be highly concentrated in a dense particulate subfraction (below 1.2*M* sucrose) of the primary mitochondrial fraction. The relative specific activity (RSA = percent tissue renin/percent protein) in this subfraction is 2.69. Such highly enriched renin activity has been found also in parallel experiments with renal cortex, where the RSA of this same subfraction was 2.39 (RSA of primary mitochondrial fraction is 1.00). Unspecific binding of renin was ruled out as the explanation of the occurrence and subcellular localization of brain tissue enzymatic activity by experiments with addition of renin to the homogenate, hypo-osmotic shock of the primary mitochondrial fraction to release particle-bound enzyme, and in vivo infusion of renin before fractionation.

Tissue renin is significantly decreased in the caudate nucleus by aldosterone and increased by progesterone administration (Fig. 1). These results indicate that brain tissue renin activity can be altered by administration of hormones that change tissue excitability and electrolyte balance (*11*).

An "excitability-determining enzyme" in the brain has been postulated to be induced by a decrease in intracellular sodium (*12*). This enzyme could well be an isoenzyme of renin which yields angiotensin. Angiotensin is known to promote the entry of sodium into the cell (*13*).

Renin and angiotensin have similar effects when injected into the brain (*1*). This is consistent with the finding in our laboratory that renin substrate is present in brain tissue. Enzyme converting angiotensin I also has been found in brain tissue (*14*). Thus it appears that the components of the renin/angiotensin system are available at the level of the brain cell itself.

The known effects of angiotensin on the homeostatic control of electrolyte balance by the central nervous system could possibly be explained as effects of locally released angiotensin. The local tissue renin might then be partly responsible for cellular sodium regulation and, thereby, for regulation of cell excitability and hydration (*15*).

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Hypolipidemia in a Mutant Strain of "Acatalasemic" Mice

Abstract. *Mutant "acatalasemic" mice have an unstable catalase in hepatic and renal peroxisomes that is readily degraded, apparently into peroxidase subunits. The hypothesis that an in situ cataloperoxidase would affect serum lipids was tested in these mutants and serum triglycerides and cholesterol were found to be significantly lower than in the wild strain. This finding is in accordance with reports that a hypolipidemic response to the injection of peroxidase subunits of hepatic catalase occurs in humans and rabbits.*

Peroxisomes (microbodies) are a group of cytoplasmic organelles that are enriched in oxidases that generate hydrogen peroxide and catalase, an enzyme that rapidly degrades hydrogen peroxide (*1*). Catalase represents approximately 16 percent of the peroxisomal protein (*2*). Although peroxisomes occur in plants, protozoa, and yeast, as well as in liver and kidney of higher animals, little is known of their function (*1*). A link to lipid metabolism is suggested by the effect of ethyl-α-*p*-chlorphenoxyisobutyrate (Clofibrate-CPIB), a hypolipidemic drug that is used clinically. Administration of this drug to male rats and mice results in a proliferation of peroxisomes and increased synthesis of catalase (*3*, *4*). However, studies by Svoboda *et al.* (*4*) suggest that the peroxisome effect is not related to the hypolipidemic action of the drug. Nevertheless, other evidence points to an association between catalase and lipid metabolism. Puig-Muset *et al.* (*5*) and Caravaca *et al.* (*6*) have reported that injection of bovine hepatic catalase produces a hypocholesterolemic response in humans (*5*) and rabbits and inhibits hepatic cholesterol synthesis (*6*). Caravaca and May subsequently noted that the lyophilized catalase used in their experiments had been prepared in a fashion that partially degraded the enzyme to peroxidase subunits and then demonstrated that the peroxidase induced the hypolipidemic response (*7*). Tanford and Lovrien, and Inada *et al.*, had previously shown that alkaline denaturation of catalase results in the breakdown of the molecule to smaller sub-

Table 1. Triglycerides in serums of mutant and wild mice. (See text for details.)

Expt. No.	Animals (No.)	Age (mo)	Triglycerides (mg/100 ml) (*19*)		Significance
			Mutant*	Wild	
1	3†	6	17	103	
2	5	7	14 ± 4.12	46 ± 6.69	$P < .01$
3	5	2	64 ± 9.86	92 ± 8.29	$P < .1$
4	3†	6	35	50	

* In the course of these experiments animals were screened for catalatic activity by adding three drops of blood to a test tube containing a 3 percent solution of hydrogen peroxide. One mutant, unexpectedly, reacted in the same fashion as the wild strain, indicating the presence of a normal blood catalase. This aberrant mutant was set aside and is not listed in Tables 1 and 2. Its serum triglyceride concentration proved to be the highest recorded in these experiments, 159 mg/100 ml. Serum cholesterol in this animal was 95.9 mg/100 ml. † One determination of pooled serums.

Table 2. Cholesterol in serums of mutant and wild mice. (See text for details.)

Expt. No.	Animals (No.)	Cholesterol (mg/100 ml) (*20*)	
		Mutant*	Wild
1	3†	90	110
2	3†	97	141
	1	93	102
	2†		114
3	1	98.5	89.6
	1	91.1	113
Average of all determinations		93.9‡ ± 1.65	111.6‡ ± 6.95

* See first footnote to Table 1. † One determination from pooled serums. ‡ The difference between the two means is significant ($P < .05$).

units with high peroxidase activity (*8*). Recent cytochemical studies have shown that after *alkaline incubation* (*9*) or *pretreatment* (*10*) peroxisomes can be optimally stained in benzidine-type media that apparently demonstrate the peroxidatic activity of the altered catalase.

We have recently investigated the peroxisomes of a strain of mutant, "acatalasemic" mice (Cs^b) (*11*). These mice have an unstable catalase that is readily degraded and inactivated with a loss of catalatic activity (*12*). Our cytochemical studies showed that the peroxisomes of these animals, unlike those of the wild strain (Cs^a), had high levels of peroxidatic activity that can be demonstrated *without alkaline treatment*, indicating that the degraded catalase may be functioning as an active peroxidase (*13*). For example, in the mutant, peroxisomes are readily stained in a 3,3′-diaminobenzidine medium when incubated at *p*H 7 and 37°C (Fig. 1a). In the wild strain (as in the rat, chicken, toad, and human) peroxisomes cannot be made visible under these conditions (Fig. 1b) but require incubation at an alkaline *p*H (*p*H 9 to 10) at 37°C to demonstrate the peroxidatic activity of catalase (*13*).

We speculated that the presence of an in vivo peroxidase in the peroxisomes of mutant animals might be reflected in lower concentrations of serum lipids. This was tested in three experiments in which serums from 32 mutant and wild strain mice (all males) were assayed for triglycerides. Serum cholesterol concentrations were determined in 20 animals.

The results listed in Tables 1 and 2 demonstrate that serum triglyceride levels are significantly lower in the acatalasemic mice than in the wild strain. Serum concentrations of cholesterol are also reduced. It is of interest that the differences are more pronounced in older animals (experiments 1, 2, and 4) than in mice that had just reached maturity (experiment 3).

Whether the hypolipidemia is related to the abnormal catalase or to some other metabolic defect is not established. No other enzymatic abnormality has been described in these animals.

The work of Caravaca *et al.* (*7*, *14*), demonstrating that the injection of subunits of degraded catalase (hepatic cataloperoxidase) results in hypolipidemia, suggests that these two phenomena are also related in the mutants. It may be pertinent that administration of allylisopropylacetamide, an agent that blocks catalase synthesis (*15*), has recently been shown to produce hyperlipidemia (*16*). In plant cells interesting data relating peroxisomes (glyoxysomes) to lipid metabolism comes from recent studies of endosperm of fatty castor bean seedlings. A striking increase in the number of peroxisomes and synthesis of catalase and other enzymes associated with these particles occurs at germination when stored lipids are converted to carbohydrate (*17*). Human acatalasemic patients have been studied extensively and the only clinical manifestations are reported to be gangrenous lesions of the mouth (*18*). To our knowledge serum lipids have not been studied in these patients.

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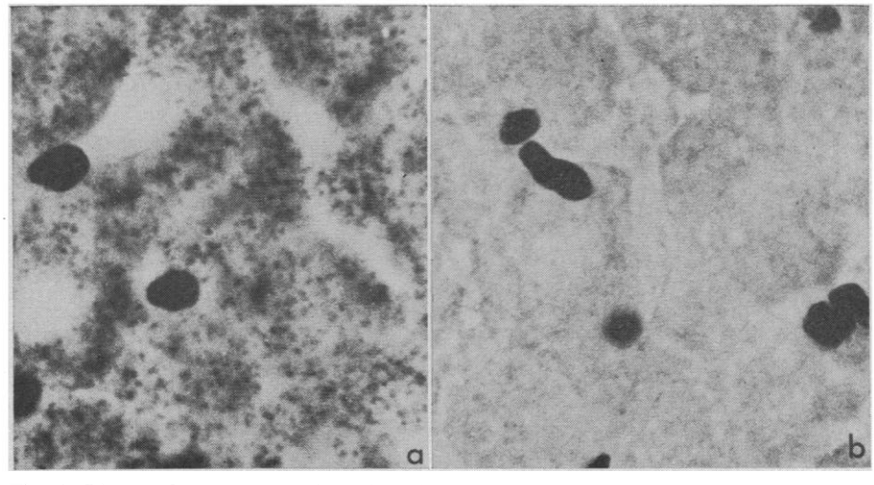

Fig. 1. Livers of mutant acatalasemic (a) and wild (b) strains of mice. Frozen sections (10 μm) of tissues were fixed overnight in cold neutral formaldehyde and incubated for 60 minutes in a 3,3′-diaminobenzidine medium at *p*H 7, 37°C. Under these conditions peroxisomes are stained throughout the cytoplasm in the mutant but are not reactive in the wild strain. Erythrocytes are stained in both specimens (× 1000).

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