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THE EFFECT OF ETHANOL ON ENZYME SYNTHESIS AND SECRETION IN ISOLATED RAT PANCREATIC LOBULES

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Abstract—This study investigates the effect of ethanol on enzyme synthesis and secretion in rat pancreatic lobules. Ethanol caused a dose-dependent inhibition of ^{3}H-leucine incorporation into total protein. Examination of the time dependence showed that ethanol inhibited protein synthesis at each time point. Removal of ethanol partially reversed this inhibition. An autoradiograph of the newly synthesized proteins separated on SDS-PAGE showed that ethanol inhibited synthesis of all proteins. ^{14}C-cycloleucine uptake was not altered by ethanol, excluding inhibition of amino acid uptake as the mechanism for the decreased protein synthesis induced by ethanol. Electron microscopy revealed no ultrastructural damage. Ethanol had no effect on the stimulated release of (i) amylase from zymogen granules nor (ii) newly synthesized pulse labelled enzymes.

Acetaldehyde had no inhibitory effect on enzyme synthesis or secretion indicating that ethanol *per se* and not its metabolite is inhibitory.

The decreased synthesis after acute exposure to ethanol with preservation of exocytosis would limit the autodigestive potential of pancreatic tissue. This may explain why isolated toxic doses of ethanol are rarely if ever associated with pancreatitis.

There is strong clinical evidence implicating chronic ethanol abuse as an aetiological factor in both acute and chronic pancreatitis [1]. However, potentially fatal serum levels of ethanol (170–340 mM) that follow the acute ingestion of large amounts of ethanol are not usually associated with pancreatitis [2–4].

Depending on the conditions and animal model ethanol has been variously shown to increase [5–14], decrease [5–7, 15–23], or have no effect [11, 13, 19, 20, 24–26] on pancreatic secretion. Some of the differences may be related to species, the route of alcohol administration, the blood level achieved, hormonal influence, charges in blood flow, duration of study and whether the studies were performed *in vivo* or *in vitro*. However, pancreatic secretion itself is a composite process involving exocytosis of enzymes stored in zymogen granules and secretion of newly synthesized protein. Previous studies have not distinguished between these two components.

The acute effect of ethanol on pancreatic protein synthesis has received little attention. Two previous studies have shown ethanol to have no effect on pancreatic protein synthesis. In one of these studies only a low concentration of ethanol (50 mM) was used in the incubation medium [27] while in the other the blood ethanol levels achieved were not stated [28].

The aim of this study using rat pancreatic lobules was to investigate *in vitro* the acute effects of ethanol on (i) enzyme synthesis and (ii) exocytosis of preformed enzyme from zymogen granule and newly synthesized pulse labelled protein.

MATERIALS AND METHODS

Female Sprague–Dawley rats 250–300 g, fed on a regular Chow diet, were fasted for 24 hr prior to sacrifice, the pancreas excised and pancreatic lobules prepared as described by Scheele and Palade [29]. This involved injecting buffer into the gland, separating the connective tissue layers to allow excision of lobules with fine scissors. Lobules weighted 6–8 mg and from 10 to 12 lobules were incubated in each vial.

Protein synthesis studies. Pancreatic lobules were incubated in Krebs–Ringer Hepes buffer, pH 7.4 containing 1% BSA, 0.4 mM amino acid mixture, ^{3}H-leucine ± ethanol or acetaldehyde in 100% O_2 atmosphere at 37°. ^{3}H-leucine incorporation into total protein was linear for 2.5 hr (Fig. 1). After a 2 hr incubation the lobules were homogenized in ice-cold saline using an ultra turrex homogenizer and then sonicated. After three trichloroacetic acid (TCA) washes the protein precipitate was dissolved in 1 ml of 1 M hyamine hydroxide, neutralized with

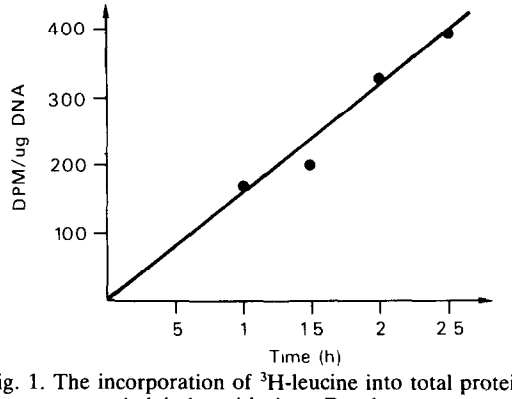

Fig. 1. The incorporation of ^{3}H-leucine into total protein in rat pancreatic lobules with time. Results are expressed DPM/μg DNA for one animal.

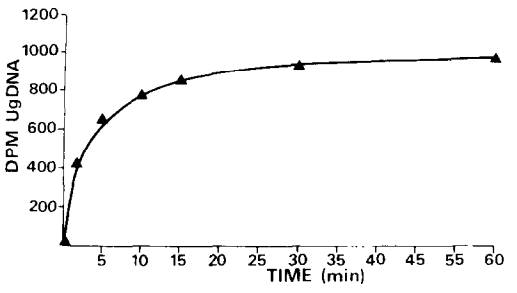

Fig. 2. The uptake of ^{14}C-cycloleucine into rat pancreatic lobules (DPM/μg DNA) with time. Line of best fit determined by a linear regression analysis of 2 experiments (r = 0.95).

1 ml of 1 M hydrochloric acid and scintillant added. Total radioactivity was measured in a Beta counter and appropriate quench corrections made.

Amino acid uptake experiments. ^{14}C cycloleucine a non-metabolizable amino acid (0.5 μCi/ml) was used to study the effect of ethanol (680 mM) on amino acid uptake. Initial experiments showed rapid initial uptake which plateaued at 30 min (Fig. 2). Cold leucine 4 mM and 40 mM inhibited ^{14}C cycloleucine uptake by 25% and 52% respectively. In subsequent experiments a 2 min and 60 min incubation in buffer containing 0.4 mM amino acid, ^{14}C cycloleucine (0.5 μCi/ml) ± ethanol (680 mM) were used to study rate of uptake and uptake capacity respectively. The reaction was terminated by washing with 50 ml of ice-cold saline. Additional uptake experiments were performed after preincubating the lobules in ethanol (680 mM) or buffer for 10 min prior to the 2 min incubation as above. The lobules were homogenized and radio-activity expressed per μg of DNA. DNA was determined by a direct technique using the Hoechst Reagent [30]. The extracellular volume of pancreatic lobules incubated in buffer ± ethanol (680 mM) was estimated using ^{14}C sucrose. At 10, 30 and 60 min there was no significant difference between ethanol and control.

Pulse labelling experiments. Lobules were incubated with ^{3}H-leucine for 3 min, washed 3 times in buffer containing excess cold leucine and transferred to the final incubation medium [31]. Cholecystokinin 0.5 U/ml ± ethanol or acetaldehyde was added to the medium and incubated for 1.5 hr.

Amylase activity in the medium and homogenate was measured using (starch polymer) Phadebas tablets [32]). Four millitres of distilled water was added to 200 μl of sample diluted in buffer ($CaCl_2$ 20 mM NaCl 0.9% BSA 2%) then preincubated for 5 min in a water bath at 37°. A Phadebas tablet was added, the tubes vortexed and then incubated in a water bath for exactly 15 min. The reaction was terminated by the addition of 1 ml sodium hydroxide (0.5 M) and then the tubes centrifuged at 4000 r.p.m. for 5 min. The absorbance of the clear blue supernatant was read in a spectrophotometer (Gilford 500 T) at 620 nM after settling the reading to zero with a blank.

Autoradiographic studies. SDS-PAGE [33, 34] was performed on lobules incubated in medium containing ^{3}H-leucine and cold leucine (0.04 mM). A TCA precipitate of the homogenate was dissolved in SDS sample buffer containing mercaptoethanol and run with standards of known molecular weight on a gradient gel 5–20%. After fixing and drying the gel was autoradiographed.

Ultrastructural studies

After a 2 hr incubation ± alcohol (680 mM) pancreatic lobules were immediately placed in 2.5% glutaraldehyde and cacodylate buffer, pH 7.3 for 1 hr at room temperature and washed for a further 30 min in cacodylate buffer. The tissue was post fixed in 2% osmium tetroxide in cacodylate for 30 min, washed and block stained in 2% uranyl acetate for a further 20 min, dehydrated through an alcohol series and then embedded in Spurr's resin. Pale gold sections cut onto collodion coated 200 mesh copper grids stained with lead citrate were viewed in a JEM 100 B electron microscope at 80 kV.

Materials

L (4–5^{3}H) Leucine (Amersham)

1-Amino cyclopentane-1-(^{14}C) carboxylic acid (cycloleucine) was supplied by (New England Nuclear).

Cholecystokinin (33 Amino Acid) was supplied by Sigma.

Phadebas Tablets were supplied by Pharmacia Diagnostics Uppsala Sweden.

Scintillant—(Toulene, 1332 ml; Triton X-100, 666 ml; PPO, 11 gm; Dimethyl POPOP, 1 g).

RESULTS

Ethanol caused a dose dependent inhibition of ^{3}H-leucine incorporation into rat pancreatic lobules (Fig. 3). Examination of the time dependence showed that ethanol inhibited protein synthesis at each time point (Fig. 4). Similarly preincubation in ethanol (680 mM) before the addition of the ^{3}H-leucine decreased synthesis compared to lobules preincubated in buffer alone (Table 1). The inhibitory effect was found to be partly reversible in that lobules incubated in buffer alone, after preincubation in ethanol, showed increased synthesis over those exposed to alcohol in both periods (Table 1).

To explore the mechanism of the inhibition the effect of ethanol on amino acid uptake was studied. A non-metabolized amino acid ^{14}C cycloleucine was quantitated in the homogenate of pancreatic lobules after a 2 min and 60 min incubation (Table 2). However, ethanol did not affect the rate of uptake or uptake capacity of the amino acid (Table 2).

In keeping with many *in vitro* studies we also observed that cholecystokinin inhibited enzyme synthesis. Cholecystokinin 0.005 U/ml and 0.5 U/ml inhibited protein synthesis by 10% and 40% respectively ($P < 0.01$ paired t-test). Autoradiographic studies were performed to see if the inhibitory effects of alcohol and cholecystokinin were generalized or specific to a particular protein. Ethanol and cholecystokinin inhibited new synthesis of all proteins (including enzymes) (Fig. 5).

Ethanol had no effect on the release of preformed enzyme (amylase—Table 3), newly synthesized protein (pulse labelled with ^{3}H-leucine—Table 3) nor basal amylase release (results not shown).

Table 1. The effect of ethanol preincubation on the incorporation of ^{3}H-leucine into total protein in rat pancreatic lobules

A	B	C
156.7 ± 48.3 DPM/μg DNA* ± SD	86.7 ± 12.9 DPM/μg/DNA ± SD	166.3 ± 52.1 DPM/μg DNA† ± SD

A. Lobules were preincubated in buffer for 30 min then incubated in buffer containing ^{3}H-leucine and ethanol (680 mM) for 1 hr.
B. Lobules were preincubated in ethanol (680 mM) for 30 min then incubated in buffer containing ^{3}H-leucine and ethanol (680 mM) for 1 hr.
C. Lobules were preincubated in ethanol (680 mM) for 30 min washed and then incubated in buffer containing ^{3}H-leucine without ethanol for 1 hr.

Four animals were studied.

* $P < 0.05$ AB (paired t-test).
† $P < 0.05$ CB (paired t-test).

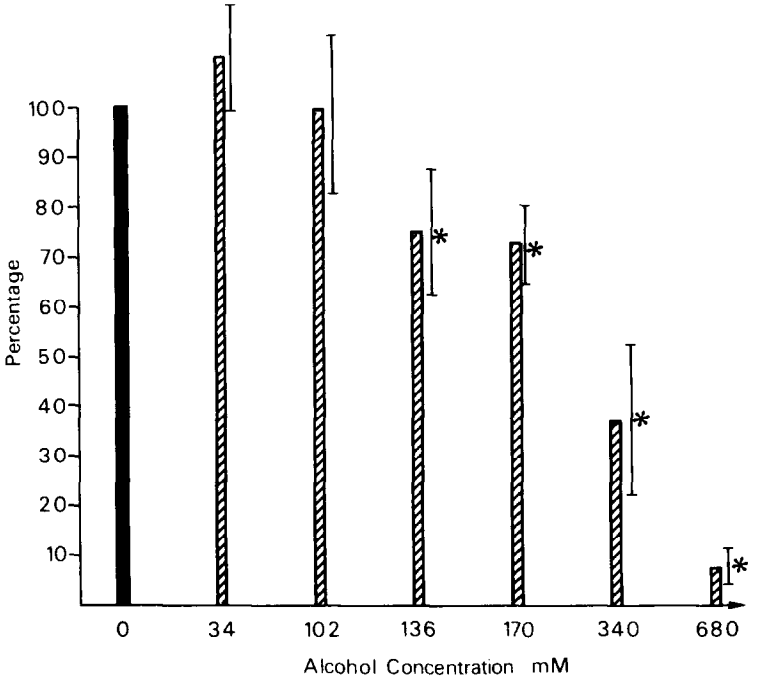

Fig. 3. The effect of increasing concentrations of ethanol on the incorporation of ^{3}H-leucine into total protein in rat pancreatic lobules expressed as a percent of control (100%) ± SD. * Denotes significant inhibition of synthesis by ethanol ($P < 0.01$) paired t-test. Incubation period 2 hr.

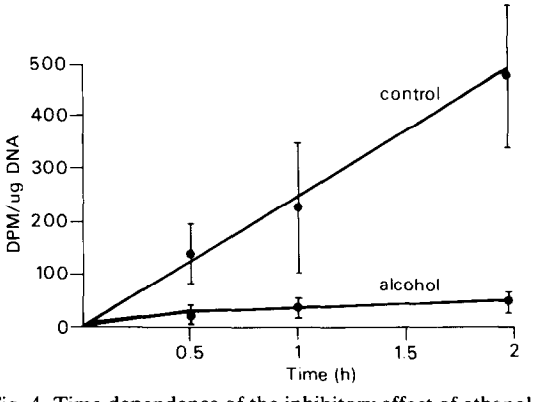

Fig. 4. Time dependence of the inhibitory effect of ethanol (680 mM) on the incorporation of ^{3}H-leucine into total protein in rat pancreatic lobules. (N = 3; mean ± SD).

Table 2. The effect of ethanol on amino acid uptake (^{14}C-cycloleucine) in rat pancreatic lobules

	Control DPM/μg DNA ± SD	Ethanol (680 mM) DPM/μg DNA ± SD
A	324 ± 96	332 ± 29.5*
B	313 ± 50	357 ± 52*
C	817 ± 112	892 ± 198*

A. Lobules were incubated in ^{14}C-cycloleucine for 2 min.
B. Lobules preincubated in ethanol (680 mM) or buffer for 10 min then incubated as in A.
C. Lobules were incubated in ^{14}C-cycloleucine ± ethanol (680 mM) for 60 min.

Three animals were studied.

* Not significant paired t-test.

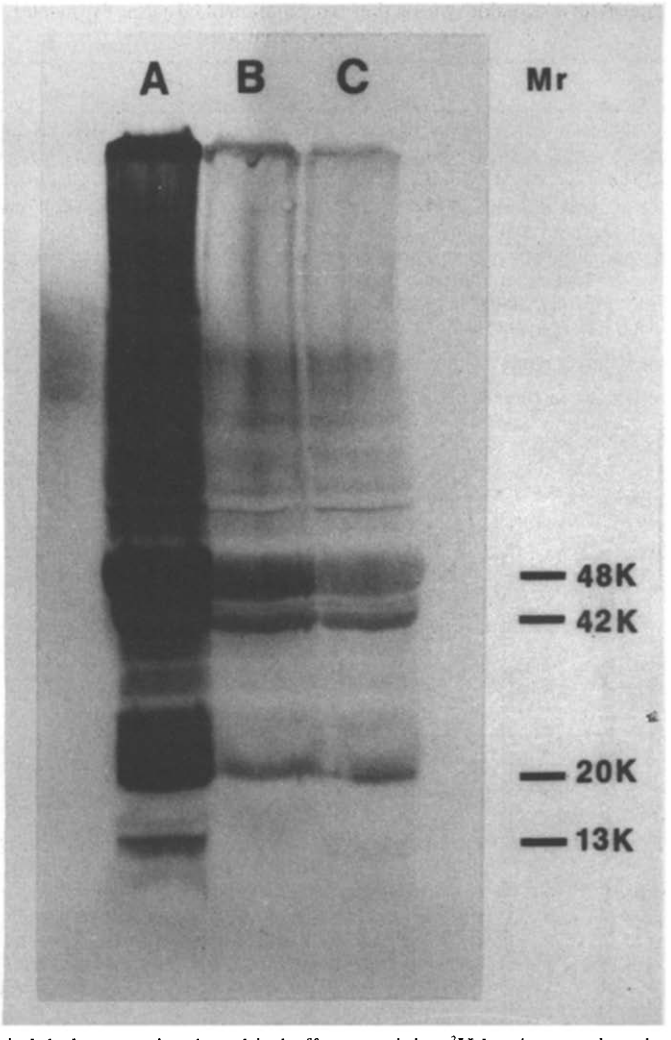

Fig. 5. Pancreatic lobules were incubated in buffer containing ^{3}H-leucine ± ethanol or CCK for 2 hr. A TCA precipitate of the homogenate was subjected to SDS-PAGE. Equal amounts of protein were loaded on each strip. Ethanol (340 mM) and CCK (0.5 U/ml) respectively caused a 75% and 55% inhibition of ^{3}H-leucine incorporation into total protein. The autoradiograph shows uniform inhibition of ^{3}H-leucine incorporation into all proteins. Lane A = control; lane B = CCK; lane C = ethanol.

Table 3. The effect of ethanol on the release of newly synthesized protein

Conditions	Amylase $\% \frac{M}{M+H}$	Pulse labelled $\% \frac{M}{M+H}$
Control	8.7 ± 1.5	1.3 ± 0.8
CCK	20.3 ± 2.2	8.6 ± 2.7
CCK + Eth. (10 mM)	19.5 ± 3.91*	9.4 ± 3.2*
CCK + Eth. (34 mM)	19.5 ± 2.8*	10.0 ± 4.3*
CCK + Eth. (68 mM)	20.9 ± 3.7*	9.1 ± 3.3*
CCK + Eth. (170 mM)	19.5 ± 2.6*	8.9 ± 3.6*
CCK + Eth. (340 mM)	19.3 ± 0.9*	8.4 ± 3.1*
CCK + Eth. (680 mM)	21.6 ± 3.1*	8.7 ± 3.5*
	(N = 4)	(N = 5)

Lobules were pulse labelled with ^{3}H-leucine and then incubated for 1.5 hr in medium containing CCK 0.5 U/ml. The radioactivity incorporated into total protein in the medium is expressed as a percentage of that in the medium (M) and homogenate (H)

$\% \frac{M}{M+H} \pm SD.$

CCK = cholecystokinin; Eth. = ethanol.
N = number of animals.
* Not significantly different from CCK alone (paired *t*-test).

Table 4. The effect of acetaldehyde on the incorporation of ^{3}H-leucine into total protein in rat pancreatic lobules

Control DPM/μg DNA	Acetaldehyde	DPM/μg DNA	N	Paired *t*-test
653 ± 84	0.07 mM	677 ± 58.9	3	NS
624 ± 181	0.18 mM	766 ± 228.1	3	NS
693 ± 67	1.06 mM	820 ± 72.3	4	$P < 0.02$
589 ± 77	2.12 mM	603 ± 101.3	4	NS

Incubation period 2 hr.
All experiments in duplicate.
N = number of animals studied.

Electronmicroscopy of pancreatic lobules incubated in ethanol (680 mM) showed no ultrastructural changes (Fig. 5).

As ethanol dehydrogenase activity has been detected in pancreatic tissue [27, 34, 35] the effect of acetaldehyde on synthesis and secretion was studied. Acetaldehyde neither inhibited protein synthesis (Table 4) nor secretion (Table 5). In fact acetaldehyde slightly increased synthesis; however, this increase was significant at only one concentration (Table 4).

DISCUSSION

In this study rat pancreatic lobules were incubated *in vitro* to study the direct effects of ethanol on (i) enzyme synthesis and (ii) exocytosis of preformed and nascent pulse labelled proteins.

A dose-dependent inhibition of protein synthesis was observed (Fig. 3); however, this inhibitory effect was observed only at high concentrations of ethanol. Similar results have been observed by other workers. Schreiber *et al.* [38] found that ethanol (330 mM) inhibited protein synthesis by 40% in isolated rat heart while Estival *et al.* [27] observed no effect on rat pancreatic protein synthesis *in vitro* with an ethanol concentration of 50 mM. Ethanol is known to alter membrane function [39] and in isolated liver studies a dose dependent decrease in amino acid uptake was observed [40]. However, in this study even at the ethanol concentrations which inhibited synthesis by 90% no change in amino acid uptake occurred (Table 2). That the inhibitory effect of ethanol and cholecystokinin on synthesis was common to all protein bands precludes a differential effect of ethanol on a particular enzyme class (Fig. 5). Liebow demonstrated reuptake of enzymes into pancreatic tissue [41] and inhibition of pancreatic enzyme synthesis [42]. As CCK stimulates enzyme release into the medium feedback inhibition might explain the decreased synthesis observed with this hormone (Fig. 5). However, as ethanol even at high concentrations did not alter the secretion of pancreatic enzyme into the incubation medium feedback inhibition would be unlikely to account for the decreased protein synthesis.

Solomon *et al.* [43] found that ethanol decreased the levels of ATP in rabbit pancreas incubated *in vitro*, an observation that could explain the decreased synthesis observed. However, exocytosis which is also an energy dependent step was not altered in this study although exocytosis may in rela-

Table 5. The effect of acetaldehyde on the secretion of amylase and newly synthesized protein

	Amylase $\% \frac{M}{M+H}$	Pulse labelled $\% \frac{M}{M+H}$
Control	7.6 + 1.5	0.9 + 0.4
CCK	20.5 + 3.2	7.7 ± 2.5
CCK + Ac (0.07 mM)	22.8 ± 4.8*	10.1 ± 3.4*
CCK + Ac (0.18 mM)	22.2 ± 3.2*	8.4 ± 4.6*
CCK + Ac (1.06 mM)	22.0 ± 4.2*	6.9 ± 2.8*
CCK + Ac (2.12 mM)	20.9 ± 5.9*	7.0 ± 2.9*
	(N = 5)	(N = 4)

Lobules were pulsed labelled with ^{3}H-leucine and then incubated for 1.5 hr in medium containing CCK 0.5 U/ml plus acetaldehyde. The radioactivity incorporated into total protein, and the amylase levels in the medium are expressed as a percent of that in the medium (M) and homogenate (H)

$$\% \frac{M}{M+H} \pm SD.$$

N = number of animals.
CCK = cholecystokinin; Ac = acetaldehyde.
* Not significantly different from CCK alone (paired *t*-test).

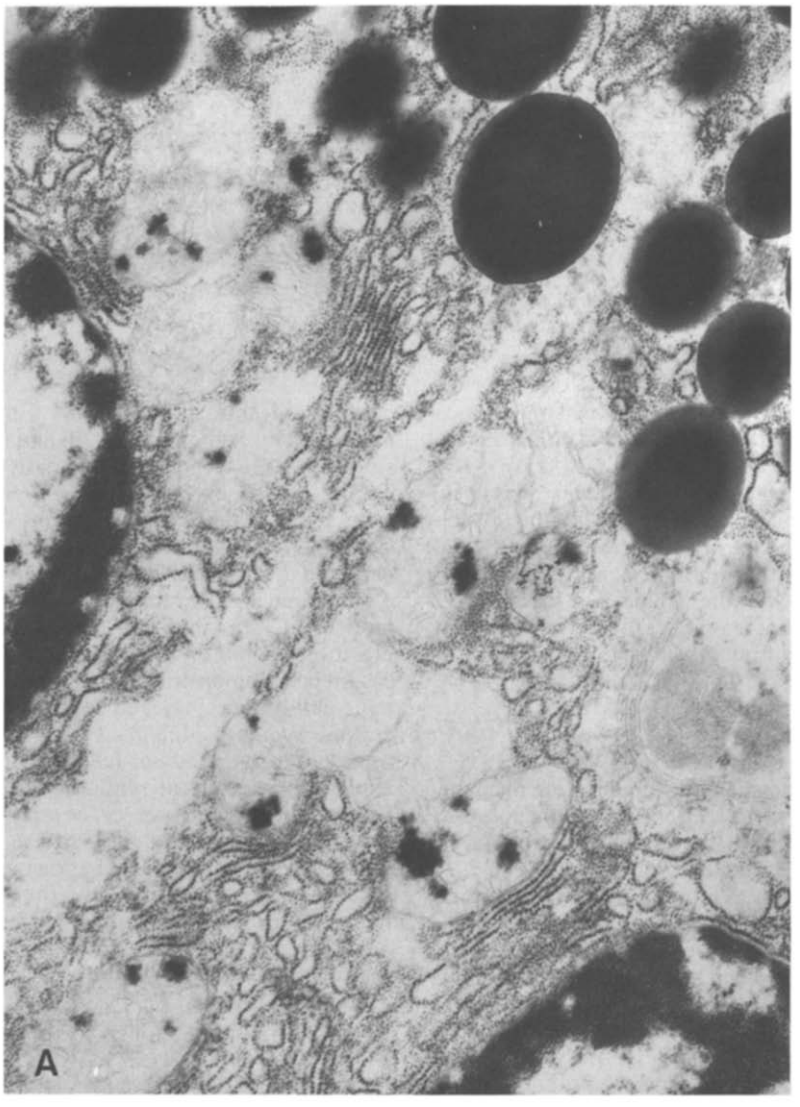

Fig. 6. (*continued on facing page*).

tive terms have a lower energy requirement. The exact mechanism by which ethanol inhibits synthesis remains therefore undefined although the preservation of exocytosis with normal amino uptake would be compatible with an inhibitory effect in the endoplasmic reticulum.

Electron microscopy on pancreatic lobules after incubation in ethanol showed no ultrastructural changes (Fig. 6) which is in keeping with the reversibility of the inhibitory effect of ethanol on synthesis. Morphological changes in mitochondria and endoplasmic reticulum have only been described after chronic ethanol intake [44, 45]. Alcohol dehydrogenase has been demonstrated in pancreatic tissue although at much lower concentrations than in liver [27, 34, 35]. Thus the inhibitory effect of ethanol may have been in part mediated by acetaldehyde. Blood ethanol concentrations of 200 mg/100 ml (44 mM) have been shown to result in acetaldehyde levels of 2.65 μg/ml (0.06 mM) in rats [46]. Based on this data acetaldehyde concentrations from 0.07 mM (3.1 μg/ml) to 2 mM (93.6 μg/ml) were used in this study. However, acetaldehyde neither inhibited pancreatic enzyme synthesis nor secretion (Tables 5 and 6). In fact at some concentrations there was a slight increase in synthesis (Table 5). Demol *et al.* [19] found that physiological concentrations of acetaldehyde 0.01 mM (4.6 μg/ml) had no effect on basal enzyme secretion in rats although pharmacological concentrations 1.5 mM (65.7 μg/ml) were inhibitory. Similarly, other workers found a pharmacological concentration of acetaldehyde 45 mM inhibited cholecystokinin and calcium ionophore (A23187)-stimulated secretion in pancreatic acini isolated from rats [23, 47].

In summary, ethanol caused a dose dependent inhibition of enzyme synthesis without effecting exocytosis of preformed or newly synthesized protein. This was a direct inhibitory effect of ethanol and non mediated via its metabolite acetaldehyde.

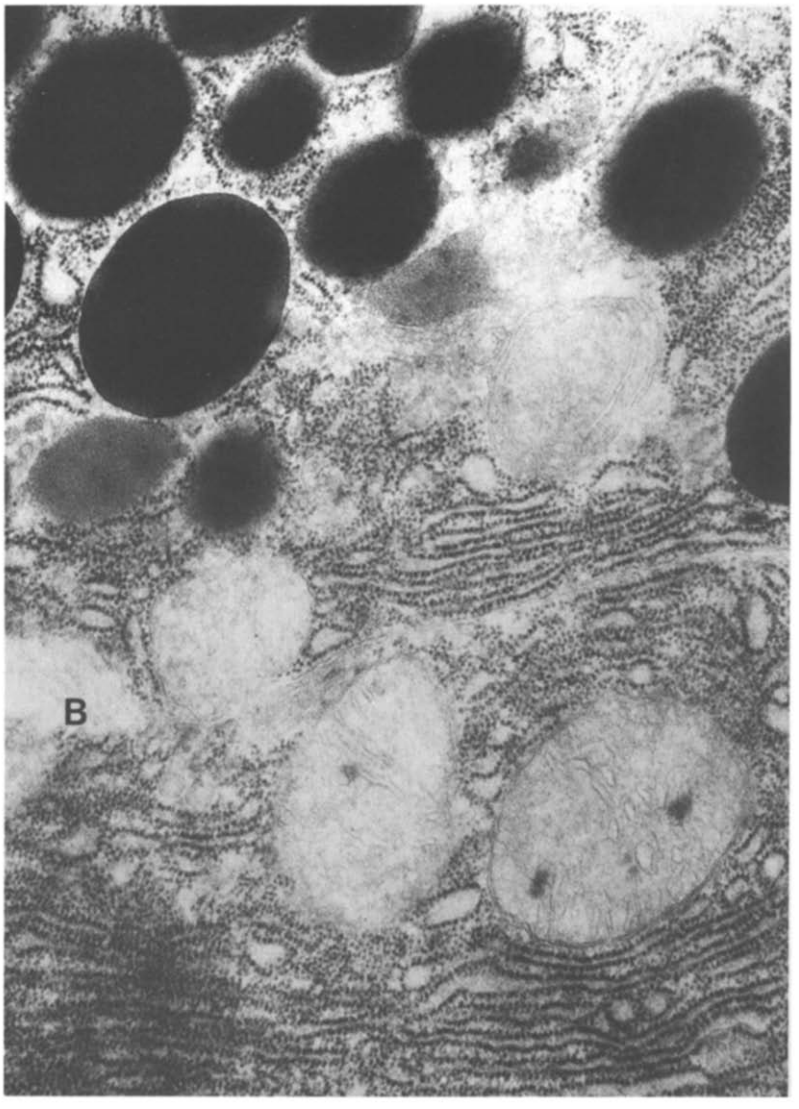

Fig. 6. Electron microscopic studies of pancreatic exocrine cells after incubation in buffer ± ethanol (680 mM) for 2 hr. No ultrastructural changes were observed with ethanol. A = control; B = ethanol. Magnification (×29,000)

Pancreatitis involves autodigestion of pancreatic tissue; however, the mechanism resulting in pancreatic enzyme activation *in situ* is not known. Chronic ethanol ingestion, an aetiological factor in pancreatitis, has been shown to increase the protease concentration of pancreatic juice and decrease the concentration of trypsin inhibitor [45, 48]. By contrast acute ethanol ingestion, even in amounts that produce toxic blood levels (170–340 mM), rarely if ever is associated with pancreatitis [2–4].

Decreased enzyme synthesis as observed in this study with preservation of exocytosis would thus limit the autodigestive potential of pancreatic tissue after acute alcohol intoxication.

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