produced by a Welsbach ozonator style T816 at an air pressure of 5.6 kg/cm^2 and a flow of 2.1 l/min. Ozone level is controlled with a Dasibi monitor model 1003-AH which gives a reading every 30 sec.

Results and discussion. As can be seen in figure 1, most of the unstable protoplasts have burst before the first 30 min (unpublished data from the M.C. show that enzymatically obtained protoplasts are heterogenous as to their resistance to bursting under an osmotically produced strain). After 30 min, the rate of bursting becomes low, so as to be almost imperceptible after 180 min.

This initial bursting cannot be attributed solely to the possible action of macerating enzymes on the plasmalemma, because earlier mechanical methods of obtained protoplasts also showed the same phenomenon.

With the 3-h delay, and with open Petri dish fumigation which prevents further damage by air bubbling, the results gathered in figure 2 clearly show that ozone acts on the plasmalemma to weaken its mechanical properties and cause bursting. Normally the plasmalemma of plant cells show elastic properties[16]; it can expand and shrink again[17,18]. Quantitatively, unpublished data by M.C. show that the surface of the plasmalemma of a protoplast can expand 58% in 1 h upon submitting immobilized protoplasts in 0.15% agar to a change of osmotic concentration from 0.40 M to 0.30 M mannitol in T_o, without apparent disorganization of the external membrane or of the internal structure of the cell. It is possible that still more strain can be put on some protoplasts without irreversible changes. In order to explain the loss of elasticity, one could refer to ozone action on 2 classes of the constituents of the plasmalemma.

1. It has been suggested that critical sulfhydryl groups[9] of sulfur containing proteins of the cell are affected. There is some reason to believe that the phenomenon includes the enzymes floating on and within the lipid layer, resulting in the unfolding of the molecules, modifying their enzymatic properties as well as their relationship to the lipid layer. It is of interest to recall here that Phan and his team[19,20] have found an ozone-induced enhancement of various 'lysosomal' enzymes, which have been detected in the vicinity of the plasmalemma and the cell-wall.

2. The lipids most affected seem to be triunsaturates having chains of 16 and 18 carbons[16], which become more rigid upon being transformed into epoxides. Although the affected fatty molecules represent only between 0.7 and 1.7% of the total lipids of the cell[16], it can be assumed that they may constitute stiffened and breakable sites within the membrane, thus creating zones where it can rupture.

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HCO_3^--ATPase activity distribution in rat liver cell fractions prepared by zonal centrifugation[1]

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Summary. Plasma membrane sheets prepared by zonal centrifugation of a premicrosomal pellet obtained from a rat liver homogenate are devoid of HCO_3^--ATPase activity. Since the microsomal fraction is also lacking in this ATPase activity, it can be concluded that the HCO_3^--ATPase is not involved in the secretion of HCO_3^- into bile.

A HCO_3^--stimulated Mg-ATPase activity supposedly responsible for HCO_3^- or H^+ transport has been reported in both microsomal and plasma membrane fractions prepared from many tissues. However, the existence of a plasmalemmal HCO_3^--ATPase has not been demonstrated in a definitive manner for any tissue except erythrocytes of the rabbit[2,3] and rat[4]. Sachs et al.[5] appeared to have demonstrated the existence of a plasmalemmal HCO_3^--ATPase in a microsomal fraction isolated from dog gastric mucosa, but these results have recently been disputed by Soumarmon et al.[6] and Bonting et al.[7]. One tissue in which definitive cell fractionation is possible is the rat liver. We previously tested for HCO_3^--ATPase activity in microsomal fractions prepared from rat livers, and found that with careful tissue homogenization, microsomal fractions without HCO_3^--ATPase activity could be prepared[8]. Since the HCO_3^--ATPase activity of the homogenate could be accounted for in the premicrosomal pellets, we concluded that any HCO_3^--ATPase activity in the microsomal fraction arose from mitochondrial contamination. However, there is another possible explanation for this finding. Microsomes are a collection of vesicles with heterogeneous sites of origin, one of which is the cell surface membrane. The contribution of the various cell structures to the microsomal population is not constant and can vary with homogenization procedures. In particular, gentle homogenization techniques are known to leave a large portion of the plasmalemma intact as large fragments that sediment with the nuclei[9]. Also, the number of plasmalemmal vesicles formed under these conditions will be decreased, and the possibility exists that they would not be a significant portion of the microsomal population. Thus, the negative results obtained in our previous study might also have resulted from the fact that the extremely gentle homogenization procedures that were utilized

resulted in the formation of large amounts of plasma membrane fragments that pelleted in the premicrosomal pellets and insignificant numbers of plasmalemmal vesicles that pelleted in the microsomal fraction. This possibility was earlier thought to be unlikely. However, in light of the recent findings in red cells[2-4], a report by Ivashenko et al.[10] that a plasmalemmal HCO_3^--ATPase is present in plasma membrane fractions prepared from Ehrlich ascites tumor cells, and persisting reports of plasmalemmal HCO_3^--ATPase activity in various tissues[11-13], it became necessary to re-examine this possibility. This was done by characterizing the enzymic properties of large fragments of plasma membranes isolated from a low speed nuclear pellet.

Materials and methods. Preparation of the plasma membranes. The plasma membranes were prepared from fed Sprague-Dawley rats (150–250 g) using the procedure described by Evans[14]. Zonal centrifugation of the resuspended nuclear pellet was performed at 5 °C in a type A-XII zonal rotor using an International PR 2 centrifuge. An Isco Dialagrad gradient pump was used to form the sucrose gradient (6–54%). Mg^{2+}-ATPase and HCO_3^--ATPase activities were measured as was previously described[8]. 5′-nucleotidase (5′-AMPase), glucose-6-phosphatase (G-6-Pase) and acid phosphatase (acid-Pase) were measured as described by Evans[14] except that 50 mM paranitrophenylphosphate or 50 mM β-glycerolphosphate were used as substrate in the acid phosphatase assay. Phosphate liberation in all cases was linear over incubation periods of from 15 to 60 min. Succinate dehydrogenase (SDH) activity was measured as previously described[15] and monoamine oxidase (MAO) activity was measured using the method of Tabor et al.[16] with benzylamine as substrate. Enzyme assays were converted to specific activity utilizing protein concentrations determined by the Lowry technique[17]. The 650 nm O.D. of each fraction were measured using round curvettes (19 × 105 mm) and Coleman, Jr, II spectrophotometer. Urate oxidase activity was measured using the method of Beaufay et al.[18]. The sucrose density measurements were based on index of refraction measurements obtained with a Bausch and Lomb refractometer at room temperature.

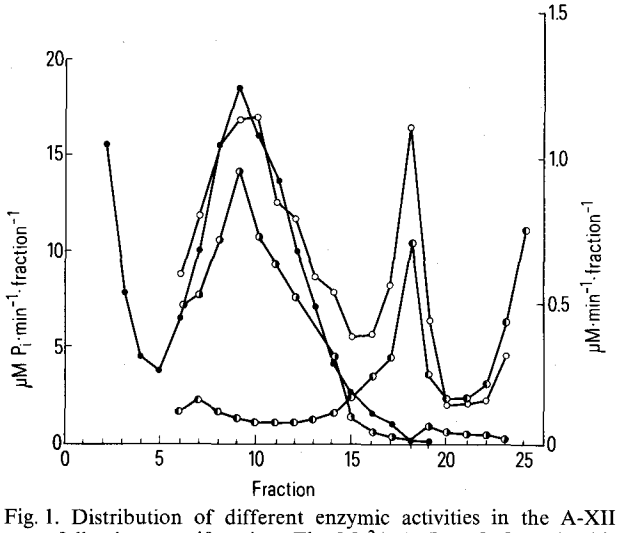

Fig. 1. Distribution of different enzymic activities in the A-XII rotor following centrifugation. The Mg^{2+}-ATPase 0, 5′-nucleotidase ◐, HCO_3^--ATPase ◑, and succinate dehydrogenase ●, were measured as described in methods. The succinate dehydrogenase activity is expressed as μmoles of substrate utilized per min per fraction as shown on the right hand border. The units for the other enzymes are shown on the left hand border.

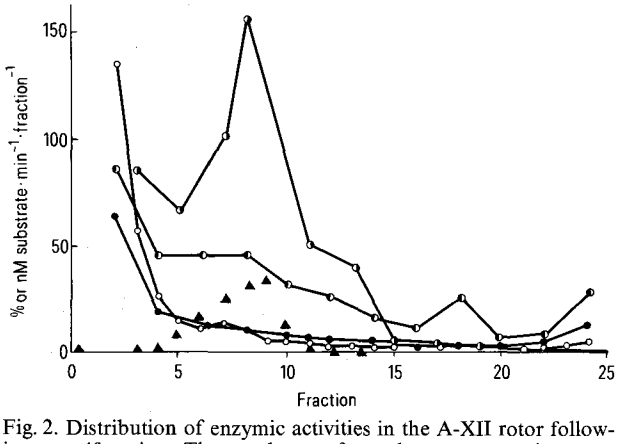

Fig. 2. Distribution of enzymic activities in the A-XII rotor following centrifugation. The results are from the same experiment as used in figure 1. The monoamine oxidase ◑, glucose-6-phosphatase ○, acid paranitrophenylphosphatase ●, acid β-glycerolphosphatase ◐, and urate oxidase ▲ activities were measured as described in methods. The monoamine oxidase activities can be read directly off the ordinate. The acid paranitrophenylphosphatase and glucose-6-phosphatase activities were divided by 100 to fit the given scale. The B-glycerolphosphatase activity was divided by 10 to fit the present scale. The urate oxidase activity is expressed as the percentage in each fraction of the total activity.

Results and discussion. Band pattern and marker enzyme distribution. 4 bands of material were visible after 1 h of centrifugation. The subcellular constituents of these bands were analyzed using marker enzyme analysis. The inner most band was the most prominent and enzyme analyses indicated that it consisted mainly of endoplasmic reticulum vesicles and mitochondrial fragments (figure 1, SDH; and figure 2, acid-Pase, G-6-Pase, MAO). The 2nd band was more diffuse and enzymic analysis indicated that it was a mixture of mitochondria and microbodies (figure 1, SDH; and figure 2, MAO and urate oxidase). Low levels of endoplasmic reticulum were also present as indicated by above baseline G-6-Pase readings (figure 2). The 3rd band was thin and contained the plasma membrane fragments as indicated by 5′-AMPase. Previous electron microscopy showed these plasma membrane fragments to be sheets with desmosomes and microvilli readily identified[19]. The outermost band was dense and was also previously morphologically characterized as consisting of cell debris, nuclei and nuclear debris. These marker enzyme results are similar both qualitatively and quantitatively to the results of Evans[14] except that additional enzymic activities have been examined here. The sucrose densities of the mitochondria and plasma membrane containing fractions were approximately 1.08 and 1.17 g/ml, respectively.

The distribution of HCO_3^--ATPase activity and Mg^{2+}-ATPase activity are considered together since the HCO_3^--ATPase is actually a HCO_3^--stimulated, Mg^{2+}-ATPase. The Mg^{2+}-ATPase activity showed a bimodal distribution (figure 1). The inner peak was associated with the mitochondrial fraction and the outer peak was correlated with the plasmalemmal fraction. The HCO_3^--ATPase activity displayed a single peak and its distribution was similar to that of the mitochondrial marker enzymes and the first of the Mg^{2+}-ATPase activity peaks.

The conclusion to be drawn from these data is that a HCO_3^--stimulated, Mg^{2+}-ATPase is associated with mitochondria prepared from the rat liver but not with the plasma membrane fragments. Our previous study with rat liver microsomes showed that plasma membrane vesicles also did not contain HCO_3^--ATPase activity. Thus, we conclude that the rat liver plasma membrane does not contain a HCO_3^--ATPase. This conclusion is valid even if there is an asymmetric distribution of HCO_3^--ATPase activity in the apical, basal or lateral cell membranes since our

previous electron micrographs[19] and those of Evans et al.[20] showed that all of these cell borders are present in typical plasma membrane fractions prepared with the present method.

The present result is of interest in light of the current hypothesis that the HCO_3^--ATPase is involved in the development of pH gradients across cell membranes of secretory organs[5]. In the liver, there are transcellular pH gradients that cannot be explained by a passive distribution of hydrogen or bicarbonate ions, and the liver is also capable of secreting a solution of high bicarbonate ion concentration and high pH[21]. Since this secretion of the liver is similar (in alkalinity) to that of the pancreas, the fact that the liver plasma membrane does not contain a HCO_3^--ATPase while that of the pancreas is thought to[22], is of physiological interest. However, the pancreas results are suspect due to mitochondrial contamination. In conclusion the present results indicate that the HCO_3^--stimulated Mg^{2+}-ATPase is not involved with HCO_3^--secretion into bile since neither rat liver p.m. fragments nor rat liver microsomal vesicles have HCO_3^--ATPase activity.

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Anthelmintic activity of tioxidazole (Sch 21480) against gastrointestinal roundworms

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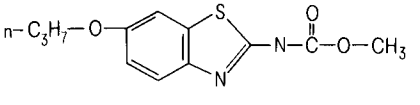
Summary. Methyl-6-propoxybenzothiazole-2-carbamate (tioxidazole) has broad spectrum activity against gastrointestinal nematodes.

We wish to report the discovery of a new anthelmintic agent with broad spectrum activity against gastrointestinal roundworm infections. Tioxidazole is methyl-6-n-propoxybenzothiazole-2-carbamate and has the chemical formula $C_{12}H_{14}N_2O_3S$ (figure). Structural similarity of tioxidazole and benzimidazole anthelmintic compounds should be noted.

Tioxidazole is prepared from 2-amino-6-n-propoxybenzothiazole and methylchlorocarbonate in pyridine as solvent. It is crystallized from ethanol as a stable, white, odorless, powder melting at 178–180 °C. It is insoluble in water and only slightly soluble in most organic solvents. Other methods of preparation have been described[1].

Tioxidazole was effective in mice against *Nematospiroides dubius* and *Syphacia obvelata* when administered at dietary levels of 0.018% to 0.037% or by gavage at 50 mg/kg for 5 days. A single 75 mg/kg oral dose was over 99% effective against a *Trichinella spiralis* infection in mice[2]. Against *Strongyloides ratti* tioxidazole was active by gavage as a single oral dose of 200 mg/kg, 100 mg/kg twice daily, or by 50 mg/kg given for 3 days or 25 mg/kg for 5 days.



Methyl-6-n-propoxybenzothiazole-2-carbamate - (tioxidazole).

In preliminary experiments in dogs, a single oral dose of 200 mg/kg was active against *Toxascaris leonina.* Daily doses of 100 mg/kg for 3 days or 50 mg/kg for 5 days were also effective against hookworms and *Trichuris vulpis.*

Single oral doses of 10–50 mg/kg administered to sheep, naturally or artificially infected, eliminated 87–100% of *Haemonchus spp., Ostertagia spp., Trichostrongylus spp., Marhallagia spp.* or *Cooperia spp.* In critical studies in horses, single oral doses of 5–25 mg/kg were effective in eliminating over 90% of *Parascaris equorum, Oxyuris equi, Strongylus vulgaris, S. edentatus* and a variety of small strongylid worms.

Dose levels up to 100 mg/kg have had no adverse effects in the horse. Further studies to evaluate oral toxicity and teratogenic potential are underway, as are investigations to delineate the compound's safety and field efficiency.

1 U.S. patent No. 4,006,242, issued February 1, 1977 to M.M. Nafissi-V.

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