Citrate Cleavage Enzyme in Livers of Obese and Nonobese Mice

Abstract. *The specific activity of the citrate cleavage enzyme is 3.3 times greater in livers of obese mice than in livers of their nonobese litter mates. The difference persists during starvation. The specific activity of the acetate activating enzyme is approximately the same in the livers of both types of animals. It is proposed that a high activity of citrate cleavage enzyme is one of the factors responsible for obesity.*

In the conversion of carbohydrate to fat, the oxidation of pyruvate to acetyl coenzyme A occurs within the mitochondria. On the other hand, the incorporation of acetyl groups of acetyl coenzyme A into fatty acids is chiefly an extramitochondrial process. Citrate is the main precursor of carbon for the extramitochondrial synthesis of fatty acids in nonruminant animals. It has been proposed that the acetyl group of intramitochondrial acetyl coenzyme A may be transferred across the mitochondrial membrane as citrate (*1*);

Intramitochondrial
acetyl coenzyme A + oxaloacetate → citrate + coenzyme A

Intramitochondrial citrate → *Extramitochondrial* citrate

Extramitochondrial
citrate + coenzyme A + ATP (2) → acetyl coenzyme A + oxaloacetate + ADP + orthophosphate

Extramitochondrial oxaloacetate → *Intramitochondrial* oxaloacetate

Net reaction:
Intramitochondrial acetyl group → *Extramitochondrial* acetyl group
and ATP → ADP + orthophosphate

The rate of citrate incorporation into fatty acids by cell-free preparations of rat liver varies according to the nutritional state of the animal. It is lowest in starved animals and highest in animals that have been starved and then fed (*3*). The activity of the citrate cleavage enzyme shows a similar pattern. For example, it is 20 times less in rats that have been starved than in rats that have been starved and fed a high carbohydrate diet for 2 days (*4*).

The formation of acetyl coenzyme A from citrate can thus be regarded as the first step in the pathway of extramitochondrial synthesis of fatty acid. In a biosynthetic pathway the first step may often be rate-determining (*5*). In other words, differences in the enzyme activity of the first step may be responsible for different rates of operation of the entire pathway.

We have measured the activities of citrate cleavage and acetate activating enzymes in cell-free extracts of livers of obese mice and their nonobese littermates. The animals, which had an average weight of 43 and 23 g, respectively, were obtained from the Jackson Laboratory, Bar Harbor, Maine. The strain (C_{57} BL/6J-ob) carries a recessive gene for obesity, individual mice homozygous for the recessive trait being obese and all others nonobese (*6*). The mice were maintained on a diet of Wayne Lab-Blox (*7*). Livers from the animals were cut into small pieces with scissors, and were then homogenized in three volumes by weight of ice-cold, 0.25*M* sucrose with the aid of a Philpot (*8*) homogenizer. The homogenate was centrifuged at an average centrifugal force of 59,000*g* for 30 minutes. The clear supernatant was withdrawn and used for the enzyme assays. Protein was measured by the method of Lowry *et al.* (*9*). Citrate-cleavage and acetate-activating enzyme activities were measured by the hydroxamate methods of Srere and Lipmann (*10*) and Lipmann and Tuttle (*11*). Each assay was performed at two or three protein concentrations to ensure proportionality between enzyme activity and protein concentration (*12*). In measurements of citrate-cleavage enzyme activity, and hydroxamate method gave the same results as the malate dehydrogenase assay (*13*).

Results of enzyme assays are shown in Table 1. The specific activity of the citrate-cleavage enzyme is 3.3 times greater in obese mice than in their nonobese littermates ($p < .001$). On the other hand, the activity of the acetate-activating enzyme is only 1.2 times greater in obese mice than in their nonobese littermates ($.02 < p < .05$). The small difference in the activity of the acetate-activating enzyme is found mainly in the livers of the obese females. The effect of starvation on the activity of the citrate-cleavage enzyme is shown in Fig. 1. The activity declines in both the obese and the nonobese animals. After 3 days of starvation the activity is about one-third of that before starvation. Over a 3-day period of starvation the activity in the obese mice remains approximately three times higher than that of their nonobese littermates.

Table 1. Citrate cleavage and acetate-activating enzyme activities of livers from obese and nonobese mice.

Age of litter mates (days)	Sex	Specific activity (μmoles/mg of protein per hr)			
		Citrate cleavage		Acetate activation	
		Non-obese	Obese	Non-obese	Obese
59	M	0.71	1.30	0.33	0.32
64	M	.49	1.58	.29	.36
75	M	.60	2.51	.36	.36
76	M	.64	1.69	.34	.41
58	F	.51	2.46	.36	.59
59	F	.56	1.98	.35	.49
67	F	.75	2.45	.44	.47
79	F	.72	2.46	.39	.50
Mean		0.623	2.054	0.358	0.438
± S.E.		±0.099	±0.482	±0.044	±0.091
		$t = 8.249$		$t = 2.296$	
		$p < .001$		$.05 > p > .02$	

These results provide a correlation between obesity and the activity of the citrate-cleavage enzyme. They raise the interesting question of whether the obese mice have a greater capacity for fatty acid synthesis than their nonobese litter mates. Liver slices from obese mice incorporate radioactive glucose into fatty acids more slowly than liver slices from their nonobese littermates (*14*). The same results were obtained with pieces of adipose tissue from both

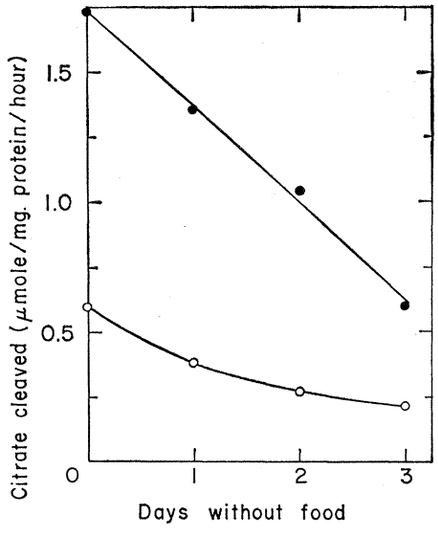

Fig. 1. Decline in activity of citrate-cleavage enzyme of liver with time of starvation. The conditions of the assay were as described in the text. The points are the averages of two determinations. Solid circles, obese animals; hollow circles, nonobese littermates.

types of mice (*15*). However, the possibility of dilution of the radioactive compound by endogenous substrates was not taken into account in these experiments. A higher rate of fatty acid synthesis in obese mice as compared to their nonobese littermates has so far been demonstrated only in vivo (*16*).

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2. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate.
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Sterol Induction of Reproduction and Stimulation of Growth of Pythium and Phytophthora

Abstract. *Sterols of fungal, plant, and animal origin induced sexual reproduction in* Pythium periplocum *and* Phytophthora megasperma *var.* sojae *and the formation of large zoosporangia in* Phytophthora parasitica *var.* nicotianae. *Some sterols, especially cholesterol, stimulated growth.*

Although considerable emphasis has been placed upon carbohydrate, nitrogen, and water-soluble vitamin nutrition of species of *Phytophthora* and other Phycomycetes which cause plant diseases, lipid nutrition has received little attention. Recently, vegetable oils were found to be better carbon sources than glucose for *Phytophthora parasitica* (Dastur) var. *nicotianae* (Breda de Haan) Tucker (*1*). Apparently, non-saponifiable compounds were involved (*2*). Triolein was a poor carbon source, and charcoal-treated oat oil supported less growth than untreated oat oil. Cholesterol and tocopherol reversed the toxicity of oleic acid. Cholesterol and a fatty acid fraction of cow liver stimulated the growth of *P. infestans* de Bary (*3*).

When *P. parasitica* var. *nicotianae* was grown on a vegetable oil-nitrate medium, zoosporangia were numerous in and near the densely colonized oil droplets (*1*, *2*). No reports of reproduction of species of *Pythium* or *Phytophthora* on defined media were found. These observations led to the studies described here of the effects of sterols on growth and reproduction of species of Pythiaceae.

Cultures used were *Pythium periplocum* (Drechs.), *Phytophthora megasperma* (Drechs.) var. *sojae* (Hilde.), and *P. parasitica* var. *nicotianae* (*4*). The medium contained 5.4 g glucose, 1.5 g $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2 ml of a 1000-ppm stock solution of thiamine-HCl, 17 g agar, and 1000 ml distilled water. The *p*H was adjusted to 6.0 before the medium was autoclaved for 10 minutes. Solutions of sterols in ether (*5*) were applied to the surface of the solidified agar (25 ml/plate) at the rate of 20 mg of sterol per liter of medium. Control plates received an equal volume (2 ml) of ether reagent. The plates were seeded several hours after the ether solutions were applied. The inoculum was produced by transferring mycelium from the stock culture (maintained on potato-dextrose agar) to 1.7 percent agar in petri plates. Disks cut with a No. 1 cork borer at the edge of the resulting colony were transferred to the edge of petri plates containing the glucose-nitrate medium. Plates were incubated in the dark at about 25°C. Linear growth was measured 3 and 7 days after seeding the *Pythium* and the *Phytophthora* isolates, respectively. The plates were examined microscopically for reproduction 10 days after seeding.

Oospores of *Pythium periplocum* and *Phytophthora megasperma* var. *sojae* were abundant on sterol media. The controls were completely vegetative. *Phytophthora parasitica* var. *nicotianae* produced numerous large sporangia and chlamydospores in the presence of sterols. In the absence of sterols, small abnormally shaped sporangia and small chlamydospores were formed. Whether these abortive-appearing sporangia were functional in producing zoospores was not investigated. There were no obvious differences in the numbers of oospores or sporangia produced per unit area on the various sterol-containing media.

Sterols varied in their capacity to stimulate growth (Table 1). Cholesterol and phytosterol consistently stimulated growth. The purity of the sterols used (*6*) was not specified. Regardless of whether the differences in growth on media containing various sterols are due to impurities or to sterol structure, the inhibition or lack of stimulation of growth by some sterols argues against the concept (*7*) that there are no fungal requirements specifically for reproduction and independent of growth effects.

To determine whether the activity was due to an impurity, equivalent amounts of digitonin and a purified grade of cholesterol (99+ percent) (*8*) were mixed as 1 percent solutions in 95 percent ethanol. The digitonide was removed by filtration, washed

Table 1. Linear growth (in millimeters) of *Pythium periplocum* (Py.p.), *Phytophora megasperma* var. *Sojae* (Ph.m.), and *Ph. parasitica* var. *nicotianae* (Ph.p.) on glucose-nitrate agar medium supplemented with sterols (20 mg/liter).

Supplement	Py.p.*	Ph.m.†	Ph.p†
Ergosterol	39.5	12.0	11.0
Phytosterol	46.5	15.5	25.0
Stigmasterol	26.5	9.0	19.0
β-Sitosterol	28.0	7.0	17.5
Cholesterol	52.5	15.5	26.5
None	38.3	6.7	13.7
Statistical data			
LSD .05 ‡	7.8	3.3	3.1
LSD .01 ‡	11.1	4.7	4.5

* After 3 days' growth. † After 7 days' growth. ‡ Least Significant Difference at the 5 or 1 percent confidence level.