sample from saline-treated rats when suspended in the isotonic buffer (A). Saline-hypertonic treatment (B) caused the increase in the percentage of echinocytes. A single β-PC 300 mg/kg - hypertonic treatment (C) significantly ($p < 0.02$) decreased the percentage of echinocytes. Repeated β-PC 100 mg/kg - hypertonic treatment (E) more significantly ($p < 0.001$) decreased the percentage of echinocytes as compared with the values for the corresponding control (D), the latter values being similar to B.

Discussion. Keeping the erythrocyte in hyperosmolarity is known to cause both a shape change of the erythrocyte from discocyte to echinocyte and a decrease in the deformability of the erythrocyte, which is reflected in the decrease in filterability[10,11]. The present finding of the preventive effect of β-PC on the increase in the filtration time of the blood suspension by hyperosmolarity suggests an improving action of the drug on erythrocyte deformability. This is further supported by the morphological evidence that β-PC at comparable doses to those in the filterability experiments also prevents the increase in percentage appearance of the echinocyte shape of erythrocytes caused by hyperosmolarity. The shape and function of the erythrocyte depend at lease in part on the concentration and composition of circulating lipoproteins; LDL interact at the exterior surface of the erythrocyte to stimulate the dephosphorylation of spectrin[5], which is supporting the shape of the eryhtrocyte[12]. LDL thus cause the shape change of the erythrocyte[4] On the other hand HDL prevent the LDL-induced activation of membrane phosphatase[5]. Therefore, both a high concentration of LDL and a low concentration of HDL are likely to decrease the deformability of the erythrocyte. β-PC lowers LDL and elevates HDL[2]. The erythrocyte deformability improving action of this drug may therefore be well explained by its effects on the circulating lipoproteins, although the plasma content of lipoproteins was not measured under the present experimental conditions. β-PC was inactive in vitro or at a single dose of 100 mg/kg p.o. but was active at the same dose when given repeatedly. These results also support the above theory, since the effect of β-PC on the lipoproteins requires some time before it becomes detectable[2]. The inferior effect of nicotinic acid on erythrocyte filterability, compared with β-PC, may come from its poor bioavailability as compared with β-PC[13,14].

In conclusion, β-PC was found to improve erythrocyte deformability, which may be explained speculatively by its LDL-lowering and HDL-elevating effects. Erythrocyte deformability has been reported to be decreased in various diseases characterized by high blood viscosity and local hyperosmolarity, especially in ischemic diseases[15] and hyperlipoproteinemia[16]. One of the rational approaches to preventing a reduced flow rate of blood is to decrease blood viscosity, and since it has been claimed that this can be achieved by improving the erythrocyte deformability[10], the present findings are believed to have a potential significance in explaining the role of β-PC in the medication of ischemic diseases and hyperlipoproteinemia.

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Thymidine: inhibitor of differentiation in the young chick blastoderm in culture

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Summary. Differentiation in the young chick blastoderm is affected by thymidine at concentrations higher than 8.2×10^{-4} M. Blastoderms at the hypoblastic island stage cultured continuously in the presence of thymidine form an atypical primitive streak which is not capable of inducing the embryonic axis. However, blastoderms with a mature streak escape the effect of thymidine and develop normally.

In the course of work on the effects of the thymidine analogue 5-bromodeoxyuridine (BUdR) on early chick blastoderm axialization (Zagris and Eyal-Giladi, in prep.), we used the thymidine to alleviate the inhibitory action of BUdR, and observed that thymidine itself had unusual effects interfering with normal morphogenesis.

Earlier work has shown that injection of deoxyriboside triphosphates into fertilized eggs of *Xenopus laevis* affected morphogenesis by prolonging the blastula stage and by synchronizing cell division at this relatively late stage[3]. In the present work, we studied the effect of thymidine on 2 representative, one pre-streak and one streak, stages of the developing young chick blastoderm.

Materials and methods. Freshly laid fertile chicken eggs were incubated for 7 h (hypoblastic island stage XI, Eyal-Giladi and Kochav[4]), or 19 h (definitive streak stage 4, Hamburger and Hamilton[5]) at 38 °C. The explanted blastoderms were cleaned of any adhering yolk in Ringer solution and were cultured in thin egg albumen (2 ml/blastoderm) according to New[6].

Thymidine (Sigma) dissolved in Ringer solution was included in the culture medium at a concentration of

1.23×10^{-3} M. Development of blastoderms was followed for a 37 h period and was compared with control blastoderms. Observations were made on a total of 20 to 25 blastoderms per group.

Blastoderms were fixed in Carnoy's fixative and embedded in paraffin. Sections (10 µm) stained with hematoxylin and eosin were examined histologically.

Results. Working with various concentrations of thymidine, we found that this deoxynucleotide interferes with normal early chick blastoderm development at concentrations higher than 8.2×10^{-4} M. In all the experiments reported in this paper 1.23×10^{-3} M thymidine was used because it was the concentration with which consistent results were obtained.

Blastoderms explanted at stage XI[4] and cultured continuously in the presence of thymidine start formation of a primitive streak (PS) which is transformed into a central cellular mass and show no signs of an embryonic axis after 12 h (fig. 1a), and after 37 h (fig. 1b) of culture. However, blastoderms of the same age cultured in medium containing thymidine for 12 h, then washed with Ringer solution several times and cultured in plain albumen for an additional 25 h did develop a shortened embryonic axis with fragmentary notochords and irregularly segmented lateral mesoderm (fig. 2).

Blastoderms explanted at stage 4[5] and cultured in the presence of thymidine for 37 h seem to have escaped the thymidine effect. Observations after 12 h (fig. 3a) and after 37 h (fig. 3b) of culture show normal development.

Control blastoderms explanted at stage XI[4] and cultured in plain egg albumen for 37 h develop normally (fig. 4).

A histological transverse section through the central cellular aggregation of the blastoderm pictured in figure 1b shows presence of mesenchyme, blood cell formation, formation

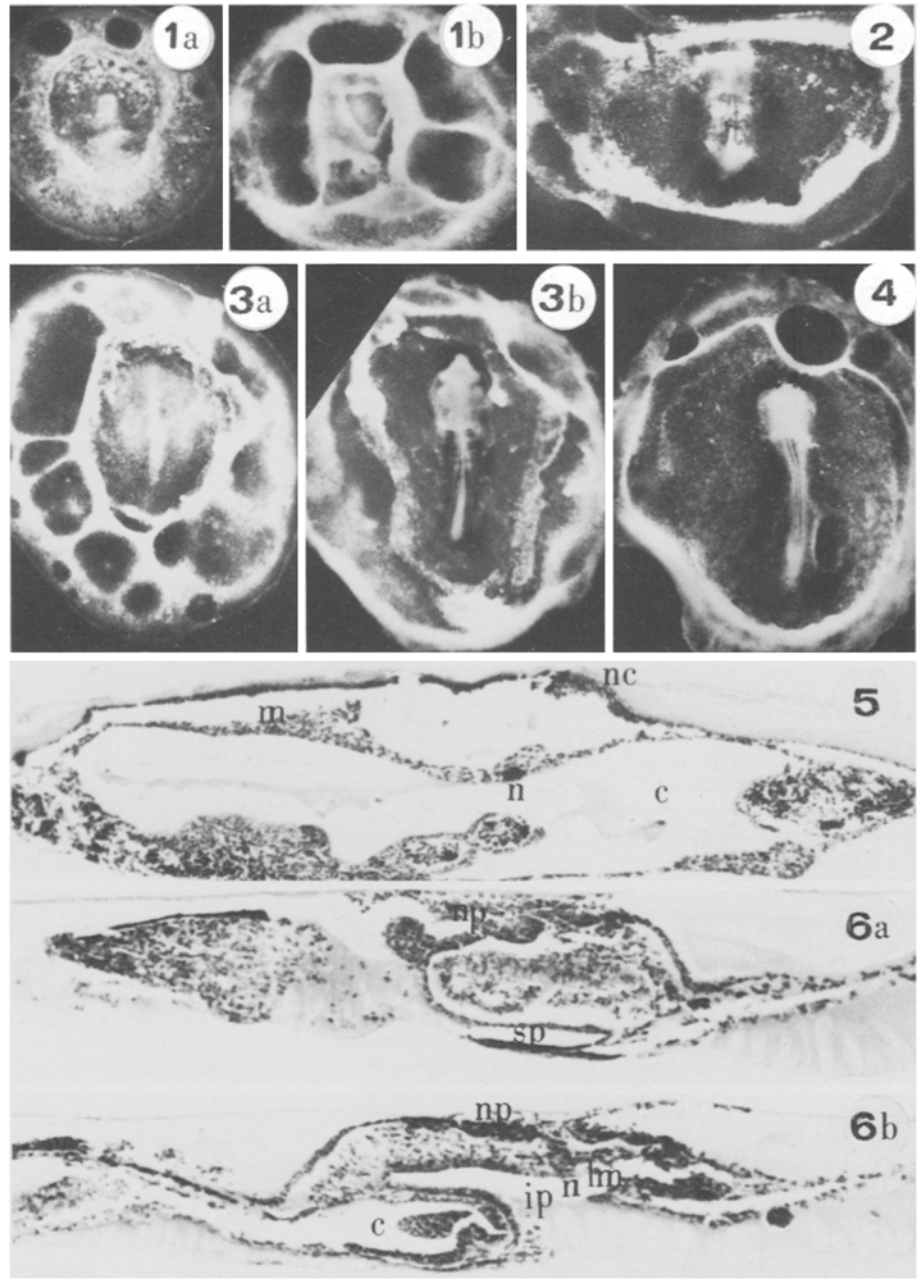

Figure 1. Chick blastoderm explanted at stage XI[4] and cultured continuously in egg albumen containing 1.23×10^{-3} M thymidine, after 12 h (a) and after 37 h (b) of culture. ×31.

Figure 2. Chick blatoderm explanted at stage XI[4] cultured in egg albumen containing 1.23×10^{-3} M thymidine for 12 h, washed in Ringer solution and cultured in plain albumen for an additional 25 h. ×31.

Figure 3. Chick blastoderm explanted at stage 4[5] and cultured continuously in egg albumen containing 1.23×10^{-3} M thymidine, after 12 h (a) and after 37 h (b) of culture. ×31.

Figure 4. Control chick blastoderm explanted at stage XI[4] and cultured for 37 h in egg albumen. ×31.

Figure 5. Transverse section through the central cellular aggregation of the blastoderm presented in figure 1b. Section (10 µm) stained with hematoxylin-eosin. ×86. c: coelom; m: mesenchyme; n: notochord; nc: neural cluster.

Figure 6. Transverse sections through head (a) and notochord (b) regions of the blastoderm presented in figure 2. Sections (10 µm) stained with hematoxylin-eosin. ×86. c: coelom; ip: anterior intestinal portal; lm: lateral mesoderm; m: mesenchyme; n: notochord; nc: neural cluster; np: neural plate; sp: subcephalic pocket.

of neural cell cluster, and of a notochord which appears in 3 sections only (fig. 5).
Transverse section through the head region of the blastoderm presented in figure 2 shows an open neural plate but no identifiable brain structure (fig. 6a). A more posterior section shows a discontinuous neural plate, a notochord, and also lateral mesoderm with some indication of segmentation (fig. 6b).
Histological examination of the blastoderms pictured in figures 3b and 4 shows normal development.

Discussion. The effect of thymidine on chick blastoderm differentiation depends on the stage of development and on the length of time blastoderms are exposed to thymidine. Our results indicate that it is the process of PS formation which is sensitive to thymidine. We assume that the severely defective neural plate is an expression of the thymidine interference with determination of the axial mesoderm and its inductivity. Blastoderms at stage 4[5] with a mature PS escape the effect of thymidine and develop normally, probably because the subsequent differentiation and inductive role of the different PS components are already determined.
It is likely that thymidine exerts its effect on young blastoderms by increasing their thymidine triphosphate pool, thus disturbing the delicate balance that must exist among the nucleotide pools and initiating a chain of events which leads to interference with differentiation.

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Proximal tubule changes in the polycystic kidney induced by methylprednisolone acetate in the newborn rabbit. A microdissection-SEM study[1]

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Summary. A single i.m. injection of methylprednisolone acetate, given to rabbits within 24 h after birth, produced dilations and modifications in the proximal tubule convolutions of the nephrons during the elongation stage. These changes were not accompanied by alterations in the surface morphology of the epithelial cells of the proximal tubule.

Dilation of the different nephron segments and also of the collecting tubules induced by repeated injections of cortisol have been reported in the young rabbit[3]. On the basis of that study Perey et al.[4] have developed an experimental model of renal polycystosis, which is produced in the newborn rabbit by a single administration of corticoids, and only 2 kinds of cystic alterations have been described to be present[5-7]: tubular cysts affecting the ampular portion of the collecting tubules and glomerular cysts consisting of dilation of Bowman's capsular space. No alterations have been reported until now in other nephron segments.
In the present work we have undertaken a SEM study of microdissected proximal tubules in corticoid-induced renal polycystosis, to assess possible structural alterations.

Materials and methods. Newborn rabbits were injected i.m. once with methylprednisolone acetate (20 mg/kg), as previously described[5]. Some animals of each litter, injected with an equal volume of saline solution, were employed as a control. Rabbits from 2 to 20 days old, anaesthetized with ether, were fixed by perfusion through the aorta with 3% glutaraldehyde made in 0.1 M cacodylate buffer at pH 7.3. Small kidney fragments were then digested by HCl and collagenase[8] and then carefully microdissected. The isolated nephrons were attached to a gelatin-coated coverslip, dehydrated in acetone, dried by the critical point method, using liquid CO_2, then ion-sputtering coated with gold and observed with a Philips SEM-501.

Results. Although all the segments of the nephron were microdissected we only report here the alterations observed in the proximal tubule.
In approximately 10% of the nephrons studied the convoluted portion of the proximal tubule, especially its terminal segment, showed a conspicuous spiral twisting with short convolutions, taking the appearance of a corkscrew (fig. 1a). This morphology contrasted with the normal appearance of this portion (fig. 1b). Alterations were never observed in the straight portion of the proximal tubule. In some instances the terminal segment of the convoluted portion displayed eccentric dilation separated by normal segments or, more rarely, by constrictions (fig. 2a, b). All these alterations were observed as early as 10 days after treatment and affected only the nephrons of the outer cortex. No evolutive changes of the lesion were observed in the older animals.
The observation of fractures of the proximal tubules did not reveal any difference between the cell morphology of the normal animals and that of the corticoid treated animals, even in the segments displaying abnormal dilation. As can be seen in figure 3a, the epithelial cells were pyramidal in shape with a brushed luminal surface and a large basal surface attached to the basal lamina. When digestion of the extracellular matrix was carried out, this basal surface showed its normal rough appearance (figs 2b and 3b). The lateral cell infoldings, described in the normal cells, were also prominent (fig. 3a).

Discussion. Our results show that in addition to the glomerular and tubular cysts described in the corticoid-induced polycystic kidney[5], alterations of the convoluted portion of the proximal tubule are also present. Similar alterations of the proximal tubule were produced by repeated injections of corticoids in older rabbits[3], but they did not develop renal polycystosis.
The location of the affected nephrons in the outer cortex of the kidney, and the fact that in the postnatal period studied an important morphogenetic process takes place, suggest