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ETHANOL AND CALCIUM METABOLISM

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ABSTRACT

Chronic alcoholism is associated with osteopaenia and increased incidence of fracture while acute ethanol administration causes hypocalcaemia in experimental animals. The present paper discusses possible mechanisms by which ethanol may affect calcium metabolism, putting emphasis on the involvements of kidney, bone and intestine. Upon reviewing previous reports and our own investigations, we find that neither calcium regulating hormones, i.e., parathyroid hormone, calcitonin and 1, 25 dihydroxy vitamin D, nor kidney plays any crucial role in mediating the hypocalcaemic action of ethanol. On the other hand, chronic but not acute ethanol administration is reported to increase bone resorption, while the latter is found to suppress calcium efflux from bone. Most studies indicate that ethanol-induced hypocalcaemia can also be partly accounted for by the decrease in intestinal calcium absorption; the mechanisms involved are discussed.

INTRODUCTION

Chronic alcoholism has been associated with a number of metabolic derangements,[1-4] including various abnormalities of managesium and phosphate metabolism,[5-10] which have been regarded as possible factors in the development of alcoholic myopathy.[5, 11, 12] Although disorder in calcium metabolism in the alcoholics has not received much attention, there have been reports of osteopaenia and increased incidences of fracture in alcoholics.[13-18]

The effect of ethanol on serum calcium levels was first noted by Peng and coworkers[19] who observed a rapid fall in both total and ionized calcium within 30 minutes after a single dose of ethanol in dogs and rats. However, acute ingestion of ethanol has no effect on total or ionized calcium levels in the serum of healthy volunteers.[20] On the otherhand, there have been reports of an increase in calcium excretion in man during the intitial 4 hours when ethanol is administered acutely, in varying doses and by way of various routes.[21-24]

Many of these studies concerning ethanol and calcium metabolism consist of results that are conflicting and generally difficult to interpret. It is often unclear whether the reported findings are due to the direct effect of ethanol or the consequences of ethanol-related complications such as cirrhosis, portal hypertension, pancreatic insufficiency, or chronic malnutrition. Furthermore, acute effects of ethanol in chronic alcoholics are not analogous to the acute effects in nonalcoholics.[25, 26] It has been shown that chronic ethanol consumption in experimental animals produces adaptive changes in membrane phospholipid composition that are protective against the acute effects of ethanol *in vitro*.[25, 26] The conflicting findings are also due to major differences in species responses, both in terms of the specific metabolic effect of alcohol and the individual species' adaptation to chronic exposure.

The present paper attempts to discuss the mechanisms by which ethanol may affect calcium metabolism. Emphasis is given to the three main target organs in calcium metabolism i.e., bone, kidney and the intestine, and how the altered functions of these organs may contribute to the observed hypocalcaemia in some experimental animals.

It is logical to first briefly describe the inherent nature of ethanol. Ethanol is moderately polar, readily forms hydrogen bonds, and is thus infinitely soluble in water. Requiring no digestion, it is absorbed freely across the gastrointestinal tract by simple diffusion. Once absorbed, ethanol is rapidly distributed. It readily diffuses across capillary walls and through plasma membranes so that intra- and extracellular concentrations equilibrate promptly. Gastric ethanol absorption is about 40% at 20 minutes after ingestion.[27] Eight percent of ethanol is metabolized by the liver which utilizes three pathways for oxidizing ethanol to acetaldehyde[28] : the major pathway, the alcohol dehydrogenase pathway of the cytosol,[29] the microsomal ethanol oxidizing system in the endoplasmic reticulum, and the catalase located in the peroxisomes which is responsible for no more than 10% of ethanol oxidation.[29, 30] Ten percent of ingested ethanol is excreted in the breath, sweat, and urine, and the remaining 10% is metabolized at other body sites. The alcohol dehydrogenase system is also found in the mucosa of the stomach, jejunum, and ileum in humans[31] and rats.[32-34] Both in men and rats, most of the ethanol ingested at a low dose is metabolized before it reaches the systemic circulation. Oxidation of ethanol which occurs mainly in the stomach accounts for the bulk of this effect.[35]

This "first pass" metabolism helps to protect against the systemic toxicity of ethanol and thus is a significant factor in determining the blood levels and systemic effects after ingestion. In this regard, attention should be given to the different routes of ethanol administration used in the experiments. After an oral or intragastric administration, maximal ethanol levels are found in the stomach, duodenum and proximal jejunum.[36, 37] High ethanol levels are sustained for up to 1 hour at these sites. The early and parallel increase of ethanol in the serum and distal intestine suggests that ethanol reaches this part from the vascular space rather than from transit along the small intestine (Fig. 1).[36, 37]

Ethanol and Plasma Calcium Concentration

Although hypocalcaemia is a feature not commonly found in chronic alcoholism, administration of ethanol rapidly lowers the serum calcium concentration in dogs[19, 38] and rats.[19, 38-40] In our investigation, the administration of 3 g ethanol/100 g body weight of rat results in a rapid onset of hypocalcaemia within 30 minutes regardless of whether it is given by an intraperitoneal injection or by an oral route. The effect is prolonged for about 8 hours. In the chronic experiment, the ethanol-treated animals show no significant change in the plasma calcium concentration.[38]

Ethanol and Renal Calcium Excretion

The observed hypocalcaemia cannot be explained by urinary calcium excretion since urinary calcium excretion is reduced by ethanol within 30 minutes.[40] This confirms the previous reports.[15] It is not known whether this suppression of urinary calcium excretion is due to a direct inhibitory action of ethanol or a consequence of the observed hypocalcaemia. A reduction in the glomerular filtration rate may be partly responsible.[19] Chronic ethanol administration is also reported to decrease the urinary excretion of calcium.[41]

In man, however, acute ethanol ingestion has been reported to markedly increase calcium excretion.[22] The diversity of results obtained from man, dogs and rats may be explained by the differences in the doses of ethanol, the lengths of investigation after administration of ethanol, and/or species differences.

Ethanol and Calcium-Regulating Hormones

The possibility that ethanol might cause hypocalcaemia by interfering with the calcium-regulating hormones, such as parathyroid hormone (PTH), calcitonin (CT) and 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D$) is also considered. However the involvement of PTH and CT is unlikely because the hypocalcaemic effect of ethanol is not altered by acute removal of the thyroid and parathyroid glands.[19, 40] We find that, after the calcium chloride infusion is given to thyroparathyroidectomized (TPTX) rats to raise the level of plasma calcium to a normal level, ethanol is still able to induce a significant hypocalcaemia.[40] Shah and co-

workers[42] have provided another evidence by demonstrating that hypocalcaemia, induced by even small doses of ethanol (0.2 and 0.4 g/kg), could not be prevented by a compensatory increase in PTH secretion. The authors explain that the decrease in serum calcium is the primary event and the increase in serum PTH is observed in response to the hypocalcaemic effect of ethanol. However, such an increase in PTH may be due to a direct action of ethanol since ethanol has been shown to induce an increase in PTH without detectable hypocalcaemia in man.[43] On the other hand, Chanard *et al.*[44] report a suppressive action of ethanol on PTH secretion even in the presence of hypocalcaemia both in the *in vivo* and *in vitro* preparations. Thus, whether ethanol stimulates or inhibits PTH secretion, one can say that alteration in PTH secretion does not directly mediate the ethanol-induced hypocalcaemia. In addition, the demonstration of ethanol-induced hypocalcaemia in TPTX rats also excludes the possibility of an involvement of CT despite a report of ethanol-induced release of CT.[45]

Chronic ethanol consumption is often associated with numerous nutritional imbalances resulting from inadequate intake and/or utilization of essential nutrients. Vitamin D-associated imbalances such as reduced circulating 25-hydroxy vitamin D,[46, 47] reduced intestinal calcium absorption,[48] reduced bone mass,[13, 49] bone density[14] and trabecular bone volume[41] have been reported in chronic alcoholics. Furthermore, suppression of 1α-hydroxylase by ethanol with a consecutive decrease of 1,25-dihydroxycholecalciferol is suggested by Kent *et al.*[50] However, the derangement of calcium metabolism is not corrected when vitamin D is administered,[51] suggesting that vitamin D does not play a significant role in this situation.

Ethanol and Bone

The stability of the plasma calcium concentration at any one time is the result of a complex balance between the influx of calcium from various tissues including intestine, bone and soft tissues, and the efflux of calcium from the plasma pool. Bone is a likely candidate for one of the target organs of ethanol-induced hypocalcaemic action, for it is the largest reservoir of calcium. Even slight alterations in calcium fluxes between the exchangeable bone pool and the vascular compartment can effect the plasma calcium concentration. It is important to mention, at this point, that results from studies of calcium transport in bone is complicated by the fact that there are many processes involved, such as bone accretion, bone resorption, and calcium fluxes across the rapidly exchangeable calcium pool in bone.

We have done some investigations on the effect of ethanol on calcium fluxes and find no effect of acute ethanol administration on the release of ^{45}Ca from prelabelled rat tibiae *in vitro*,[40] which is consistent with earlier reports.[52, 53] The studies of Peng *et al.*[19] suggest that ethanol may acutely increase the movement of calcium into bone. In support of this suggestion, Ramp *et al.*[54] have shown

that ethanol exerts a dose-related, direct stimulatory effect on bone mineral accretion in embryonic chick tibiae cultured for 4 days. This finding leads to the conclusion that inhibited net calcium efflux from bone[55] may, at least in part, cause the hypocalcaemic effect of ethanol seen *in vivo*. This conclusion is strengthened by the results of Ramp *et al.*[56] which demonstrate that PTH-stimulated net calcium efflux from bone[57] can be inhibited by ethanol. Because both PTH and ethanol have essentially no effect on net phosphate efflux within 8 hours in this system, these early changes in net calcium efflux are probably not due to changes in bone resorption. However, longer incubations do result in loss of both calcium and phosphate in response to PTH in 2 day,[58] indicating that PTH-stimulated bone resorption is also suppressed. In contrast, there is a report of increased bone resorption (release of both matrix and minerals) by chronic ethanol administration (8 weeks)[41] without ultrastructural changes in the skeletal cells of rat.[59] This is confirmed by our findings in rats (Table 1) which show that 8 weeks of treatment with 20% ethanol as drinking water significantly reduces the bone calcium content, but has no effect on the rate of calcium uptake by tibiae.[60] Farley and coworkers,[61] using release of I^3Hl hydroxyproline as an index of bone resorption, also report an increase in bone resorption after 24 hours incubation of embryonic chick embryo in ethanol solution. The discrepancies among these results are probably due to age and species differences and duration of experiments.

Although some of the results i.e. decreased net calcium efflux from ethanol treated bones and hypocalcaemia in experimental animals given ethanol[19, 38-40, 42] represent acute effect of ethanol, these acute effects may well be involved in some of the long-term effects on the skeleton. As mentioned before, osteopaenia occurs in chronic alcoholics[13-15] and chronic administration of ethanol to growing rats leads to increased bone resorption.[41] There are many possible mechanisms by which chronic ethanol may cause bone disorder. Ethanol-induced hypocalcaemia results in compensatory increase in PTH release which ultimately causes increased osteoclastic activity and thus, resorption. Ethanol-induced hypomagnesaemia could have a similar effect in rats since hypomagnesaemia was found to stimulate PTH secretion in this species.[62] Moreover, bone resorption could be stimulated by ethanol-stimulated corticosteroid secretion. Ethanol-induced reduction in calcium efflux from bone could be another factor contributing to hypocalcaemia, increased PTH secretion, and subsequent bone resorption. It should be emphasized that bone resorption would occur rather slowly and have little effect on immediate calcium homeostasis.[55, 62]

Despite the conflicting data, it could be summarized at this stage that the effects of ethanol on bone depend on the doses and duration of ethanol administration, age and species of animals. Chronic ethanol is reported to increase incidence of fracture,[63] increase bone resorption in growing rats,[41] and suppress the PTH-

stimulated bone resorption in embryonic chick bone.[58] Acute ethanol administration is reported to have no effect on calcium efflux from bone of adult rats,[40, 52, 53] to reduce the calcium efflux from bone of 8 day-old suckling rats[50] and to inhibit the PTH-stimulated net calcium efflux from embryonic chick bone.[56]

The minimal medium ethanol concentration (3 μl/ml) that reduces net calcium efflux from rat bone[56] is equivalent to a blood ethanol level in humans that is attained only in severely intoxicated individuals, and the minimal concentration of ethanol that inhibits net calcium efflux from chick tibiae (10 μl/ml),[56] is above the lethal blood level in humans. The average blood level of ethanol found in the social drinker is about 0.5 μl/ml.[43] So it seems that ethanol toxicity is less tolerated by man than by experimental animals, therefore the comparison of results obtained from animals and man should be made with care.

Ethanol and Intestinal Calcium Absorption

There is evidence to indicate that chronic ethanol administration may lead to steatorrhoea, malnutrition, or simple diarrhoea.[32] These conditions could be caused by abnormal pancreatic and hepatobiliary secretion, impaired intestinal function, or a combination of all the above.[64] It is known that chronic alcoholics exhibit calcium malabsorption, which, as suggested by many authors,[65-67] may occur indirectly as a result of steatorrhoea. However, studies in humans have been rather inconsistent. No effect of acute ethanol administration on calcium absorption was demonstrated in normal subjects,[70] although chronic alcoholics may have a reduced ability to absorb calcium.[46-51] Deficiencies in the vitamin D metabolites that regulate intestinal calcium transport may be present in alcoholics. However, results are inconsistent.[72-75] Interestingly, significantly more calcium is absorbed during wine and dealcoholized wine periods than during ethanol and deionized water periods.[72] The authors suggest that this effect is attributable to the congeners in wine, but, to our knowledge, no further study has been persued.

In the early 1970's, Krawitt,[68, 69] using an everted gut sac technique, measured ^{45}Ca transport by rat duodenum and ileum after acute and chronic administrations of ethanol. The results indicate that chronic ethanol ingestion (20% ethanol solution as drinking fluid for 12 days) suppresses duodenal net calcium absorption.[68] Acute ethanol administration (7.5 g/kg, intragastric) also inhibited the duodenal active transport of calcium, which is accompanied by necrosis of epithelium.[72] In the chronic ethanol-treated rats, the effect of ethanol on calcium absorption was, at least in part, independent of vitamin D.[49] Suggestions have been made that these effects of ethanol on calcium absorption might reflect a nonselective toxic effect on the intestine.[76-78]

Although Krawitt[68-69] demonstrated some inhibitory effects of ethanol on the net transport of calcium across the everted duodenal sacs, we chose to further

the studies by using both the *in vivo* and *in situ* techniques and to apply a lower dose of ethanol. Since calcium can also be actively absorbed[79-81] and secreted in the ileum[79, 81-83] and the effect of ethanol on ileal calcium transport has not been studied, we also investigated the effect of ethanol on calcium fluxes in this intestinal segment. We have been able to demonstrate that the inhibition of net duodenal calcium absorption by an acute administration of 3 g ethanol/kg body weight is due to a reduction in the lumen to plasma flux of calcium (Ca_{L-P}) with the plasma to lumen flux (Ca_{P-L}) being unaltered[84] suggesting that the damage may reside in the active transport of calcium (medium calcium concentration of 0.9 mM). Although the Ca-ATPase activity was not determined, it is possible that ethanol may inhibit the duodenal Ca-ATPase in a similar manner to the ethanol-induced inhibition of jejunal Na-K-ATPase.[85]

As shown in Fig. 2, in contrast to its action in the duodenum, ethanol increases the net calcium secretion in the ileum by markedly stimulating the Ca_{P-L}.[84] The different responses seen in the duodenum and ileum may be accounted for by the different properties of transport mechanisms and different concentrations of ethanol to which they are exposed (Fig. 1).[37]

The *in vivo* intestinal calcium transport is also influenced by changes in the rate of gastric emptying, intestinal motility, blood flow distribution and humoral factors. Some of these parameters are absent from the *in vitro* and *in situ* preparations. Besides, the presence of serosal and muscularis layers as potential barriers to transepithelial movement *in vitro* do not interfere with the *in vivo* transport processes.[86] Our *in vivo* study[37] demonstrates that, under control conditions, most of the exogenous calcium is absorbed in the proximal and mid-small intestine, and the luminal calcium present along the distal parts is largely represented by endogenous or secreted calcium, a large portion of which is of gastric origin (Fig. 3). Since the colon is a site of calcium absorption rather than calcium secretion,[81, 82] the endogenous calcium present in the colon probably results from a transit from the small intestine. The *in vivo* experiment (Fig. 4) also demonstrates that ethanol markedly stimulates gastric calcium secretion, and, in accordance with our *in situ* findings,[37] it also shows that ethanol suppresses calcium absorption in the proximal small intestine. The secretion of calcium in the distal small intestine is also significantly increased within 20 minutes by ethanol which evidently reaches the distal small intestine by circulation.[37]

Can this increase in intestinal calcium secretion be partly responsible for the ethanol-induced hypocalcaemia? To answer this question, we performed a series of experiments to find out the mechanism of ethanol-induced hypocalcaemia after ethanol was administered via different routes.[87] The results demonstrate that only the intragastric route of ethanol administration leads to a significant increase in calcium transport from plasma into the gastrointestinal tract (Fig. 5). Moreover, only the gastrointestinal tissues directly exposed to high concentrations of ethanol

after intragastric administration exhibited significantly elevated tissue ^{45}Ca content.[87] So it seems that, when ethanol is administered via an oral route, the ethanol-induced hypocalcaemia can indeed be at least partly explained by the increase in luminal and tissue content of calcium in the gastrointestinal tract.

Ethanol and Calcium Contents in Soft Tissues

There has been little evidence on the effects of ethanol on soft tissue calcium contents. Some work has been done on the effect of ethanol on brain calcium concentration but the findings are controversial.[88-91] Acute pancreatitis resulting from ethanol abuse deserves some comments. The complications of severe alcoholic pancreatitis include, among other abnormalities, fluid and electrolyte disturbances, hypocalcaemia, and hypomagnesaemia. The precise pathogenetic mechanism of acute alcoholic pancreatitis is unknown. The initiation of the autodigestive process in this pathological condition requires the activation of trypsinogen as the initial step, but the sequence of subsequent biochemical events is controversial. Hypocalcaemia is frequently observed in acute pancreatitis[92, 93] but its pathogenesis is not understood. Among the various mechanisms proposed are 1) deposition of calcium in areas of fatty necrosis;[94] 2) hyperglucagonaemia[89] which is capable of inducing hypocalcaemia either by releasing calcitonin[96] or by directly inhibiting bone resorption;[97] 3) PTH deficiency[98] and 4) hypomagnesaemia. However, numerous findings contrary to these observations have also been reported. So it remains to be investigated to see whether such changes in the plasma membrane could lead to soft tissue calcium accumulation. Furthermore, high levels of ethanol *in vitro* are reported to inhibit the $Na^+-K^+-ATPase$ activity,[102] so ethanol may at least indirectly influence the calcium transport by affecting the calcium and sodium exchange transport.

SUMMARY

It appears that the effects of ethanol consumption on the calcium metabolism vary among different species and depend on the amount and duration of ethanol intake. In species such as rats and dogs which clearly exhibit ethanol-induced hypocalcaemia, the hypocalcaemia cannot be accounted for by urinary calcium excretion or direct interference of PTH and CT secretion. On the other hand, ethanol-induced reduction in calcium efflux from bone could contribute to acute hypocalcaemia which eventually causes a compensatory increase in PTH secretion with subsequent bone resorption. Bone resorption occurs rather slowly and probably has little effect on immediate calcium homeostasis but it possibly leads to osteopaenia.

Reports of ethanol effects on intestinal calcium absorption in humans are rather inconsistent despite numerous reports on calcium malabsorption in chronic alcoholics. Nevertheless, studies in rats consistently show that acute and chronic

ethanol administrations suppress calcium absorption. The ethanol-stimulated secretion of calcium in the small intestine may well contribute to the observed hypocalcaemia. In addition, an accumulation of calcium in soft tissues is another possible disturbance of calcium metabolism in alcoholics but there has been little evidence on this aspect.

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บทคัดย่อ

เนื่องจากมีรายงานทางการแพทย์ว่าผู้ป่วยโรคพิษสุราเรื้อรังมีอาการของโรคกระดูกผุ และมีอัตราการเกิดกระดูกหักร้าวสูงกว่าคนปกติ และแอลกอฮอล์มีผลลดระดับแคลเซี่ยมในเลือดของสัตว์ทดลองได้อย่างเฉียบพลัน จึงเป็นที่น่าสนใจว่าแอลกอฮอล์มีผลต่อสภาวะแคลเซี่ยมในร่างกายอย่างไร เพื่อที่จะหาข้อสรุปเกี่ยวกับกลไกของแอลกอฮอล์ต่อสภาวะแคลเซี่ยม รายงานนี้ได้เปรียบเทียบผลการทดลองของกลุ่มผู้วิจัยต่าง ๆ รวมทั้งผลการวิจัยของผู้เขียนเองโดยเน้นที่ไต, กระดูก, และระบบกระเพาะอาหารลำไส้ ซึ่งพอจะสรุปได้ดังนี้ ฮอร์โมนสำคัญที่ควบคุมสภาวะแคลเซี่ยมได้แก่พาราไทรอยด์ฮอร์โมน แคลซิโทนิน และ 1,25 dihydroxy vitamin D และอวัยวะเช่นไต ไม่มีส่วนเท่าใดนักในการออกฤทธิ์ของแอลกอฮอล์ต่อสภาวะแคลเซี่ยม ในทางตรงข้ามเราพบว่าการสลายกระดูก ถูกยับยั้งโดยแอลกอฮอล์ที่ให้ติดต่อกันเป็นเวลานาน และแอลกอฮอล์มีผลเฉียบพลันต่อกระดูกคือยับยั้งการปล่อยแคลเซี่ยม (calcium efflux) จากกระดูกโดยไม่มีผลต่อ bone resorption ส่วนที่ระบบกระเพาะลำไส้นั้นพบว่าการลดลงของระดับแคลเซี่ยมในเลือดอย่างเฉียบพลันอาจเป็นผลที่เกิดจากการที่แอลกอฮอล์ออกฤทธิ์ลดการดูดซึมแคลเซี่ยมที่ลำไส้ ซึ่งข้อถกเถียงและข้อสนับสนุนต่าง ๆ ได้เสนอไว้ในรายงานนี้

TABLE 1 The effects of chronic ethanol (20%) treatment for 8 weeks on the plasma calcium concentrations, urinary calcium excretion, bone calcium content and ^{45}Ca uptake over a 2 hour period by tibiae of rats.

	Control	Ethanol
Body weight (g)	200 ± 4	182 ± 10
Plasma calcium concentration (mg/100 ml)	9.9 ± 0.02	9.6 ± 0.2
Urinary calcium excretion(μg/h/100 g)	6.7 ± 1.8	7.1 ± 1.5
Weight of tibia (g)	0.45 ± 0.01	0.45 ± 0.002
Bone calcium content (mg/g bone)	18.2 ± 1.8	10.3 ± 0.8***
^{45}Ca uptake (cpm × 10^3/g bone)	3188 ± 412	3231 ± 311

***P<0.001 (unpaired t-test). (From reference 54)

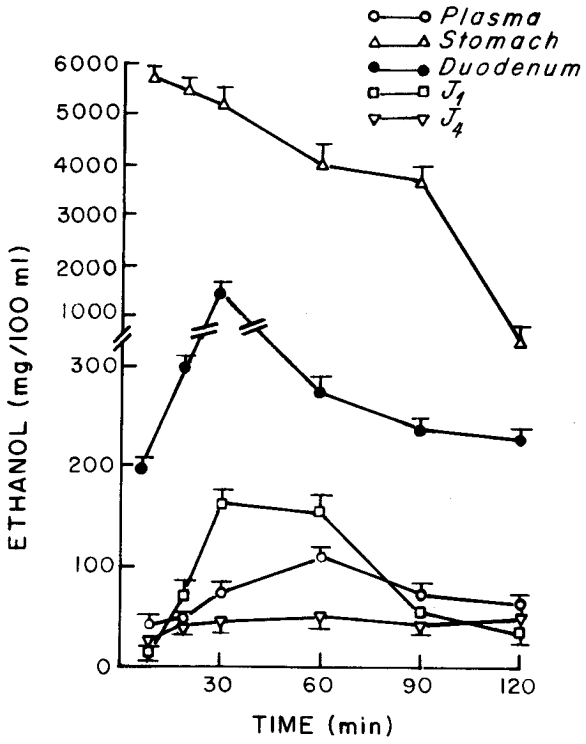

Fig. 1 Ethanol concentrations in plasma and contents from the gastrointestinal segments : the stomach, duodenum, intestinal segments J_1 (proximal jejunum) and J_4 (ileum) of fasted rats (n = 6) which had received an intragastric administration of 2 g ethanol/kg body weight. (From reference 37)

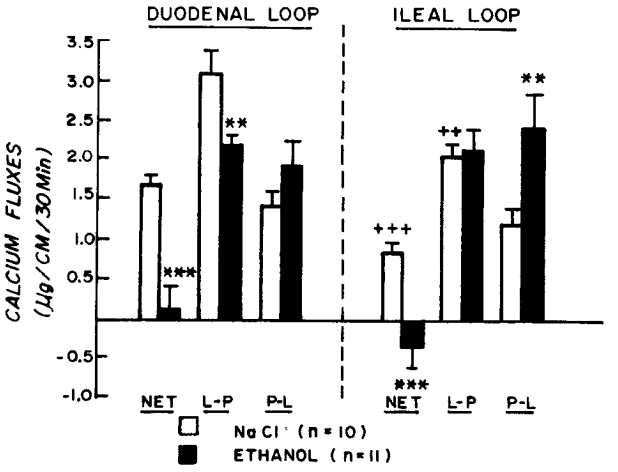

Fig. 2 The effect of an intragastric administration of 3 g ethanol/kg body weight of rat on calcium fluxes : net absorption, lumen to plasma flux (L-P) and plasma to lumen flux (P-L) in the duodenal and ileal *in situ* loops during 30 minutes incubation. Statistical comparisons were made between the duodenum and ileum (paired t-test) of the saline control group and between the control and ethanol treated group (unpaired t-test). $^{++}P<0.005$, $^{+++}P<0.0005$ (paired t-test); $^{**}P<0.005$, $^{***}P<0.0005$ (unpaired t-test). (From reference 84)

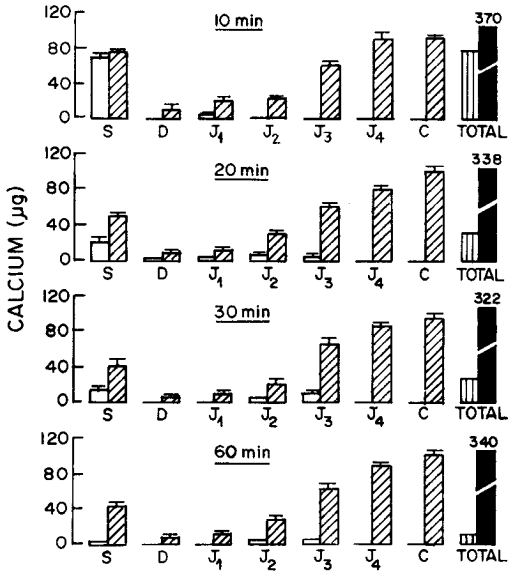

Fig. 3 Distribution of endogenous and exogenous calcium in the gastrointestinal tract at various time intervals in control rats. The contents of exogenous calcium (calculated from luminal ^{45}Ca content, □), calcium (exogenous + endogenous) along the gastrointestinal tract (▨), the total exogenous calcium (▥), and total calcium content (■) were presented at 10, 20, 30 and 60 minutes after an intragastric administration of test solution in control rats. (From reference 37)

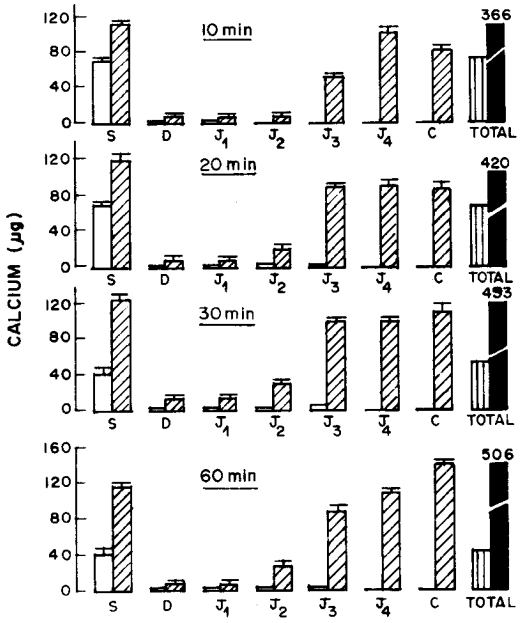

Fig. 4 Distribution of endogenous and exogenous calcium in the gastrointestinal tract at various time intervals after an intragastric administration of test solution containing 2 g ethanol/kg body weight of rat. Descriptions of symbols are the same as those in Fig. 3. (From reference 37)

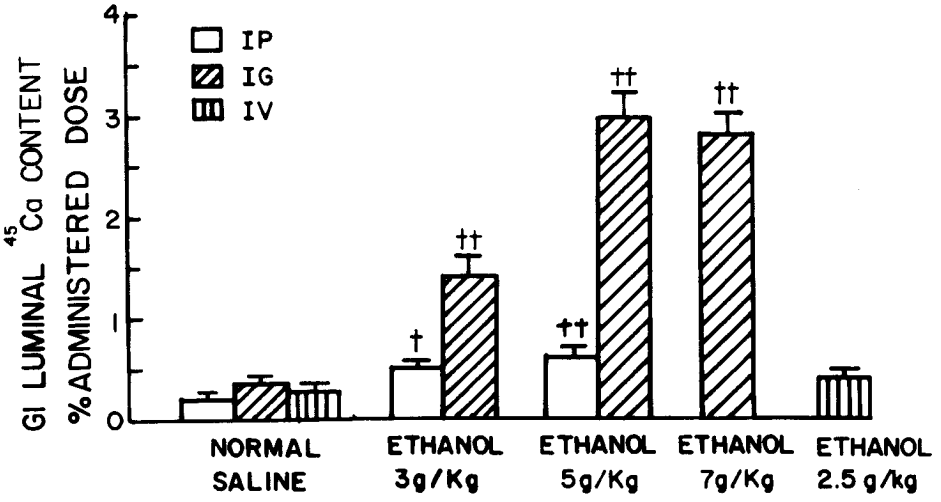

Fig. 5 The effect of normal saline or 3, 5 and 7 g ethanol/kg body weight given intraperitoneally (IP) or intragastrically (IG) or normal saline or 2.5 g ethanol/kg given intravenously (IV) on the gastrointestinal luminal content of ^{45}Ca in fasted rat at 30 minutes after ethanol administration. Randomized design ANOVA and Newman-Keuls test were used to compare the values of ethanol-treated groups with control groups that received saline by the same route of administration. $^{+}P<0.05$, $^{++}P<0.01$. (From reference 87)