Complete	+27	98.3 ± 21	18.9 ± 13	0.58 ± .07	—	1.07	—
	Sucrose	−6	80.1 ± 20	20.7 ± 11	0.74 ± .08	—	0.37	—
+14	Complete	+45	90.2 ± 26	55.7 ± 15	—	4.89 ± .3	—	8.92
	Sucrose	−12	70.6 ± 21	49.9 ± 33	—	2.13 ± .4	—	5.63
			(b) Sucrose ration started on Day -5					
+7	Complete	+19	117 ± 37	15.9 ± 8	0.50 ± .07	—	1.00	—
	Sucrose	−6	81.6 ± 37	9.5 ± 3	0.40 ± .07	—	0.26	—
+10	Complete	+24	111 ± 32	57.3 ± 26	—	2.97 ± .6	—	8.04
	Sucrose	−19	74.8 ± 10	22.5 ± 15	—	0.71 ± .2	—	5.25

[a] Control rats received a ration of compressed pellets (Rockland Mouse/Rat Diet, Teklad, Inc., Monmouth, Il). There were 4 rats/group; each rat had 6 transformation plaques. Mean values are given ± standard deviation of mean.

[b] 1 μCi/g body weight of either $Na_2{}^{35}SO_4$ or $H_3{}^{32}PO_4$ in 0.2 ml saline was injected in a caudal vein; 4 hr later, at harvest, the plaques were excised and cleansed of adherent tissues. Additional preparation of the tissues and measurement of radioactivity have been described (1); radioactivity is expressed per milligram of tissue fresh weight.

significant differences in the plaques of the two groups with reference to concentration of alkaline phosphatase or the incorporation of ^{35}S (Table I). It is noteworthy that the concentration of ^{35}S was depressed in the ensiform cartilage of S-R rats compared to controls. On Day 14, bone had formed in all the transformation plaques in both groups. Compared with the control group, there was a moderate and significant ($P < .01$) decrease (Table I) in the animals fed sucrose diet in the incorporation of ^{32}P both in transformation plaques and in diaphyseal bone. The greatest difference in the plaques was observed after 3 weeks and concerned their bone marrow. On Day 21, the transformation plaques in the control group were red and contained hemopoietic marrow (Fig. 1), whereas in the S-R rats the plaques were pale and contained gelatinous bone marrow without erythropoietic cells.

In another experiment, sucrose ration was started 5 days before the bone matrix transformant was transplanted. At harvest on Day 7, cartilage was present in all of the transformation plaques of rats in S-R group, but it was less in amount than in control group. On Day 10, bone was present in

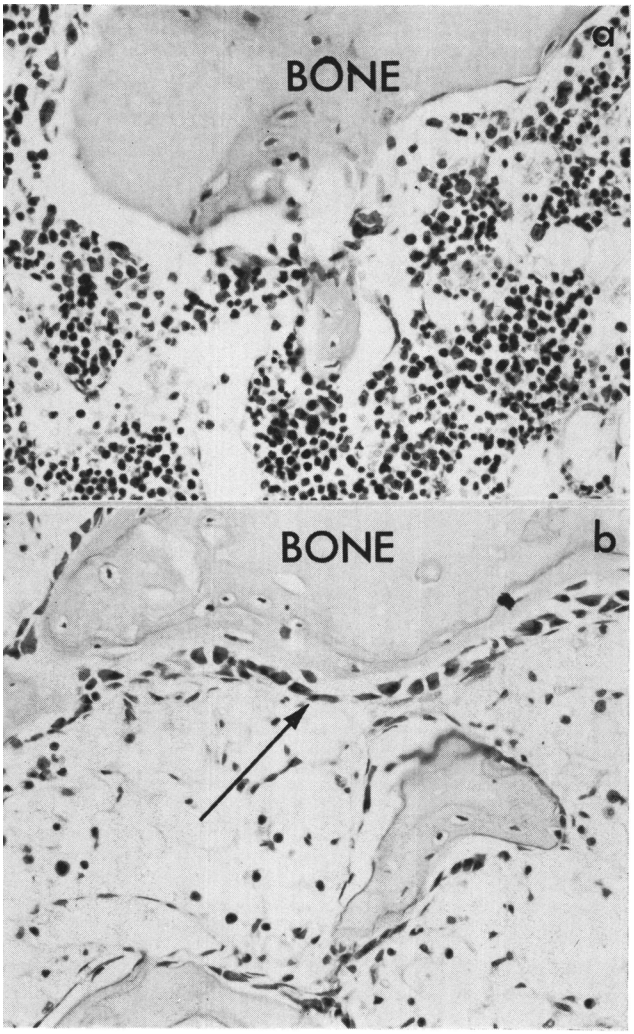

FIG. 1. Photomicrographs of transformation plaques, Day 21, from (a) rat fed the control ration showing erythropoietic bone marrow, (b) rat fed sucrose ration showing gelatinous bone marrow. The arrow points to a row of osteoblasts. 150×.

transformation plaques in both groups. The incorporation of ^{35}S in cartilage on Day 7 and of ^{32}P in bone on Day 10 was less in the sucrose-ration animals than in control groups (Table I).

Dietary intake limited to sucrose, inorganic salts, and water led to progressive emaciation with extensive tissue breakdown which was fatal within 1 month. Nevertheless, a rearrangement of products of tissue destruction took place in fibroblasts, transformed during dietary deprival, resulting in growth and synthesis in these special cells.

In conclusion, the transformation of fibroblasts by the bone matrix is obligatory since calcified cartilage and bone occurred in considerable amounts in rats fed a ration consisting exclusively of sucrose. Hemopoietic bone marrow did not form in the plaques in the rats fed in this group, and it would appear that exogenous foodstuffs are required for erythropoiesis in the ossicles.

Summary. Allogeneic transplantation of coarse powder of demineralized bone matrix in rats has a profound influence on the gene expression of responding fibroblasts. Under normal nutritional states, the fibroblasts are transformed sequentially into cartilage and bone with hemopoietic bone marrow. In rats fed sucrose exclusively, there was an obligatory transformation of fibroblasts to cartilage and bone. However, there was no evidence of erythropoiesis.

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