Effect of ethanol on the structure and function of rabbit esophageal epithelium

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Bor, Serhat, Canan Caymaz-Bor, Nelia A. Tobey, Solange Abdulnour-Nakhoul, Esteban Marten, and Roy C. Orlando. Effect of ethanol on the structure and function of rabbit esophageal epithelium. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G819–G826, 1998.—Epidermologic studies indicate a relationship between alcohol consumption and esophageal epithelial disease. We therefore sought the contribution of the direct effects of ethanol on esophageal epithelial structure and (transport and barrier) function. Epithelium from the rabbit was mounted in Ussing chambers and exposed luminally for 1 h to 1–40% ethanol. At concentrations of 1–5% potential difference (PD) increased, and at 10–40% PD decreased. The increase in PD with 1–5% ethanol was accompanied by an increase in short-circuit current (Isc), and this increase in Isc could be blocked by ouabain pretreatment. The decrease in PD with 10–40% ethanol was associated with a decrease in electrical resistance (R), and this decrease in R was paralleled by an increase in transepithelial [14C]mannitol flux. Reversibility of these changes was limited at ethanol concentrations ≥10%, and these were associated morphologically by patchy or diffuse tissue edema. Moreover, as with ethanol exposure in vitro, exposure in vivo produced dose-dependent changes in PD, Isc, R, and morphology. These observations indicate that exposure to ethanol in concentrations and under conditions reflecting alcohol consumption in humans can alter and impair esophageal epithelial transport and barrier functions. Such impairments are likely to contribute to the observed increase in risk of esophageal disease with regular consumption of alcoholic beverages.

MATERIALS AND METHODS

In vitro studies. New Zealand White male rabbits weighing between 8 and 9 lb were killed by administering an intravenous overdose of pentobarbital sodium (60 mg/ml). The esophagus was excised, opened lengthwise, and pinned mucosal surface down in a paraffin tray containing ice-cold oxygenated normal Ringer solution. The submucosa was grasped with hemostats, lifted up, and dissected free of the underlying mucosa with a scalpel. This process yielded a sheet of tissue consisting of stratified squamous epithelium and a small amount of underlying connective tissue. From this tissue, four sections were cut and mounted as flat sheets between Lucite half-chambers with an aperture of 1.13 cm2 that permitted contact with different bathing solutions for the luminal and serosal side of the tissue. Tissues were initially bathed with normal Ringer solution composed of the following (in mmol/l): 140 Na+, 119.8 Cl−, 25 HCO3−, 1.2 Ca2+, 1.2 Mg2+, 2.4 HPO42−, 0.4 H2PO4− with osmolality 268 mosmol/kg H2O and pH 7.5 when gassed with 95% O2-5% CO2 at 37°C. Tissues were exposed to varying concentrations of luminal ethanol (1–40% vol/vol) by replacing different volumes of Ringer with variable amounts of 100% ethanol in a solution in which the ion composition resulted in final luminal ion concentrations equivalent to those of the normal Ringer serosal bath. This same method was used to expose tissues to varying concentrations of serosal ethanol; however, only the replacement of normal Ringer was from the serosal bath. The chemicals used were obtained from Sigma (St. Louis, MO).

Luminal and serosal solutions were connected to calomel and Ag-AgCl electrodes with Ringer-agar bridges for measurements of potential difference (PD) and short-circuit current (Isc) by means of a voltage clamp (World Precision Instruments, Sarasota, FL). Tissues were continuously short-circuit except for 5- to 10-s periods when the open-circuit PD was read. Electrical resistance (R) was calculated using Ohm’s Law (R = PD/Isc) from the open-circuit PD and the Isc or from the current deflection to imposed voltage. Forty-five minutes after mounting, to allow stabilization of electrical parameters (equilibration period), tissues paired by R (within 25% of each other and having R > 1,000 Ω·cm2) were exposed either luminally or serosally to ethanol (as described above). After ethanol exposure, both luminal and serosal solutions were drained and replaced with normal Ringer solutions (washout). PD, Isc, and R were monitored before and after ethanol exposure. After the experiments, tissues were fixed with 3% glutaraldehyde in 0.1 mol/l phosphate buffer, pH 7.4, stained with hematoxylin and eosin, and processed for light microscopy. Junction potentials were determined for

THE HUMAN ESOPHAGUS is frequently exposed to alcohol-containing beverages such as beer and wine and more distilled spirits such as whiskey. Given that these contain ethanol in concentrations ranging from 5% for beer to 10% for wine and 40% for whiskey (19a), it is likely and we hypothesize that one or more such exposures may result directly in esophageal epithelial structural and/or functional damage. Indeed, there is a known relationship between excess consumption of alcoholic beverages and diseases of the esophageal epithelium, most notably reflux esophagitis and esophageal carcinoma (3, 5, 8, 10–12, 19, 20, 22). Although the mechanisms for such a relationship are likely to be multifactorial, the contribution to epithelial disease of the direct effects of ethanol appear to be underestimated. For this reason, we sought the direct effects of ethanol on esophageal morphology and esophageal epithelial transport and barrier functions both in vitro and in vivo and utilized rabbit esophageal epithelium as a model, since it is known to be structurally and functionally similar to the epithelium lining the human esophagus (13). Moreover, to mimic human consumption of alcoholic beverages, in vivo experiments incorporated intermittent bolus administration of ethanol into the upper esophagus and adequate drainage from the distal esophagus to ensure effective (one-pass) luminal clearance.

resistance; transport; potential difference; short-circuit current; Ussing chambers

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all solutions by a modification of the method of Read and Fordtran (14) and Tobey and Orlando (18). Junction potentials were <1 mV for all experimental conditions except for 40% ethanol, which was -2 mV. Therefore, corrections for junction potentials were only made under conditions in which 40% ethanol was present.

Mannitol fluxes. Mannitol fluxes were performed in the Ussing chamber by luminal addition of 10 mmol/l of cold mannitol and 10 mCi of radiolabeled [14C]mannitol (ICN, Irvine, CA). An initial sample from the “hot” side was obtained for calculation of specific activity, and samples from the serosal solution were obtained at 45-min intervals for calculation of fluxes using the counts obtained from a liquid scintillation counter. The reported flux value was the average for two 45-min fluxes for each tissue.

In vivo studies. Rabbits were anesthetized intramuscularly with a mixture of ketamine (35 mg/kg) and xylazine (5 mg/kg) and positioned so that they were inclined with their heads up at a 45° angle (9). A tracheotomy was performed and, after laparotomy, a cannula for drainage was inserted 2 cm into the distal esophagus and tied in place. A small catheter was passed orally into the upper esophagus for administration via syringe of boluses of ethanol or Ringer solution. Alongside the oral catheter and extending 1 cm distal to its tip was a Ringer-agar bridge that was used for measuring the transepithelial PD. A reference Ringer-agar bridge was also placed through the laparotomy site into the peritoneum so that it made contact with peritoneal fluid. The Ringer-agar bridges were each inserted into a beaker containing both a saturated KCl solution and a calomel electrode, the latter for connecting the system to a voltage clamp (World Precision Instruments) for PD recording. The solutions that were separated into boluses were heated to 37°C before administration and before experimentation, and all esophagi were flushed with Ringer solution. After the Ringer wash, a baseline PD was recorded in each animal, and animals were then pulsed via syringe with 1 ml of either ethanol (10–40%) or normal Ringer solution. Boluses were administered over 5 s every 5 min for 30 min during the experimental period, and each liquid bolus was followed by air boluses until no further solution emerged from the drainage cannula. After the experimental period, three boluses of normal Ringer solution were administered in all animals before obtaining a final transepithelial PD, free from interference by junction, diffusion, and streaming potentials. Rabbits were killed at the end of the experiments with an intravenous overdose of pentobarbital sodium. Esophageal catheter, cannula, and Ringer-agar bridge positions were verified, and tissue sections were obtained from areas of epithelium exposed to the boluses for histology and for mounting in Ussing chambers. The tissue mounted in the Ussing chamber was permitted to equilibrate in Ringer solution for 20 min before a final PD, Isc, and R were recorded for each specimen.

All in vitro and in vivo studies were approved by the institutional Animal Welfare Committee. Statistical significance was determined using Student’s t-test. Data are reported as means ± SE.

RESULTS

In vitro studies. The effects of exposing esophageal epithelium to varying concentrations of luminal ethanol (1–40%) for 1 h are shown in Fig. 1, A–C. At the lowest concentrations of ethanol, 1 and 5%, esophageal PD increased and remained elevated throughout the 1-h time period. For the highest concentration of ethanol, 40%, esophageal PD decreased and remained below control values throughout the 1-h time period. At 10% ethanol, there was a delayed response in that the PD remained stable for the first 10 min and then, as with 40% ethanol, declined progressively (Fig. 1A).

The increase in PD observed with exposure to 1 and 5% ethanol was associated with increases in Isc (Fig. 1B).
B) and either no change (1% ethanol) or a decline in electrical R (5% ethanol; Fig. 1C). The changes in PD observed with exposure to 10% ethanol were associated initially with a transient rise in $I_{sc}$ that peaked at 10 min and then progressively declined over time. With 10% ethanol, R, as with 5% ethanol, declined progressively over time. The marked fall in PD at 40% ethanol was associated initially with significant declines in both $I_{sc}$ and R, and, while R continued to decline over time, at 10 min the $I_{sc}$ began a progressive rise to levels that exceeded baseline values at 40 min (Fig. 1, B and C). After exposure to 1 and 5% ethanol for 1 h, the changes in PD, $I_{sc}$, and R were unaccompanied by morphological damage on light microscopy; however, after 10 and 40% ethanol, there was patchy edema and generalized edema, respectively (Fig. 2, A-D).

Because exposure to all tested concentrations of ethanol altered esophageal epithelial function, the reversibility of the changes was assessed by removal of the ethanol-containing luminal baths and replacement with normal Ringer solution. As shown in Fig. 3B, by 1 h after washout, the reduction in R by 5% ethanol returned to baseline after exposures of 5–30 min but not after exposures lasting 60 min. Notably, the reduction in R upon exposure to 10% ethanol failed to recover to baseline, even with exposures lasting as little as 10 min, and recovery was impaired to a greater extent with exposures that were longer than 10 min. Similarly, exposure to 40% ethanol lowered R to an extent that it failed to recover even after exposure times of only 5 min (Fig. 3B). As shown in Fig. 3A, exposure to 5% ethanol increased $I_{sc}$, and this increase could recover to baseline (>100%) within 1 h of washout irrespective of the length of time of exposure, i.e., 5–60 min. However, the increase in $I_{sc}$ associated with exposure to 10% ethanol did not return to baseline, even with exposure times of only 5–10 min. Notably, the delayed increase in $I_{sc}$ associated with exposure to 40% ethanol (Fig. 1B) was followed after washout not only by the abolition of this increase but with the unmasking of an $I_{sc}$ that was almost abolished by 40% ethanol exposure (Fig. 3A).

In another set of experiments, we sought to determine if the increase in $I_{sc}$ associated with low concentrations of luminal ethanol was dependent on active (transcellular) Na$^{+}$ transport. This was done by pretreating tissues serosally for 1 h with ouabain (10$^{-4}$ M). After treatment with ouabain, $I_{sc}$ declined ~75%, in keeping

![Fig. 2](http://apgpj.physiology.org/)

**Fig. 2.** Representative light micrograph of rabbit esophageal epithelium exposed in Ussing chambers for 1 h to normal Ringer (A), 5% ethanol (B), 10% ethanol (C), and 40% ethanol (D). Hematoxylin and eosin stain; magnification $\times$400. Note that tissues exposed to 10% ethanol exhibit mild cell edema while those exposed to 40% ethanol show diffuse edema.
with effective inhibition of Na\(^+-\)K\(^+-\)ATPase activity. Subsequently, the increase in \(I_{sc}\) associated with exposure to 1% ethanol was shown to be effectively abolished (Fig. 4). In another set of experiments, we sought to determine if the observed decrease in \(R\) with ethanol exposure, an exposure associated with at worst tissue edema but not tissue necrosis, was the result of an increase in permeability via the paracellular (as opposed to transcellular) pathway. This was done by performing lumen-to-serosal [\(^{14}\)C]mannitol fluxes in tissues paired by \(R\) and exposed to either 10% ethanol or normal Ringer solution. As shown in Table 1, the reduction in \(R\) by 10% ethanol was accompanied by a significant increase in mannitol flux compared with the Ringer control.

The addition of ethanol to the luminal bath creates an environment that is hyperosmolar with respect to the serosal bath. Therefore, a set of experiments was performed to determine if this change in luminal osmolality contributes to the effects observed for ethanol. This was accomplished by exposing tissues to luminal solutions of similar osmolality (1,200 mosmol/kgH\(_2\)O), i.e., 6% ethanol or 1 M mannitol. Compared with luminal ethanol, which increased \(I_{sc}\) and decreased \(R\), hyperosmolar mannitol increased \(R\) and had little effect on \(I_{sc}\) (Fig. 5, A and B).

Because ethanol is a rapidly diffusible substance, the possibility was also considered that part or all of its luminal effects were mediated (after absorption) from the serosal side (1). This was tested by exposing esophageal epithelium for 1 h serosally to ethanol at concentrations ranging from 0.06 to 5%. The concentration of 5% ethanol was used because luminally it clearly increased \(I_{sc}\) and decreased \(R\), and the lower concentrations were used to reflect the achievable levels of blood ethanol in the alcohol-consuming live subject. As shown in Fig. 6, A and B, 5% serosal ethanol had the opposite effect of 5% luminal ethanol, i.e., it decreased \(I_{sc}\) by 60% and increased \(R\) by 50%, and, although the data are not shown, this same pattern was seen for the lower concentrations of ethanol in a dose-dependent manner.

In vivo studies. The prior chamber experiments were done in vitro and using continuous exposures to ethanol. Because this clearly is not reflective of what takes

![Fig. 3. Reversibility of \(I_{sc}\) (A) and \(R\) (B) of the changes in rabbit esophageal epithelium exposed luminally to 5, 10, or 40% ethanol (EtOH) for various time periods. Pretreatment baseline is indicated by the horizontal line, and values presented represent those achieved by 1 h after ethanol washout (ethanol replaced by normal Ringer). Values are means ± SE; nos. of rabbits are shown in bars; *\(P < 0.05\) compared with initial baseline values.](image)

![Fig. 4. Effect of ouabain on \(I_{sc}\) of rabbit esophageal epithelium mounted in Ussing chambers and its ability to inhibit the rise in \(I_{sc}\) associated with exposure to 1% ethanol. Values are means ± SE; \(n = 4\). *\(P < 0.05\) compared with preethanol \(I_{sc}\).](image)

**Table 1. Effect of luminal ethanol on electrical resistance and transepithelial mannitol flux in rabbit esophageal epithelium in Ussing chamber preparation**

<table>
<thead>
<tr>
<th></th>
<th>Resistance, (\Omega \cdot \text{cm}^2)</th>
<th>Mannitol Flux, (\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Rx</td>
<td>After Rx</td>
</tr>
<tr>
<td>Ethanol (10%)</td>
<td>2.671 ± 785</td>
<td>1.821 ± 68*</td>
</tr>
<tr>
<td>Control (Ringer)</td>
<td>2.543 ± 204</td>
<td>3.054 ± 275</td>
</tr>
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Values are means ± SE; \(n = 4\) rabbits in each group. Tissues with similar pretreatment electrical resistances (\(R\)) were exposed to ethanol or normal Ringer. During exposures, mannitol fluxes were performed, and the final \(R\) of the tissues was reported as posttreatment for each group. *\(P < 0.05\) compared with Ringer controls.
place in vivo, in vivo experiments were designed to
determine the extent to which the in vitro findings were
representative of what occurs under conditions more
representative of human alcohol consumption. The in
vivo experiments were performed in anesthetized rab-
bits that were placed on a 45° incline in the head-up
position. A cannula was inserted orally into the upper
esophagus for the intermittent administration of etha-
nol boluses, and the distal esophagus was cannulated
to permit efficient, one-pass drainage of the adminis-
tered boluses. One-milliliter boluses of ethanol, 10–
40%, were subsequently administered every 5 min for
30 min, and in control animals 1-ml boluses of normal
Ringer solution were administered. After each liquid
bolus, an air bolus was administered to ensure ade-
quate drainage, and after ethanol boluses were com-
plete, three boluses of normal Ringer were adminis-
tered to enable the transepithelial PD to be recorded
in the absence of any interference by junction, diffusion,
or streaming potentials.

Baseline esophageal PD was similar for all groups of
animals. As shown in Fig. 7, boluses of normal Ringer
solution had no effect on PD. However, boluses of 10% 
ethanol increased PD for the first 5–10 min, and then 
PD began to decline, falling to baseline by 30–35 min.
Notably, this pattern of PD change is similar to that 
observed in vitro with continuous exposure to 5% 
ethanol (Fig. 1A). Moreover, similar to continuous 
exposure to 10% ethanol in vitro, boluses of 20% 
ethanol in vivo initially produced no change in PD, but 
after 10 min PD progressively declined. In addition and 
in keeping with its noxious potency, in vivo boluses of 
40% ethanol, as with in vitro exposure to 40% ethanol, 
resulted in a rapid and progressive decline in PD with 
time, the PD in vivo being effectively abolished by 10
min (Fig. 7). After these experiments, tissue sections 
were mounted in Ussing chambers for determination of 
their electrical parameters. As is evident in Table 2, 
ethanol exposure in vivo was paralleled by a dose-
dependent lowering of R in vitro, with R reaching 
significance for tissues exposed intermittently in vivo

Fig. 5. Comparison of effects of an equihyperosmolar (1,200 mosmol/ 
kgH2O) luminal solution of mannitol (1 M) to 6% ethanol on Isc (A) 
and R (B) of rabbit esophageal epithelium mounted in Ussing 
chambers. Ethanol is noted to increase Isc and lower R, whereas 
mannitol has no effect on Isc and increases R. Values are means ± SE; 
n = 4 for each group. *P < 0.05 compared with ethanol.

Fig. 6. Effect of 5% serosal ethanol on Isc (A) and R (B) of rabbit 
esophageal epithelium. Serosal ethanol decreases Isc and increases R 
compared with concurrently studied Ringer controls. Data are ex-
pressed as % of initial values before ethanol exposure. Values are 
means ± SE; n = 5. *P < 0.05 compared with normal Ringer controls.
to ethanol resulted, even at low concentrations, in alterations in ion transport and/or barrier function, whereas at higher concentrations such alterations were also associated with morphological changes. The effect of ethanol on ion transport was documented by increases in $I_{\text{sc}}$, both during and, for 1 and 5% ethanol, after ethanol exposure. Moreover, as shown for 1% ethanol, the increase in $I_{\text{sc}}$ associated with ethanol was abolished by pretreatment with ouabain. This suggests that, at low concentrations, ethanol has a stimulatory effect on active ion (presumably Na$^+$) transport in this predominantly Na$^+$-transporting epithelium) transport in this tissue. Such an effect, however, is not observed in all moist squamous epithelium, since it has been reported that mucosal ethanol (2–6%) inhibited rather than stimulated $I_{\text{sc}}$, a reflection of net Na$^+$ transport, in frog skin (2). Similar to the lower concentrations of ethanol, 10% ethanol initially increased $I_{\text{sc}}$, but this was transient, and after 10 min $I_{\text{sc}}$ began to decline. Moreover, at 40% ethanol, the initial effect on $I_{\text{sc}}$ was inhibitory. However, this gave way after 10 min to a dramatic rise in $I_{\text{sc}}$ to levels that rose above pretreatment baseline, a rise that was completely abolished after ethanol removal, unmasking a marked inhibition of $I_{\text{sc}}$ by 40% ethanol. These observations are compatible with the fact that at low concentrations ethanol stimulates active transport and that at high concentrations (10–40%) ethanol inhibits active transport. The rise in $I_{\text{sc}}$ during, but not after, exposure to 40% ethanol is compatible with the generation of diffusion and/or streaming potentials induced by damage to the tissue at this high concentration. In keeping with this latter interpretation was the presence of generalized tissue edema in the specimens exposed to 40% ethanol. Yorio and Bentley (21) have also observed the toxic effects of 40% ethanol in toad bladder, finding that, at 40% but not at lower concentrations, there was marked inhibition of oxygen consumption (21).

The experiments in vitro also showed a detrimental effect of continuous exposure to ethanol on esophageal epithelial barrier function. This was apparent in that concentrations of ethanol >1% were accompanied by a dose-dependent decline in electrical $R$. Moreover, because the decline in $R$ could occur even at concentrations having minor effects on esophageal morphology (5–10% ethanol), it was likely that this damaging effect on the barrier resulted from an alteration of the intercellular junctions. Consistent with this was the finding that the decline in $R$ associated with exposure to 10% ethanol resulted in an increase in transepithelial mannitol flux, this increase reflecting an increase in paracellular permeability. Although these data indicate that at lower concentrations the barrier-breaking effects of ethanol are the result of altered intercellular junctions, at the highest concentration studied (40%) there was enough morphological damage to suggest that the increase in epithelial permeability may include both transcellular and paracellular routes. In keeping with these conclusions, Yorio and Bentley (21) observed an increase in paracellular permeability in toad bladders exposed to 9% ethanol. Boyett and Brug-
ges (2) also noted an increase in permeability to chloride when frog skin was mucosally exposed to 2.4% ethanol; however, the route for this increase was not established in their study (2).

The mechanisms by which ethanol alters esophageal epithelial barrier and transport physiology are complex and largely unstudied. However, the present results showing alterations in esophageal epithelial $I_{\text{sc}}$ and $R$ are consistent with a direct effect of ethanol on both the cells themselves and their intercellular junctional complexes. Moreover, the inability of ethanol exposure serosally or 1 M mannitol luminally to mimic the changes produced by luminal ethanol indicates that such effects are independent of both ethanol absorption and the capacity of ethanol to increase luminal osmolality.

Because it is clear that the effects of continuous ethanol exposure in vitro may not be representative of what occurs in humans consuming alcoholic beverages in vivo, an additional set of in vivo experiments was performed. These experiments mimicked human consumption by intermittently giving ethanol as boluses and by ensuring one-pass esophageal exposure through effective bolus clearance. The results showed that intermittent exposure to ethanol in vivo mimicked the effects of continuous exposure to ethanol in vitro but required somewhat higher concentrations. Thus, for example, the effects of 10% ethanol in vivo mimicked the effects on PD, $I_{\text{sc}}$, and $R$ of continuous ethanol exposure to 5% ethanol in vitro, and the effects of 20% ethanol in vivo mimicked the effects of 10% ethanol in vitro. Furthermore, 40% ethanol in vivo, presumably because of the great magnitude of its toxicity, resulted in the same effects as that observed in vitro. Specifically, ethanol exposure in vivo initially produced a biphasic dose-dependent effect on PD, with the lowest concentration, 10% ethanol, increasing PD, 20% ethanol producing no change, and the highest concentration (40%) decreasing PD. Together these observations indicate that in vivo higher concentrations of ethanol are required to achieve the same effects as described above for lower concentrations of ethanol in vitro, yet it is important to recognize that, even though requiring higher concentrations, the concentrations remain well within the range of those to which the human esophagus is exposed. The likely reason that higher concentrations in vivo are required to give the same effects as lower concentrations in vitro is that efficient luminal clearance left less ethanol to diffuse into the tissue, and the ethanol that does diffuse is effectively reduced in potency by dilution within the luminal and epithelial aqueous environments.

After the tissues were exposed to ethanol in vivo, they were mounted in Ussing chambers or sent for morphology. Tissues mounted in the chamber demonstrated that the changes in PD induced in vivo were the result of altered ion transport and/or barrier properties of the esophageal epithelium. Specifically, impairment of barrier function was evident in that a dose-dependent decline in $R$ was associated with ethanol exposure, and altered ion transport was evident in that the lower concentrations of ethanol (10 and 20%) were observed to increase $I_{\text{sc}}$ and the higher concentration (40%) to inhibit $I_{\text{sc}}$. Further tissue edema was apparent in those esophagi intermittently exposed to 20 or 40% ethanol in vivo. Consequently, all of the effects observed for continuous ethanol exposure in vitro were observed in vivo. Consistent with our findings were those of Rubinstein and colleagues (15) who observed a significant reduction in esophageal PD in humans in vivo after consumption of wine and whiskey, and prior studies in rabbit or canine esophagus (4, 16, 17) showed that luminal concentrations as low as 5% ethanol can alter esophageal PD and increase epithelial permeability.

In summary, the results of this investigation document that esophageal exposure to ethanol in amounts and under conditions relevant to the consumption of
alcohol in humans can significantly alter both esophageal morphology and esophageal epithelial transport and barrier function. Furthermore, because the impaired functions are important for epithelial defense (13), these initial changes likely predispose the tissue to subsequent injury upon exposure to other potentially noxious luminal substances, e.g., refluxed gastric acid. With respect to the latter, it is well established that the combination of acid and ethanol is far more injurious to the esophagus than either substance alone (4, 16, 17).

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