The effect of chloroquine upon the developing lens[1]

L. Barr-Nea, Y.R. Barishak and S. Riklis

The Department of Histology and Cell Biology, and the Mitrani Foundation Eye Pathology Laboratory at the Chaim Sheba Medical Center, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv 61999 (Israel), 31 August 1978

Summary. Applied to the developing lens of the 14-day-old chick embryo, in organ culture conditions, chloroquine prevented the elongation of the primary lens fibres, destroyed the equatorial ones and provoked vacuolisation and/or destruction in the epithelial cells.

The ophthalmic literature contains some clinical information upon the controversial effect of chloroquine upon the lens[2,3], but no experimental work dealing with this. Moreover, it has been shown that chloroquine accumulates in fetuses[4,5]; the question arose whether chloroquine given to a pregnant woman might affect the developing lens of the fetus. To study this problem, we cultivated in vitro whole lenses of chick embryos. We want to emphasize that this technic permits us to know the exact concentration of the drug coming into contact with the lens.

Material and methods. The 14-day-old chick embryo has been chosen as a source of material, since at this age the embryonal lens fibres are still able to elongate[6]. 8 series of experiments have been performed. The eyes were opened at the limbus, the lens removed in toto and cultured in vitro by a modification of the millipore filter organ culture method[7] already used in our laboratory for the study of the retinal pigment epithelium[8]. The culture medium contained Eagle BME (Gibco, Long Island, NY), alone or with the addition of 50 or 100 µg of chloroquine phosphate per ml. (Assia Laboratory, Jerusalem). The cultures were kept in the incubator at 37 °C for 5 days. Lenses in culture medium without chloroquine were used as controls. Both control and chloroquine treated lenses were removed at the end of the experiment, fixed in formalin, embedded in Bioloid and cut serially. Sections were stained with hematoxylin and eosin and PAS.

Results. After 5 days in culture, control lenses were transparent and presented a normal thickness and a biconvex shape, while those treated with chloroquine were opaque and flattened (figure 1,a) In normal lenses, the primary fibres underwent full elongation, and as a result the nuclear bow was near the anterior epithelium, as it is in vivo. Some fibre bundles presented a granular appearance and the anterior epithelium was multilayered. Chloroquine at the dosis of 50 µg/ml prevented the elongation of the primary fibres and caused a moderate flattening of the lens (figure 1, b). The epithelium cells showed severe vacuolisation and/or destruction (figure 2). In cultures treated with 100 µg/ml of chloroquine, the lenses were more flattened, specially at the equatorial area (figure 1,c). The equatorial fibres were destroyed, while the central ones showed a granular appearance (figure 3). The epithelial cells showed a more advanced vacuolisation and destruction than those described in the previous group. The lens capsule was not affected in any group.

Discussion. Chloroquine induced pathological changes in the developing lens of the 14-day-old chick embryo culti-

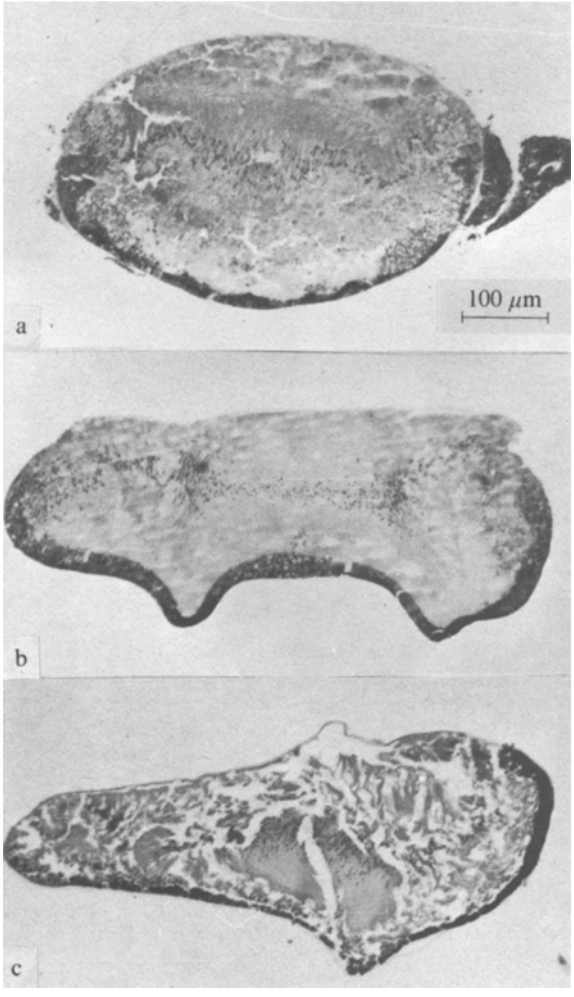

Fig. 1. Flattening of the chick embryonic lens treated with chloroquine in organ culture condition. *a* Control lens, *b* lens treated with 50 µg/ml of chloroquine, *c* lens treated with 100 µg/ml of chloroquine. × 120.

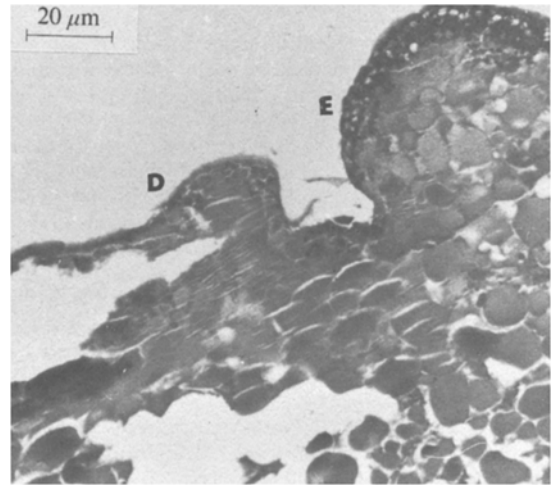

Fig. 2. Lens treated with 50 µg/ml of chloroquine. Normal and destroyed epithelium. Fibre bundles in different stages of destruction. × 480.

vated in vitro and the changes induced by the dosis of 100 µg/ml were more severe and more widespread than those induced by 50 µg/ml. Experiments were performed with the dosis of 25 µg of chloroquine per ml, but the results were inconstant and therefore the results of these experiments have not been reported. Chloroquine at the dosis of 150 µg/ml caused a complete destruction of the lens.

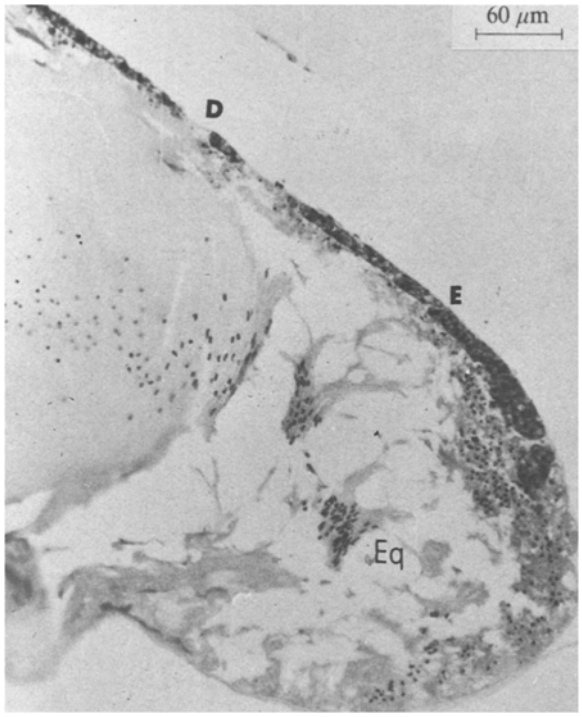

Fig. 3. Lens treated with 100 µg/ml of chloroquine. Severe destruction of equatorial fibres. × 160.

Ciak and Hahn[9] showed that chloroquine blocks protein synthesis by inhibiting DNA and RNA polymerases. This mechanism might explain the lack of elongation of lens fibres. As to the destruction of lens fibres, this might be explained by the formation of toxic vacuoles as described by Fedorko et al.[10]. The results reported above suggest that chloroquine treatment of women during the period of fertility should be undertaken with caution, in spite of the fact that susceptibility of the developing lens of the chick embryo to chloroquine could not be compared to that of human embryo.

1 This work was supported by a grant from the office of the Chief Scientist, Ministry of Health, Israel.
2 H.E. Hobbs, S.P. Eadie and F. Sommerville, Br. J. Ophthal. *45*, 284 (1961).
3 P. Henkind and N.F. Rothfield, N. Engl. J. Med. *269*, 433 (1963).
4 N.G. Lindquist and S.E. Sjostrand, Nature *227*, 1257 (1970).
5 N.G. Lindquist, Acta radiol. *325*, 1 (1973).
6 J. Piatigorsky and S.S. Rotschild, Dev. Biol. *28*, 382 (1972).
7 M.O. Tso, D. Albert and L.E. Zimmerman, Invest. Ophthal. *12*, 554 (1973).
8 Y.R. Barishak, L. Barr-Nea and Y. Messer, Ophthal. Res. *8*, 17 (1976).
9 J. Ciak and F.E. Hahn, Science *151*, 347 (1966).
10 M.E. Fedorko, J.G. Hirsch and Z.A. Cohn, J. Cell Biol. *38*, 392 (1968).

^{125}I-Insulin is preferentially internalized in regions of the hepatocytes rich in lysosomal and Golgi structures

J.-L. Carpentier, P. Gorden[1], P. Freychet[2], A. Le Cam[2] and L. Orci[3]

Institute of Histology and Embryology, Geneva University School of Medicine, CH-1211 Geneva 4 (Switzerland), 28 February 1979

Summary. ^{125}I-Insulin initially localizes to the plasma membrane of isolated rat hepatocytes but is subsequently internalized and preferentially associates with lysosomal structures. In the present study, we show that this preferential association to lysosomes occurs in regions of the cell rich in lysosomal and Golgi structures.

We have demonstrated that ^{125}I-insulin initially localizes to the plasma membrane of isolated rat hepatocytes[4-6]. A portion of the labelled material is subsequently internalized by the cell as a constant function of binding and preferentially associates with lysosomal structures intracellularly[7,8]. Qualitatively it appeared that not only were intracellular autoradiographic grains preferentially localized to lysosomal structures but, in addition, grains appeared to be localized in regions of the cell rich in lysosomal and Golgi structures. In the present study we have quantitatively verified this observation.

Materials and methods. Hepatocytes were isolated from normal 6-8-week-old Wistar rats fed ad libitum, using a modification of the method described by Seglen[9]. The percentage of parenchymal cells in the purified cell suspension exceeds 95%; the viability of the cell suspension estimated by the trypan blue exclusion and by morphological criteria exceeds 85-95%. ^{125}I-insulin was prepared at a sp. act. of 250 µCi/µg by a modification of the chloramine-T-method[10]. The labelled insulin was purified by filtration on G-50 Sephadex at 4 °C prior to each experiment. Hepatocytes (1×10^6 cells/ml) were incubated in 0.5 ml of modified KRB (pH 7.7) containing 25 mg/ml bovine serum albumin (fraction V) and 0.8 mg/ml of bacitracin with 5×10^{-10} M (3 ng/ml) ^{125}I-insulin at 37 °C for a maximum of 60 min. Incubation, fixation and processing for autoradiography were carried out as previously[5,7,11]. Samples were examined in a Philips EM 300 electron microscope (Philips Instruments Eindhoven, The Netherlands) and photographs taken at a magnification (× 9000) calibrated with a reference grid 2160 lines/mm (Fullam Inc., Schenectady, N.Y.). For each experimental condition tested (unincubated cells, cells incubated for 5 min at 20 °C and cells incubated for 30/60 min at 37 °C) 2 types of pictures were taken: pictures with autoradiographic grains and random pictures. Pictures with grains were taken in cells that were