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THE EFFECT OF LSD ON THE SURFACE OF NEUROEPITHELIAL CELLS

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SUMMARY

The neuroepithelial cells of 10 control chick embryos and of 22 exposed to lysergic acid diethylamide (LSD) (50 μg/ml) were examined in scanning electron microscopy (SEM). In specimens exposed to LSD, the cells are swollen and their surface loses its cytoplasmic projections. Labelling techniques applied in transmission electron microscopy (TEM) show that ruthenium red attaches to the surface of the neuroepithelial cells in the form of a continuous dark line in both controls and treated specimens. However, when cationized ferritin or lanthanum is used, the label appears in the form of a continuous line in the controls whereas it is discontinuous in specimens exposed to LSD. These observations suggest that LSD alters the components of the neuroepithelial cell surface in the young chick embryo.

INTRODUCTION

The teratologic and cytotoxic effects of LSD have been investigated in a wide variety of biological models [1—6]. Yet, as part of the ongoing search for an understanding of its mode of action at the cellular level, relatively few studies have considered the effect of the drug on the cell structure [7,8]. Moreover, we know of no morphological studies attempting to deal directly with the possibility that LSD interferes with the constituents of the cell surface.

In the chick embryo growing in vitro, it has been shown that concentrations of LSD as low as 0.5 and 2.5 μg (per ml of culture medium) delay the segmentation of paraxial mesoderm [9] and that doses of 50 and 100 μg/ml

Abbreviations: LSD, lysergic acid diethylamide; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

disturb the fusion of the neural folds [10]. Recently, using the sensitivity and accuracy afforded by stereological methods, we were able to show that the drug induces an increase in the volume fraction of the cytoplasm of the neuroepithelial cells in the chick embryo [11]. This finding led us to suspect that LSD might modify the nature of the cell surface. The present study was undertaken to clarify this point. In an attempt to detect the changes that might occur in the components of the cell membrane as a result of exposures to LSD we use SEM to study the morphological aspect of the cell surface and use labels in TEM.

MATERIALS AND METHODS

Scanning electron microscopy

Twenty-two chick embryos (*Gallus domesticus*) aged approx. 30 h (at stages 7—9 according to Hamburger and Hamilton [12]) were explanted and cultured for 5 h at 38°C on Spratt's [13] medium containing 50 μg of LSD per ml of culture medium. Ten control embryos of comparable stages were cultured indentically on unmodified Spratt medium. The embryos were fixed for 20 h in 2.5% glutaraldehyde in phosphate buffer. A wash in the buffer followed, during which the embryos were cut transversally just beneath the hind brain with cactus needles as described by Seymour and Berry [14]. The pieces were post-fixed for 1 h in phosphate buffered osmium tetroxide (1%), dehydrated in a graded series of ethanol, passed through freons and critical point dried in a Bomar apparatus (The Bomar Co., P.O. Box 225, Tacona, Wash. 98401, U.S.A.). The dried specimens were attached on stubs with conductivity paint and coated with gold/palladium (60/40) in a Diode Sputtering System Type E5000 (Polaron Equipment, 60162 Greenhill Crescent, Watford, Hertfordshire, England). They were examined with a JEOL 50A SEM microscope.

Labelling and transmission electron microscopy

Control and experimental embryos were cultured and treated as described above. Their vitelline membrane was always removed before labelling. Since a correlation has been reported to exist between the state of the cell surface material and the degree of organisation during neurulation [15], great care was taken to use only those specimens that had reached exactly the same stage of development when control and experimental embryos were compared.

Ruthenium red

Following a 4-h growth in vitro, the embryos were fixed for 5 min in 1.75% cacodylate-buffered glutaraldehyde and then fixed for 3 h at 6°C in a similar glutaraldehyde solution containing ruthenium red (0.17%). Afterwards, the specimens were washed three times in the cacodylate buffer, stored overnight at 6°C and then fixed for 1 h in a 0.7% cacodylate-buffered osmium tetroxide solution containing ruthenium red (0.17%). Ten controls and 6 LSD-treated embryos were thus prepared according to this procedure described by Shigematsu and Dmochowski [16].

Cationized ferritin

Ten controls and 4 experimental embryos, following a 4-h growth in vitro, were immersed for 30 min, at room temperature, in a solution made of 4 ml of sodium barbital buffer and 1 ml of cationized ferritin (Miles—Yeda Ltd., Rehovoth, Israel). They were rinsed in the same buffer, fixed for 1 h in phosphate buffered glutaraldehyde and stored overnight in the phosphate buffer. Finally, the specimens were fixed for 1 h in phosphate buffered osmium tetroxide. Our technique is based on Ackerman's [17].

Lanthanum

Following a 5-h growth in vitro, 14 controls and 12 experimental embryos were fixed for 1 h at room temperature in a 2.5% glutaraldehyde solution buffered according to Millonig [18] and containing 0.5% alcian blue. The specimens were then washed in the same buffer and post-fixed for 30 min in a 1% buffered osmium tetroxide solution to which 1% lanthanum nitrate was added. This procedure derives from Moran and Rice's [15].

Labelling and fixation having been completed, all embryos were dehydrated in ethanol solutions after which they were embedded in Epon. The thin sections, obtained with an LKB ultrotome, were stained at room temperature, first with 1% aqueous uranyl acetate for 20 min and then with lead citrate for the same period. However, the specimens labelled with cationized ferritin were stained for 5 min in a 50% solution saturated with uranyl acetate and for 4 min with lead citrate. The grids were examined either with a Siemens 1 A or with a Philips 200 electron microscope.

RESULTS

In SEM, the normal embryos examined, which were cut just beneath the hind brain, regularly showed a neuroepithelium that has invaginated and closed into an approximately round-shaped neural tube (Fig. 1). On the contrary, in specimens exposed to LSD, a collapse of the roof of the neural tube was often observed. This caving-in of the upper part of the neuroepithelium, in most cases, closed the neurocoele entirely (Fig. 2).

The cells making up the neuroepithelium of the normal chick embryo are highly elongated and form a pseudostratified columnar epithelium (Fig. 3). In most cases, each cell was seen to possess a slender cytoplasmic process, usually extending to the neurocoele, and a broad portion containing the interphase nucleus which was usually located towards the base of the epithelium (Fig. 3). It is well known that, as part of the phenomenon of interkinetic nuclear migration, these nuclei move up and down in the epithelium. Ordinarily, it is possible to determine where the nucleus is situated as a bulge appears along the cell body (Fig. 3). By far, most often, such is not the case in embryos exposed to LSD. Indeed, here, the cells seem turgid, they are swollen and exhibit almost straight walls. This loss of a distinctive cellular contour usually prevents the location of the nucleus, as no bulge is evident (Fig. 4).

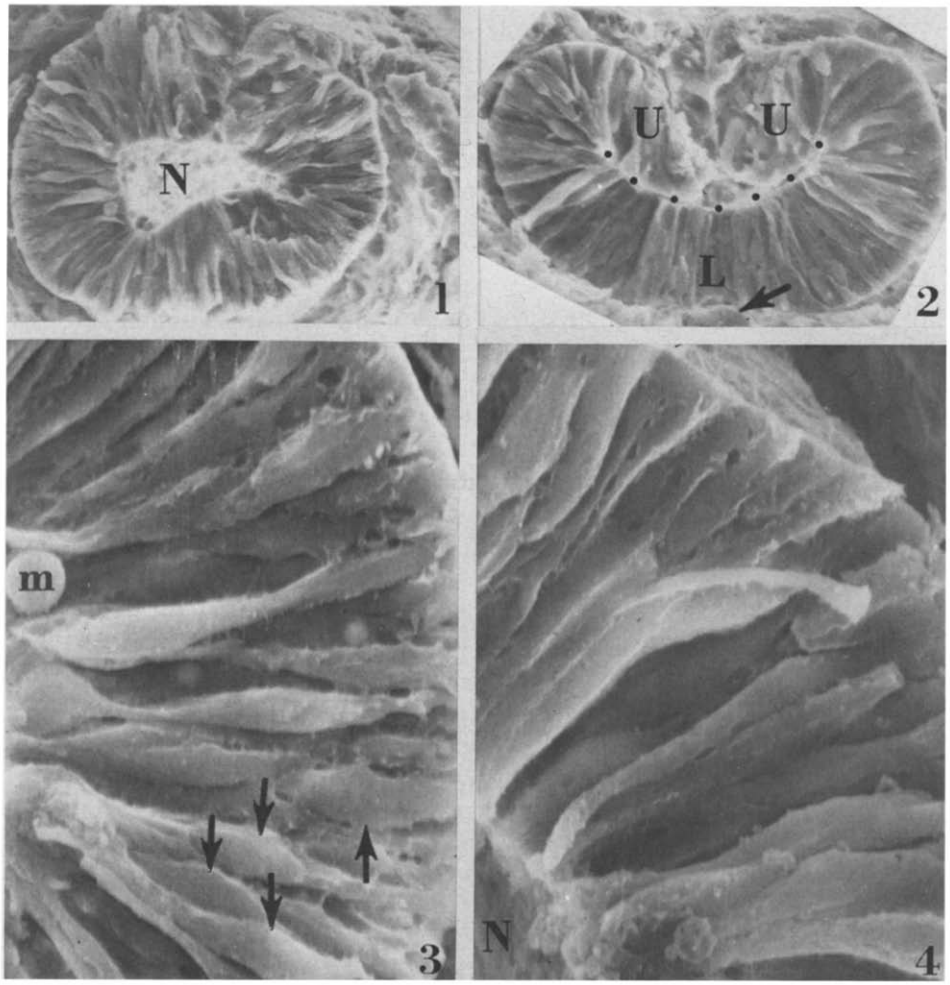


Fig. 1. Scanning electron micrograph showing the general features of the neural tube of an embryo cultured on the unmodified medium and sectioned beneath the hind brain. The neuroepithelium has rolled up into a tube endowed with a lumen, termed the neurocoele (N). × 380.

Fig. 2. SEM micrograph of the neuroepithelium of a specimen exposed to LSD (50 μg/ml). The upper portion (U) of the neuroepithelium has come to touch the lower part (L) of the epithelium. This collapse of the roof of the neural tube closes the neurocoele (dotted line) almost entirely. The notochorde (arrow) is shown. × 428.

Fig. 3. Enlarged portion of Fig. 1. The cells are highly elongated, they possess a characteristic contour and often show a slender process. Bulges, along the cell body (arrows), presumably indicate location of nuclei. The neurocoele, not shown, is towards the left hand side; mitosis (m). Approx. × 570.

Fig. 4. Neuroepithelial cells from an embryo exposed to LSD. These cells have lost their distinctive contour. They appear turgid. Usually, the nucleus cannot be located because of the generalized swelling. Neurocoele (N). Approx. × 1520.

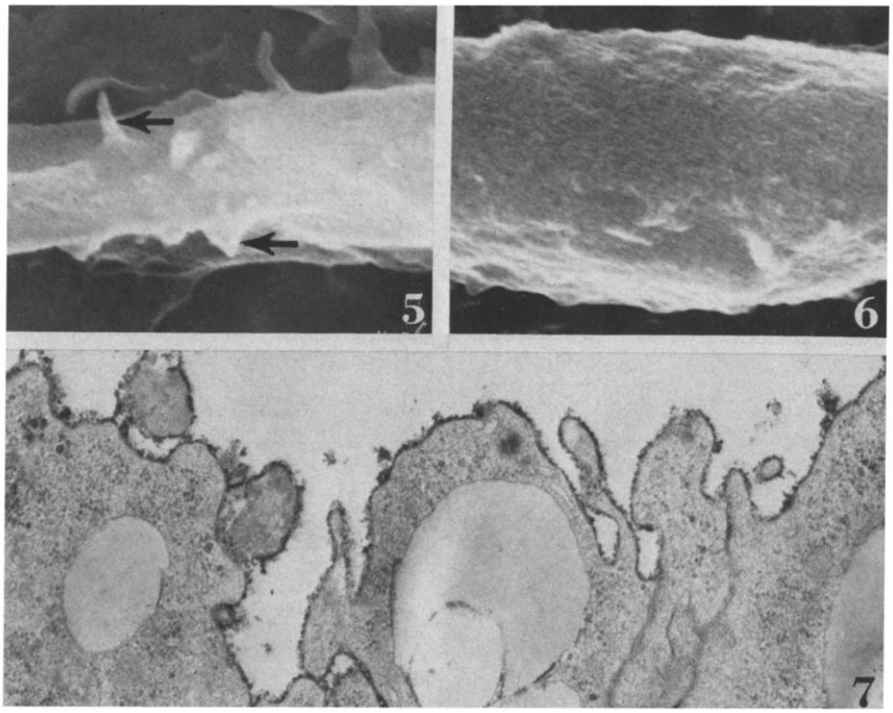


Fig. 5. Portion of a cell body from a control embryo showing cytoplasmic projections (arrows). × 9500.

Fig. 6. Portion of a cell body from an LSD-treated embryo. The surface is devoid of cytoplasmic projection. × 9500.

Fig. 7. Apical portions of a neuroepithelial cell from an embryo grown on the unmodified medium and labelled with ruthenium red. The marker is seen in the form of a thin and continuous dark line. × 28 500.

Finally, in embryos grown on the unmodified medium, the surface texture of the cell was relatively smooth; with some ruffling. Also, short fringe-like or slender cytoplasmic projections were seen to protrude, at random, from the surface of the cells (Fig. 5). Following LSD treatment, the majority of cells had lost most, if not all, of their slender cytoplasmic projections (Fig. 6).

In TEM, the use of ruthenium red as a cell surface marker gave comparable results whether it was applied to controls or to LSD-treated specimens. In both cases, the label was attached to the surface of the apical membrane of the neuroepithelial cells in the form of a continuous dark line (Figs. 7 and 8). However, cationized ferritin, which was attached in a continuous fashion at the surface of the cells in the control embryos (Figs. 9 and 10), was found in the form of a discontinuous line in embryos exposed to LSD (Fig. 11). Similarly, in controls, lanthanum is bound to the cell surface in the form of

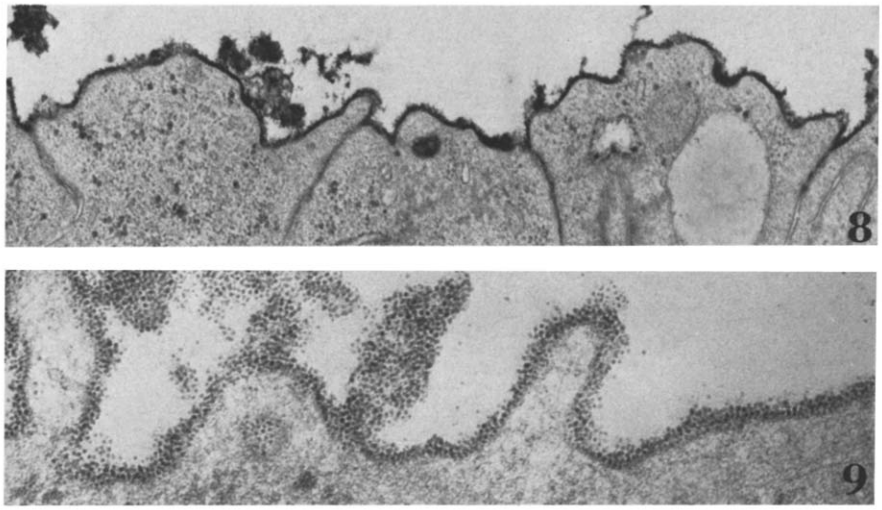


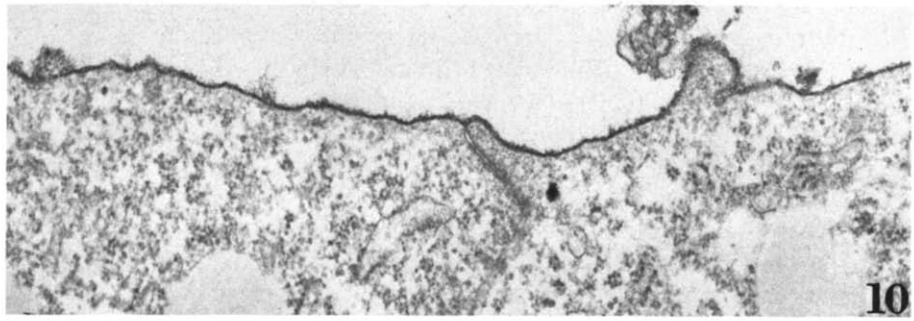
Fig. 8. Apical portions of neuroepithelial cells from an embryo cultured on a medium added with LSD. The label used, ruthenium red, forms a continuous dark line at the surface of the cells. × 28 500.

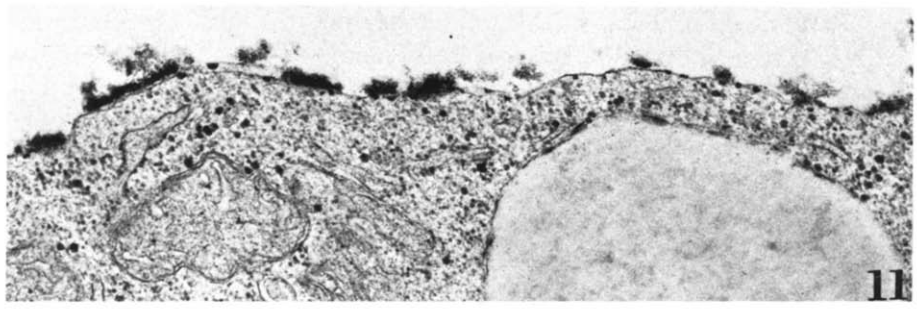
Fig. 9. Moderately high magnification of the apical region of cell from a control embryo labelled with cationized ferritin. The ferritin molecules are easily recognized as small dark dots; the marker forms a continuous line at the cell surface. × 66 500.

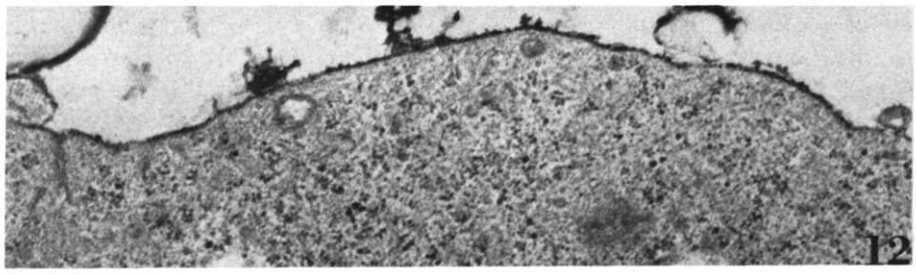
an uninterrupted dark line (Fig. 12) whereas, in the treated embryos, the labelling appears as a discontinuous dense line (Fig. 13).

DISCUSSION

Cultured mouse cerebellar neurons exposed to LSD show marked changes of lysosomes ultrastructure [7]. Similarly, rat spinal ganglia cultures maintained in a solution containing LSD reveal alterations in the lysosomes, Golgi complexes, endoplasmic reticulum, mitochondria and multivesicular bodies [8]. These cytoplasmic organelles always exhibit conspicuous abnormalities of the membranous components. In the chick embryo, thus far, only the ultrastructure of nuclei is definitely affected by LSD [9]. The changes in the volume of mitochondria, that may occasionally be observed in chick embryos exposed to LSD [9], turn out to be statistically insignificant when submitted to the stringent morphometric methods of stereology [11]. Stereology also established that, in the chick embryo, the endoplasmic reticulum is not disturbed by LSD [11]. It appears, therefore, that the membrane-rich organelles of the chick embryo suffer less from LSD exposures than do those of other biological models. However, in the present study, it is shown that the surface membrane of the neuroepithelial cells is affected in chick embryo grown on LSD-containing medium. Firstly, the components of the plasma membrane are disturbed as evinced by the modifications in the labelling







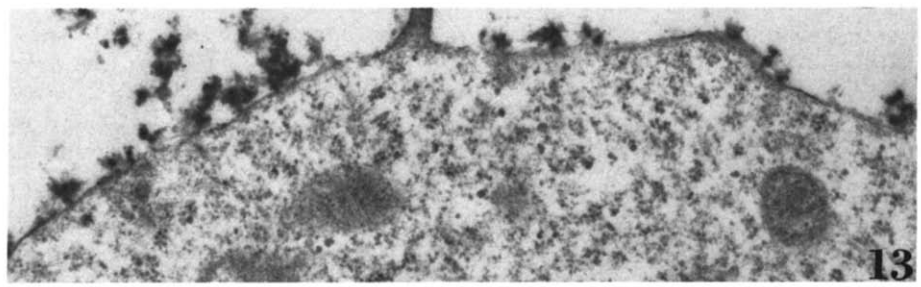


Fig. 10. Cationized ferritin, forming a continuous line at the surface of cells from a control embryo. × 28 500.

Fig. 11. Cationized ferritin, forming patches of label, at the surface of a neuroepithelial cell from an LSD-treated embryo. × 28 500.

Fig. 12. Lanthanum label attached in the form of a continuous line at the surface of a neuroepithelial cell from a control embryo. × 28 500.

Fig. 13. Lanthanum label forming patches at the surface of a neuroepithelial cell from an LSD-treated embryo. × 28 500.

pattern observed and, secondly, the cells lose their cytoplasmic projections, an event reflected as a smoothening of the cell surface in scanning electron micrographs. In our study, the region of the cell surface analysed differs according to the approach selected. On the one hand, the labelling procedures relate only to the apical membrane of the cell since the labels used do not permeate the cell junctions. On the other hand our SEM analysis applies only to the lateral membranes. The results obtained, through one or the other approach, ought not be generalized; more work is needed before conclusions are drawn concerning the response of the cell surface as a whole.

In the last decade, methods have been developed for the detection of cell surface components in TEM. Such methods include the ruthenium red reaction. It makes use of a small polyanion (ruthenium red) that reacts specifically with acid mucopolysaccharides, acid phospholipids (mainly phosphatidyl ethanolamine) and polyglutamic acid [19], all of which are incorporated in the glycocalyx or cell coat. This cell coat is viewed by Bennett [20] as an integral part of the cell membrane. The results presented here indicate that the components ruthenium red reacts with have not been disturbed by exposure to LSD. Such is not the case, however, when cationized ferritin or lanthanum is used.

Cationized ferritin is a polycationic derivative of ferritin known to be a selective compound for labelling negative charges on the cell surface [21]. These negative charges are largely due to the carboxyl groups of neuroaminic acid [22]. It is thought that, in our material, the distribution of the negative charges is modified since cationized ferritin attaches to the cell surface of the neuroepithelial cells in a continuous fashion in the controls, while it appears in the form of a discontinuous line in embryos exposed to LSD. Similarly, in embryos grown on the LSD-containing medium, lanthanum is bound in a discontinuous fashion. Lanthanum has been reported to bind to phospholipids, proteins and polysaccharides [23,24]; an alteration in the distribution of these components, separately or in any combination, may be responsible for the modified labelling pattern observed. It should be stressed, however, that the labelling procedures used do not allow us to ascertain which specific component of the glycocalyx, or plasma membrane, is disturbed by LSD. It is only evident that the membrane is somehow affected.

Numerous studies (e.g.[25,26]) suggest that a specific make up of the cell surface is important during cell differentiation and tissue organisation. For instance, the changes observed in the adhesiveness of cells during morphogenetic movements [27,28] point to programmed modifications in the chemical composition of the cell surface as being important in normal cell differentiation. Thus, it is felt, that the alterations in the membrane components, which are borne out by labelling procedures, could possibly account for the observed collapse of the roof of the neural tube and for the abnormalities in the closure of the neural tube which are known to occur in chicks exposed to LSD [6].

The use of SEM confirms the observation obtained earlier, through a totally different approach [11], that the neuroepithelial cells swell under

LSD exposures. Furthermore, the technique is invaluable in sparing us serial sectioning to show the disappearance of the cytoplasmic projection along the lateral cell membrane. This loss is of consequence as it points to a reduction in (a) cell plasticity, (b) cell intercommunication and (c) possible changes in the molecular make up of the membrane, all of which have bearing on organogenesis. Evidently, very little is available that would permit an appreciation of the extent to which the observations reported here reflect a specific response to LSD. Consequently, it is worth mentioning those effects of cytochalasin B that may be relevant to the present work. Cytochalasin B is not related to hallucinogenic drugs; it is a metabolite of the mold *Helminthosporium dematioideum*. Its effects on cell behaviour, physiology, chemistry and structure are well documented though its mode of action is quite controversial [29]. Finally, the substance inhibits neurulation in the chick embryo [30,31]. A SEM study of the effect of cytochalasin B on the surface membrane of the neuroepithelium of chick embryos showed that it (a) smoothens the cell surface and (b) induces the formation of numerous craters, or cavities [32]. Cavities have not been observed as a result of LSD exposure. On the other hand, specimens treated with cytochalasin B and labelled with ruthenium red or lanthanum show a labelling pattern entirely comparable to that which is found in controls (unpublished observations). It follows that, in the chick embryo, LSD elicits a response which is different from that produced by another chemical, tested on the same system and analysed in the same way.

In conclusion, the extrapolation to humans of the observations reported here is hazardous. Yet, the alterations noted in membrane components are important and suggest more work should be done. For instance, it would be appropriate to investigate, in a well suited system, whether the changes in the membrane charges observed here can induce derangements in the excitability characteristic that would alter the firing pattern of nerve cells as was observed in the brain [33,34].

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