from the edges of dermal plate ossicles and extend outwards from the central axis of the holothurian at about a 45° angle (figure). This posture permits the pointed hooks of the anchors to be brought to bear against the substratum or, in the case of wounding, set into the sea cucumber's own flesh.

When wounded either in the field or the laboratory, *O. spectabilis* immediately folds its torso towards the wound opening so that the anterior and posterior edges of the wound channel are pressed against one another. Next, peristaltic muscle waves arising from anterior and posterior ends of the animal apply additional force on the appressed sides of the wound closure and set the pointed hooks of anchor ossicles from both edges of the wound into adjacent flesh in a fashion similar to a 'Velcro' clothing fastener. Following completion of self-suturing, *O. spectabilis* resumes normal posture and feeding activity.

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Damage by ozone to the mechanical integrity of the protoplast plasmalemma[1]

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Summary. Ozone acts on the plasmalemma as to weaken its mechanical properties. This results in the bursting of protoplasts.

Pinpointing the action of ozone on cell constituents and cell organelles was started in 1954 when Geise and Christensen[2] demonstrated lipid peroxidation in yeast. However, in order to find the first cell organelle attacked by ozone, attention must be focussed on the plasmalemma, which is considered as the first essential living barrier for ozone to traverse in order to penetrate the cell. Work along this line was only begun in 1963 when oxidative damage to cell plasmalemma of *E. coli* was demonstrated[3]. More specific exploration of the primary target of ozone action on the plasmalemma was achieved by studies on permeability[4-12]. No mention is made in the literature of the action of ozone on the mechanical properties of the plasmalemma, beyond a vague statement that ozone modification of both critical sulfhydryl groups and fatty residues may cause changes in the membrane's fluidity[9]. Thus it has been felt by the authors that investigation of the effect of ozone on the mechanical properties of the plasmalemma might give a new approach to the knowledge of what actually happens.

Materials and methods. Protoplasts from the radish tuber (cultivar 'longs à feuilles courtes') are selected because of the ease with which they are obtained[13], and because they are non-photosynthetic, a feature which eliminates one variable in laboratory light. The stability of protoplasts immediately after washing in T_0 according to Bourgin et al.[14] was also checked. 10-ml suspensions of freshly washed protoplasts from 4 different harvests were placed in 6-cm Petri dishes. Counting of cells was done immediately and thereafter every 30 min for a period of 3 h. In order to work with stable populations (results shown in figure 1), treatment with ozone was always done at least 3 h after harvesting and washing. However, ozone fumigation was always done on the same day, because protoplasts rapidly start regenerating their cell walls and after 3 days provoke a characteristic folding of the external membrane[15].

The classical method of fumigating cell suspensions by a bubbling gas flow, as used for example on *Chlorella*[6], cannot be used with radish protoplasts because preliminary experiments showed that mechanical agitation resulting from the passage of air bubbles was in itself a cause of disruption of the plasmalemma of protoplasts. We therefore spread 10-ml aliquots of the protoplast suspension in T_0 with mannitol as the osmoticum in 6-cm Petri dishes. Prior to fumigation, the number of protoplasts present in the suspension is evaluated by counting them with a Levy-Neubauer hematocimeter. 10 separate counts are made per Petri dish, and the figures are averaged. Half of Petri dishes are then placed in the ozone fumigation chamber while the other half are left exposed to laboratory air. After the fumigation period, the protoplasts are again counted both in the experimental and the control dishes. Ozone is

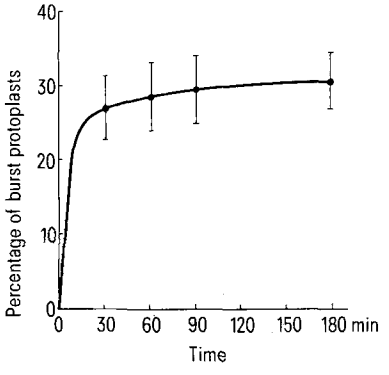

Fig. 1. Rate of bursting of protoplasts immediately after harvesting and washing.

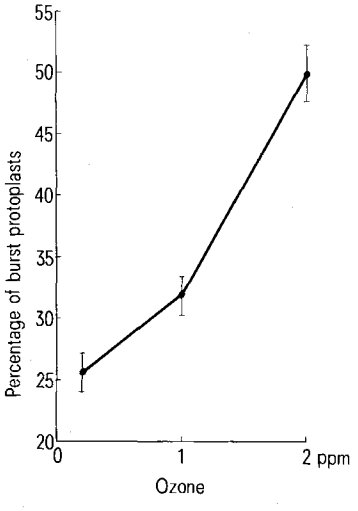

Fig. 2. Percentage of burst protoplasts resulting from a 30-min ozone fumigation at 0.2, 1 and 2 ppm in air.

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