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Effect of Chronic Ethanol Ingestion on Mitochondrial Protein Synthesis in Sinclair(S-1) Miniature Swine (38687)

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Chronic ethanol ingestion has been reported to produce numerous hepatic metabolic alterations (1, 2). One such alteration is the resulting damage to membrane of hepatic mitochondria. Kiessling and Tilander (3) concluded that, in rats given ethanol for 10–12 mo, hepatic mitochondrial membranes were disrupted. An *in vitro* increase in membrane permeability was found to occur sooner than in mitochondria from controls (4). Also, morphologic evidence of ethanol induced injury to mitochondrial membranes was obtained in rats (5) and chronic human alcoholics (6).

Rubin *et al.* (7) found ethanol to interfere with *in vitro* incorporation of ^{14}C-leucine into rat liver mitochondrial membranes. Hepatic mitochondria, obtained from rats fed ethanol for 24 days, also displayed decreased incorporation of radioactive leucine. Quastel (8), studying the effect of ethanol on ^{14}C-glycine incorporation into brain slices, showed that 0.9 *M* ethanol reduced incorporation approximately 50%. Jarlstedt (9) reported that *in vivo* incorporation of ^{3}H-leucine into protein from rats chronically ingesting ethanol for 8 mo was depressed in cerebellum and cortex cerebria and stimulated in hepatic subcellular fractions. The present study was conducted to determine the effect of chronic ethanol ingestion on the rate of hepatic mitochondrial protein synthesis in Sinclair(S-1) miniature swine and to evaluate the labeling pattern of proteins synthesized by mitochondria and possible effects of ethanol on this process.

Materials and Methods. Sixteen 7-wk old Sinclair(S-1) miniature swine were assigned randomly to four treatments, i.e., four pigs were allowed access to ethanol for 3 wk, four pigs were fed isocalorically for 3 wk, four pigs were allowed access to ethanol for 6 wk, and four pigs were fed isocalorically for 6 wk. A 10% ethanol in orange juice solution was given *ad lib.* to pigs in the two experimental groups, while pigs in the two control groups were given water *ad lib.* Water also was available *ad lib.* to pigs in the experimental groups, thus the ethanol solution was present on a free choice basis.

After 3 wk on test, four experimental and four control pigs were sacrificed. Also, after 6 wk on test, four experimental and four control pigs were sacrificed. Livers were excised and placed immediately in ice cold sucrose–Tris–EDTA buffer. Hepatic mitochondria were prepared under sterile conditions in a medium containing 0.25 *M* sucrose, 0.01 *M* Tris-HCl, pH 7.8, and 0.001 *M* EDTA-Na by the method of Beattie (10). This yielded a pellet 3% contaminated with microsomal protein as determined by glucose-6-phosphatase activity.

Amino acid incorporation into mitochondrial protein was determined using mitochondria obtained under sterile conditions (11). Mitochondria were incubated in an incubation medium containing 30 μCi of ^{3}H-4,5-L-leucine according to the method of Beattie and Ibrahim (11). After a 30 min incubation at 30° in a metabolic shaker, 10 m*M* unlabeled leucine was added and mitochondria were reisolated. The mitochondrial pellet was washed twice in the buffer medium containing 10 m*M* unlabeled leucine. The pellet from the second washing was resuspended in buffer medium and an aliquot taken for counting. The remaining suspension was sonicated at maximum output for 15 sec in a model 150 Virsonic Cell Disrupter and centrifuged at 164,000 *g* for 30 min. The sonicated pellet was resuspended in buffer medium and an aliquot taken for counting. The

TABLE I. EFFECT OF VOLUNTARY ETHANOL CONSUMPTION ON MITOCHONDRIAL PROTEIN SYNTHESIS IN MINIATURE SWINE THREE WEEKS ON TESTS.

Specimen No.	Treatment	Intact mitochondria cpm/mg	Total counts	Proteolipid fraction cpm/mg	Total counts
2546	Ethanol	25,791	206,328	102,164	40,866
2550	Ethanol	22,367	178,936	87,480	26,244
2551	Ethanol	20,060	160,480	81,240	28,434
2568	Ethanol	27,169	217,352	106,676	37,337
	Mean	23,847		94,390	
	±SEM	1616		6000	
2573	Control	23,734	189,872	96,938	33,928
2574	Control	24,654	197,232	98,616	34,516
2582	Control	35,132	281,056	130,500	52,200
2586	Control	24,260	194,080	96,060	33,621
	Mean	26,945		105,528	
	±SEM	2735		8341	

TABLE II. EFFECT OF VOLUNTARY ETHANOL CONSUMPTION ON MITOCHONDRIAL PROTEIN SYNTHESIS IN MINIATURE SWINE SIX WEEKS ON TEST.

Specimen No.	Treatment	Intact mitochondria cpm/mg	Total counts	Proteolipid fraction cpm/mg	Total counts
2553	Ethanol	24,656	197,248	99,628	39,851
2554	Ethanol	25,471	203,768	100,880	29,255
2558	Ethanol	31,527	252,216	125,108	47,541
2579	Ethanol	33,850	270,800	130,050	46,818
	Mean	28,876		113,916	
	±SEM	2258		7956	
2572	Control	42,200	337,600	150,800	60,320
2575	Control	45,628	365,024	172,600	55,232
2577	Control	43,920	351,136	165,700	62,966
2585	Control	40,047	320,376	166,176	61,485
	Mean	42,949		163,819	
	±SEM	1194		4616	

remaining suspension was used for electrophoresis or for proteolipid extraction.

Proteolipid extractions were performed on sonicate mitochondria by the method of Soto *et al.* (12). Extracts from this procedure were dried under a stream of nitrogen at 50° and dissolved in 1% SDS for protein determination or in the solvent system used for gel electrophoresis.

Gel electrophoresis was performed according to the method of Weber and Osborn (13) with a modification according to the method of Tzagoloff (14). Gels were sliced into 2 mm portions with a model B-100 Gilson Aliquogel Gel Slicer, H_2O_2 was added and the slices heated at 50° overnight or until dissolved. Toluene counting solution was added and radioactivity determined. Protein concentrations were determined on whole mitochondria, the sonicated pellet and the proteolipid fraction by the method of Lowry *et al.* (15). Protein fractions were washed and prepared for counting by previously described methods (16).

Results. Mean ethanol consumptions for experimental pigs sacrificed after 3 or 6 wk on test were 5.1 or 6.4 g ethanol/kg body wt/day, respectively.

In vitro incorporation of ^{3}H-leucine into mitochondrial protein of pigs on test for 3 wk are listed in Table I. There were no significant ($P > 0.05$) differences in rates of

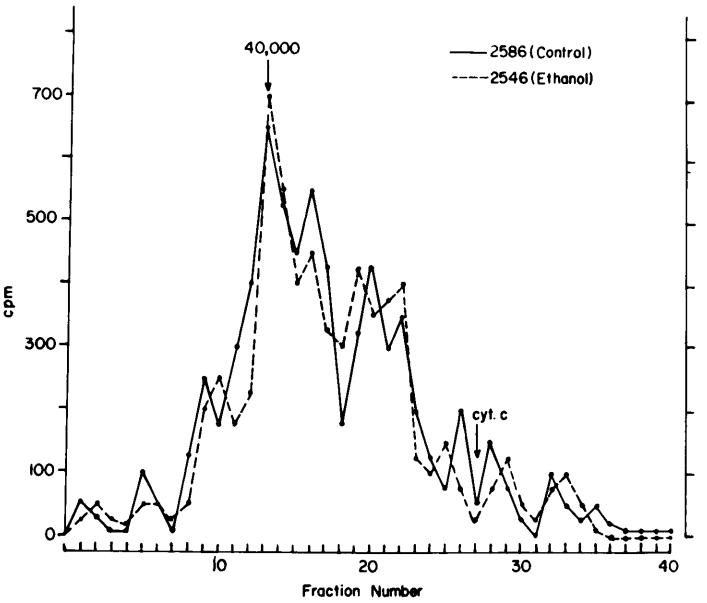

FIG. 1. SDS-gel electrophoresis profiles of whole mitochondrial membrane from hepatic tissue of one control (No. 2586) and one ethanol consuming pig (No. 2546) on test for 3 wk.

incorporation between livers of control or experimental pigs. Mean (±SEM) rates of amino acid incorporation for livers from ethanol consuming and control pigs sacrificed after 3 wk on test were 23.8 ± 1.6 and 26.9 ± 2.7 × 10^3 cpm/mg protein, respectively.

The rate of hepatic mitochondrial protein synthesis was significantly ($P < 0.01$) less in livers from pigs consuming ethanol for 6 wk than for corresponding control pigs (Table II). Rates of amino acid incorporation were decreased 33% in livers from experimental pigs as compared to control animals. Mean (± SEM) rates of incorporation for livers from ethanol consuming and control pigs were 28.9 ± 2.2 and 42.9 ± 1.2 × 10^3 cpm/mg protein, respectively.

Proteolipid proteins have been shown to be synthesized by mitochondria and to be located in the inner mitochondrial membrane (17). Rates of synthesis of these compounds also were examined with no significant ($P > 0.05$) difference observed between hepatic tissues from experimental or control pigs on test for 3 wk (Table I). However, as compared with controls, ethanol consuming pigs on test for 6 wk were found to have a 30–35% decrease in incorporation of ^{3}H-leucine into proteolipid protein (Table II). This decrease was similar to that observed for whole mitochondrial protein.

Gel electrophoresis profiles from livers of ethanol consuming and control pigs were examined to ascertain if there were differences in labeling patterns. Total membrane profiles from sonicated pellets of a control and an experimental pig on test for 3 wk are depicted in Fig. 1. There was a similar pattern of labeling, with seven to eight peaks, ranging from 48,000 to 8000 mol wt. However, for membrane profiles of pigs on test for 6 wk (Fig. 2), there was a 50% decrease in labeling of the 40,000 mol wt protein for experimental pigs, as compared with controls. The remaining peaks appeared to be unaffected. Analyses of membrane profiles for proteolipid protein fractions revealed similar results with those observed for whole membrane profiles. After 3 wk on test, livers from control and ethanol consuming pigs had similar patterns of labeling, with three to four peaks (Fig. 3). After 6 wk on test, there was a significant ($P < 0.01$) decrease in labeling of the 40,000 mol wt peak (Fig. 4) for experimental pigs as compared with controls.

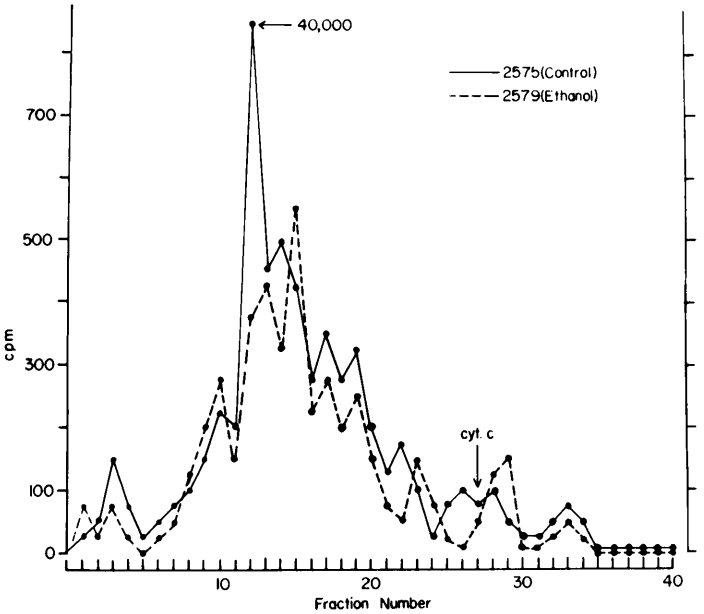

FIG. 2. SDS-gel electrophoresis profiles of whole mitochondrial membrane from hepatic tissue of one control (No. 2575) and one ethanol consuming pig (No. 2579) on test for 6 wk.

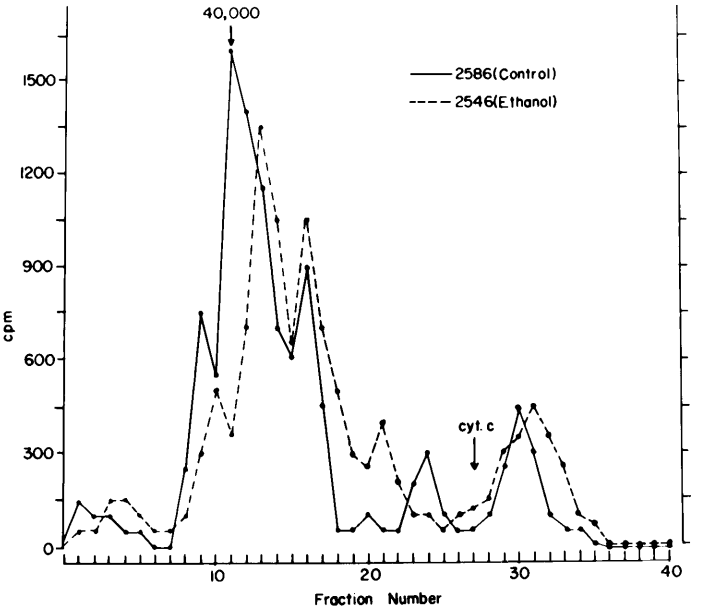

FIG. 3. SDS-gel electrophoresis profiles of hepatic mitochondrial proteolipid protein of one control (No. 2586) and one ethanol consuming pig (No. 2546) on test for 3 wk.

There were no significant ($P > 0.05$) differences in labeling of the remaining peaks.

Discussion. We have demonstrated that chronic ethanol ingestion caused a chemical derangement of hepatic mitochondria in Sinclair(S-1) miniature swine. One of the causes of this derangement appeared to be interference of hepatic mitochondrial pro-

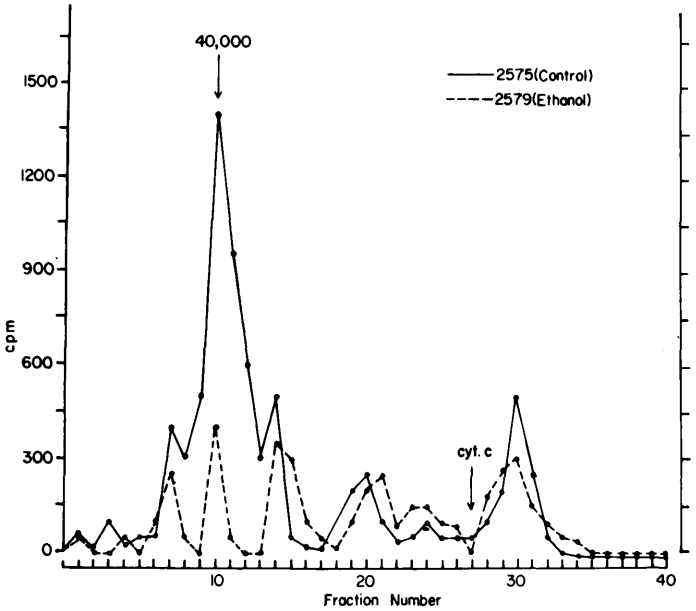

FIG. 4. SDS-gel electrophoresis profiles of hepatic mitochondrial proteolipid protein of one control (No. 2575) and one ethanol consuming pig (No. 2579) on test for 6 wk.

tein synthesis caused by chronic ethanol ingestion. Our findings were consistent with those reported by Rubin *et al.* (7) who found a decrease in the rate of mitochondrial protein synthesis in rats chronically ingesting ethanol for 24 days. Although we found no decrease in rate of protein synthesis after 3 wk on test, the different species of mammal involved, as well as the mode of administration of ethanol, may have been responsible for the delay in effect of ethanol on hepatic tissue in miniature swine.

The decrease in the rate of protein synthesis observed in the 6 wk study was the result of damage due to chronic ethanol ingestion and not to the continued presence of ethanol in tissue. Swine were sacrificed 6–12 hr after their last exposure to ethanol. Furthermore, mitochondria prepared for protein synthesis assays were washed routinely four times before being incubated in assay medium.

SDS-gel electrophoresis profiles of hepatic mitochondrial membranes and proteolipid protein from ethanol consuming swine on test 6 wk revealed a different labeling pattern than that of controls. In both whole membrane and proteolipid fractions, there was a selective decrease in labeling of the large molecular weight peak (approximately 40,000 mol wt), while the remaining peaks in both fractions remained unaffected. It might also be added that both light and electron microscopic examination revealed no discernable damage to liver tissue or liver mitochondria in either the control or ethanol consuming swine. The cause of this selective decrease in the labeling pattern is unknown and is the subject of further research in our laboratory.

Summary. The effect of chronic ethanol ingestion on hepatic mitochondrial protein synthesis in miniature swine was investigated. After 3 wk on test, there was no decrease in the rate of amino acid incorporation between livers of control and ethanol consuming pigs. After 6 wk on test, there was a 33% decrease in rate of hepatic mitochondrial membrane protein synthesis in livers of experimental pigs, as compared with controls. When analyzed by SDS-gel electrophoresis, a decrease in labeling of the 40,000 mol wt peak, from both whole membrane protein and proteolipid protein profiles, was observed for livers from ethanol consuming pigs as compared with controls.

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