to examine the immunologic properties of the Non-TBI. Anti-horse spleen ferritin failed to cross-react with fractions of pig liver or duodenal mucosa which presumably contained ferritin. However, ferritin is the only iron-containing compound of animal tissue origin known to have the properties which characterize the Non-TBI.

Ferritinemia is known to occur in human subjects with severe hepatocellular disease(7). However, clinical, biochemical and histologic evidence of liver disease in the pigs in this and in earlier studies(4,8) has been limited to centrilobular fatty infiltration. Therefore, it seems unlikely that in these animals the ferritinemia is secondary to liver disease.

A defect in heme synthesis is known to be present in pyridoxine deficiency. This is associated with a large non-heme iron pool in circulating erythrocytes(9). It is interesting to speculate that a red cell-to-plasma ferritin pathway may be present in such animals and account for the presence of the Non-TBI fraction in plasma. Since large iron pools are present in normoblasts, liver and other tissues, these may contribute to the plasma Non-TBI pool as well.

The demonstration of two functionally and perhaps kinetically distinct iron compartments (TBI and Non-TBI) in plasma will modify considerably the interpretation of ferrokinetic data in pyridoxine-deficient animals(10) since such calculations are based on the assumption of a single transferrin-bound plasma iron pool.

Summary. The hypersideremia which developed in swine deficient in pyridoxine was found to be due to the presence in plasma of non-transferrin-bound iron as well as to an increase in transferrin-bound iron. The non-transferrin-bound iron fraction was adsorbed on magnesium carbonate, not precipitated by exposure to 65°C for 5 minutes, not transferable to apotransferrin, and excluded by Sephadex gel G-200 (mol. wt. greater than 200,000). It is suggested that the non-transferrin-bound iron was present as ferritin.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.

Ethanol Damage to Canine Oxyntic Glandular Mucosa.* (32532)

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The purpose of the work reported here was to determine the threshold concentration at which ethanol damages the oxyntic glandular area of the canine gastric mucosa, to discover whether ethanol is more damaging in acid than in neutral solution, to measure the rate of ethanol absorption over a wide range of concentrations, and to distinguish between effects of a high concentration applied to the mucosa for a short time and those of a lower concentration applied for a longer time. Changes in the barrier function of the mucosa (1,2) were used to assess the damaging effects of ethanol.

In the course of this work, acid secretion

* Supported by USPHS grant AM-08716.

was found to be stimulated by applications of ethanol to the oxyntic glandular mucosa. Absorbed or intravenously administered ethanol stimulates secretion, probably by releasing gastrin from the pyloric glandular mucosa(3). It is possible that topical ethanol stimulates secretion by releasing histamine within the oxyntic glandular mucosa(4). An attempt was made to discover whether ethanol does release histamine from the oxyntic glandular mucosa.

Methods. The dogs, the method of irrigating their pouches, and the analytical methods have been described in detail(1,2). Briefly, 3 unanesthetized dogs in good health, each having a separated vagally denervated (Heidenhain) pouch of the oxyntic glandular area of the stomach were used. The dogs were deprived of food but not of water 18-20 hours before use. At the beginning of each experiment the pouch was thoroughly washed with a solution of 100 mM HCl plus 54 mM NaCl. The pouch was emptied, leaving in it a known residual volume between 3.0 and 5.3 ml. Forty ml of the same solution was placed in the pouch, thoroughly mixed and sampled, leaving an initial volume between 33 and 36 ml in the pouch. Thirty minutes later the fluid was removed for analysis, again leaving a known residual volume. The initial masses of Na^+, K^+ and H^+ in the pouch calculated by multiplying the initial volume by the analytically determined concentrations, were substracted from the final masses, and the results in microequivalents per 30 minutes were taken to be the control fluxes of the ions across the undamaged mucosa. The fluxes of Na^+ and K^+ were always positive, indicating net movement from animal to luminal fluid, and net flux of H^+ in the control periods was usually negative. The fact that the control values always fell within the control ranges established over many months of work with these dogs showed that the mucosa had recovered from any damage inflicted in previous experiments.

The pouch was then rinsed once with a solution of ethanol in 100 mM HCl plus 54 mM NaCl and emptied. Forty ml of the same ethanol solution was placed in the pouch, mixed and sampled. Thirty minutes later the fluid was removed, measured and analysed. The pouch was rinsed with the 100 mM HCl-54 mM NaCl solution, and it was filled once more with 40 ml of this solution which was mixed and sampled. At the end of 30 minutes the fluid was removed, measured and analysed. The fluxes of Na^+, K^+ and H^+ found in the first control period were subtracted from those found in the second and third periods, giving *changes* in fluxes occurring during or after ethanol application to the mucosa. Positive values mean that more Na^+ entered or less H^+ left the lumen during the second and third periods than entered or left during the control period, and negative values mean that less Na^+ entered or more H^+ left the lumen during the second and third periods than entered or left during the control period. Variations on this protocol are described in the appropriate part of *Results.*

Na^+ and K^+ were measured by internal standard flame photometry, and H^+ was determined by titration. Ethanol was measured by the alcohol-dehydrogenase-DPN method of Bonnichsen and Theorell(5, Sigma Chemical Co.). All ethanol concentrations are expressed as weights per 100 units volume (w/v). The concentrations used were approximately 0.5, 1.5, 5, 8, 14 and 27%. The exact concentration of a particular solution is specified below. For comparison a domestic beer, an imported cocktail sherry and a martini cocktail made of 4 parts gin and 1 part vermouth stirred over ice exactly 60 seconds before being decanted were analysed and found to contain 3.9, 16.5 and 25.1% ethanol respectively.

There were no systematic differences among dogs, and the data were pooled.

Results. Ethanol in 100 mM HCl. Changes in net fluxes of Na^+ and H^+ occurring during 30 minutes irrigation of the pouches with ethanol in 100 mM HCl are shown in Fig. 1. (Fluxes of K^+ are omitted, for they added nothing.) At 8.2% or less ethanol had no effect on net Na^+ flux. At 27% there was a large net positive flux of Na^+ and a corresponding large disappearance of H^+, both characteristic of damage to the mucosa(1,2). The effect of 14% was intermediate, and the threshold for damage must lie between 8.2 and 14%.

Ethanol in 30 mM phosphate buffer. Acetyl-

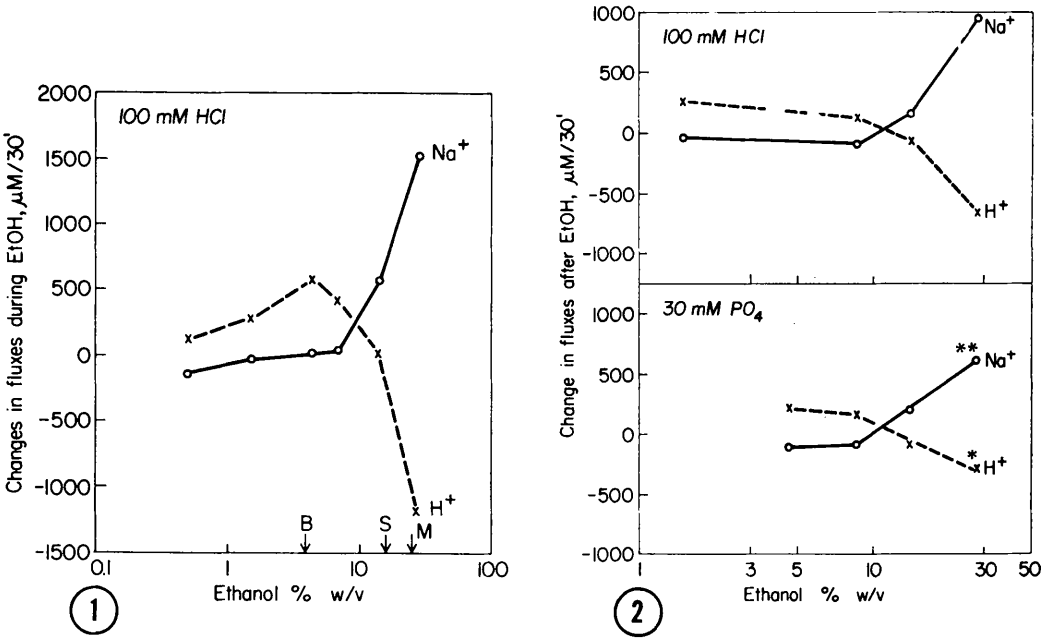
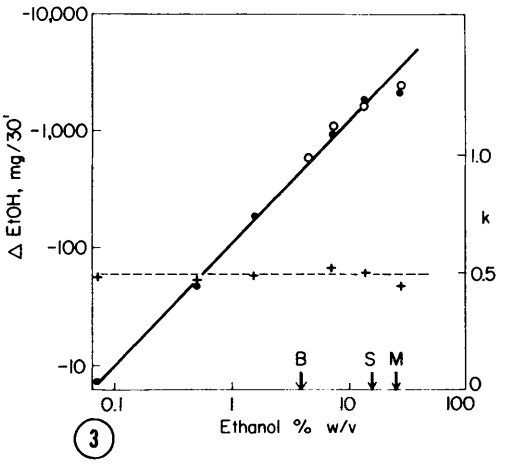


FIG. 1. Changes in fluxes of Na^+ and H^+ across the mucosa of the canine oxyntic glandular area during irrigation with solutions of ethanol in 100 mM HCl plus 54 mM NaCl. Points represent means of 3 or 4 observations using 3 dogs. B, S and M indicate ethanol concentrations of beer, sherry and a martini cocktail made of 4 parts gin and 1 part vermouth.

FIG. 2. Changes in fluxes of Na^+ and H^+ across the mucosa of the canine oxyntic glandular area following irrigation with solutions of ethanol in 30 mM phosphate buffer, pH 7.5. Points represents means of 3 or 4 observations using 3 dogs. The fluxes marked with asterisks are significantly different from corresponding ones when the mucosa was previously irrigated with ethanol in acid solution, $P<0.05$.

salicylic and salicylic acids in 100 mM HCl cause mucosal damage similar to that produced by 27% ethanol(6). However, salicylates in neutral solution have little if any effect upon the mucosa(7), probably because they are only slowly absorbed from solutions in which they are ionized(8). Absorption of ethanol is not pH-dependent, and ethanol might be expected to have the same effect in neutral as in acid solution. This was tested in experiments in which the pouch of each dog was irrigated for a 30 minute control period with a solution of 100 mM HCl plus 54 mM NaCl. The pouch was washed 3 times with 154 mM NaCl and once with a solution of ethanol in 30 mM sodium phosphate buffer, pH 7.5. The pouch was filled with 40 ml of the buffered ethanol solution which was mixed and sampled. After 30 min the solution was removed from the pouch, measured and analysed. Its pH was never less than 5.9. The pouch was washed 3 times with the 100 mM HCl-54 mM NaCl solution and tested for 30 minutes with the same solution. Changes in fluxes of Na^+ and H^+ after irrigation of the pouches with ethanol in phosphate buffer are compared in Fig. 2 with those observed after irrigation with ethanol in 100 mM HCl. Ethanol in concentrations up to 17% in neutral solution has the same effects as ethanol in acid solution. At 27% ethanol in phosphate buffer caused slightly less damage than the same concentration in acid.

Absorption of ethanol. The rates of absorption of ethanol from neutral and acid solutions are given in Fig. 3. In order to relate absorption to the mean concentration of ethanol in the pouch an absorption coefficient k was calculated from the equation $k = (\Delta EtOH)/\overline{[EtOH]}$. $\Delta EtOH$ is the rate of absorption in mg per min, and $\overline{[EtOH]}$ is the mean concentration of ethanol in the luminal fluid in mg per ml obtained by dividing the



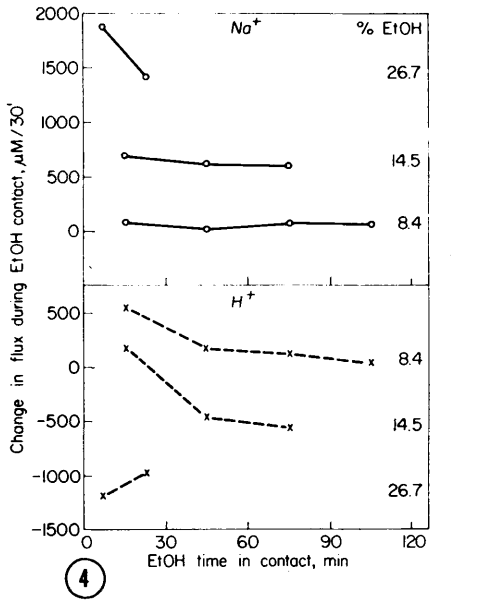


FIG. 3. Rates of absorption of ethanol from pouches of the oxyntic glandular area plotted against the initial concentration of ethanol in the fluid irrigating the pouch. The constant k (indicated by +) = $\Delta EtOH/\overline{[EtOH]}$ where $\Delta EtOH$ is rate of absorption and $\overline{[EtOH]}$ is mean concentration of ethanol in the pouch. At the highest concentrations k is significantly smaller than at other concentrations, p 0.05. Filled circles represent absorption from 100 mM HCl, and open circles represent absorption from 30 mM phosphate buffer, pH 7.5. Points represent mean values of from 3 to 18 observations using 3 dogs.

FIG. 4. Changes in fluxes of Na^+ and H^+ during irrigation of pouches of the canine oxyntic glandular mucosa with ethanol at concentrations shown at the right in 100 mM HCl. Each point represents the mean of 4 observations using 2 dogs.

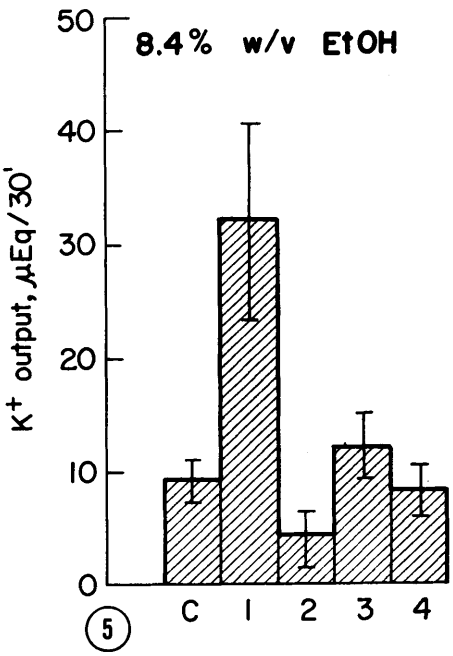
sum of initial and final concentrations by two. The units of k are ml per min. The results show that the rate of absorption over a very wide range is directly proportional to the luminal concentration. Only the constant at the highest concentration used was significantly lower than the other constants.

Time in contact with the mucosa. The fact that rate of absorption is a linear function of ethanol concentration in the lumen allows a distinction to be made between the effect of its concentration in contact with the mucosa and the effect of the amount absorbed through the mucosa. On two different occasions the mucosa of each of 2 dogs was tested by a 30 minute period of irrigation with the 100 mM HCl plus 54 mM NaCl solution. Then the pouch was irrigated for 4 successive 30 minute periods with 8.4% ethanol in acid solution. Changes in fluxes of Na^+ and H^+ were calculated, and they are presented in Fig. 4. There was no change in Na^+ flux, and the only change in H^+ flux was that the early positive flux, indicative of secretion of H^+, dropped off to zero. The experiments were repeated using 14.5% ethanol in acid solution for 3 periods following a control period. Throughout ethanol irrigation there was a steady, moderate net positive flux of Na^+ such as occurs in mild damage to the mucosa. The initial net positive flux of H^+ in the first ethanol period changed to moderate negative flux in subsequent ones. Damage was severe when 26.7% ethanol in acid was used in two 15 minute periods. Although more than twice as much ethanol is absorbed in 120 minutes from an 8.4% solution as is absorbed in 15 minutes from a 26.7% solution, the effects of the weaker solution are trivial compared with those of the stronger.

Stimulation of acid secretion. In 17 of 18 instances in which the pouch was irrigated with low concentrations of ethanol (8% or less) in acid solution, acid secretion was stimulated. The single exception was during irrigation with 0.6% ethanol. The fact that stimulation occurred when acid solutions were used demonstrates that secretion was not mediated by gastrin released from an antral fragment inadvertently left in the pouch, for gastrin release is inhibited by acid in contact with the mucosa(9).

Acid secretion during topical application of ethanol was transient. If irrigation was prolonged for 45 to 120 minutes, rates of acid secretion returned to control level in the latter part of the period. This tachyphylaxis was truly cessation of secretion, not an apparent reduction of H^+ output resulting from increased back diffusion of H^+ into the mucosa (10). The permeability of the mucosa as measured by concurrent Na^+ fluxes or by flux of H^+ immediately after removal of the ethanol solution was not increased. Output of K^+ is strongly and positively correlated with H^+ output(11), and K^+ output during prolonged irrigation with ethanol exhibited the same tachyphylaxis (Fig. 5).

Topical ethanol might stimulate by direct action upon the mucosa, and histamine might be the mediator(4). A transient burst of histamine release, similar to that found when the mucosa is damaged by acetic or salicylic acids(12), would account for the tachyphylaxis. To look for histamine release, an anesthetized dog was prepared for sampling of arterial and gastric venous blood by the method of Johnson and Overholt(12). A perforated tube was passed into the stomach by way of the duodenum, and the pylorus was tied so that gastric contents could not enter the small intestine. The stomach was washed out and then filled with 30 mM phosphate buffer, pH 7.5. After a 30 minute control period in which arterial and gastric venous blood samples were obtained the stomach was emptied and quickly filled with 14% ethanol in 30 mM phosphate buffer, pH 7.5, in the amount of 1% of body weight. Blood samples were taken and analyzed for ethanol and histamine. The histamine bioassay described by Code and McIntire(13) was used. This method is highly specific and detects less than 1 μg histamine per liter of blood. Although high concentration of ethanol was found in gastric venous blood within 30 seconds after ethanol was placed in the stomach (Fig. 6), no trace of histamine was found in any venous or arterial sample. A repetition of the experiment using 8% ethanol in phosphate buffer, a stimulating concentration, likewise gave totally negative results. These data confirm the conclusion of Irvine(14,15) that ethanol does not release detectable quantities of histamine from the stomach, but they do not prove that histamine is not the mediator. Johnson(16) gave intravenous infusions of



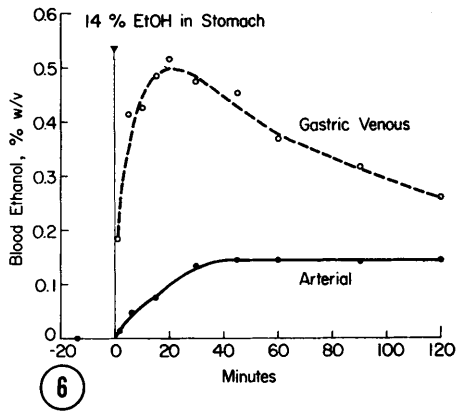


FIG. 5. K^+ output of pouches of the canine oxyntic glandular mucosa during 30 min control (C) irrigation with 100 mM HCl and during 4 successive 30 min periods of irrigation with 8.4% ethanol in the same solution. Means ± SEM; 4 to 7 observations in each period. Output in period 1 is significantly greater than that in any other period, $p<0.01$.

FIG. 6. Gastric venous and arterial ethanol concentrations. At zero time the stomach of an anesthetized dog was filled to the extent of 1% body weight with a solution of 14% ethanol in 30 mM phosphate buffer, pH 7.5. No histamine was found in any venous or arterial blood sample following ethanol instillation by a method capable of detecting at least 1 μg histamine per liter of blood.

histamine to anesthetized dogs while measuring arterial and gastric venous histamine concentrations. When histamine was infused at the low rates of 0.015 or 0.02 mg base per kg per hr, acid secretion began before there was a detectable (less than 1 μg per liter) rise in gastric venous histamine concentration.

Summary. The effect of ethanol upon the barrier function of the stomach was studied by irrigating pouches of the canine oxyntic glandular mucosa with solutions of ethanol ranging from 0.5 to 27% w/v. Solutions of 8% or less did not damage the mucosal barrier as judged by the fluxes of Na^+, K^+ and H^+. Solutions of 14 and 27% broke the barrier, for fluxes of the ions across the mucosa were greatly increased during and following ethanol application. In contrast to acetylsalicylic acid which is much more damaging in acid than in neutral solution, ethanol has essentially the same effect upon the barrier when applied in 100 mM HCl or in 30 mM phosphate buffer, pH 7.5. The rate of absorption of ethanol is a linear function of its concentration. The damaging effect of ethanol depends upon the concentration in contact with the mucosa and not upon the quantity absorbed. Low concentrations of ethanol (8% or less) stimulate acid secretion. Histamine could not be detected in the arterial or gastric venous blood of anesthetized dogs when the stomach was filled with 8 or 14% ethanol.

The author is grateful to Leonard R. Johnson and Virginia D. Davenport for help with the histamine experiments and to Morton I. Grossman for encouragement and advice.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.

Suppression of the Primary Antibody Plaque Response of Mice Following Infection with Friend Disease Virus.* (32533)

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A number of murine viruses may induce leukemia-like diseases upon injection into susceptible strains of mice(1). Several of these viruses apparently induce neoplasia only upon administration to immunologically incompetent animals, such as neonates or surgically thymectomized mice(2). On the other hand, other tumorigenic viruses, such as Rauscher Disease Virus (RDV) and Friend Disease Virus (FDV), result in rapid development of splenomegaly and leukemia-like disease when

* Supported by grants from Am. Cancer Soc. (T-328), National Science Foundation (GB 3278), and USPHS Training Grant A-1233-04.