prostaglandin-induced contraction had reached a plateau. In some experiments the antagonist drugs propranolol (1 μM) and haloperidol (1 μM) were incubated with the tissues for 30 min before relaxations were measured.

Results and discussion. The relaxant effects of dopamine and isoprenaline on renal artery and aorta were compared. Prostaglandin $F_{2\alpha}$(3 μM) produced a sustained contraction of 54 ± 8 mg tension in the renal artery and 44 ± 9 mg in the aorta (8 determinations in each case). The EC_{50} values for the dose-response curves of the relaxant effects of dopamine and isoprenaline on each tissue are shown in the table, together with the effects of incubation with the β-adrenoceptor antagonist, propranolol, alone and in combination with haloperidol, a dopamine receptor antagonist. Low figures indicate greater potency for the relaxant drugs, dopamine and isoprenaline.

Isoprenaline was more potent than dopamine in relaxing both tissues but was particularly potent on the aorta. The results show that both dopamine and isoprenaline act on β-adrenoceptors in the aorta, since the relaxation produced by each agent is antagonized by propranolol. However, in the renal artery the relaxations produced by dopamine and isoprenaline were unaffected by the presence of propranolol but inhibited by the dopamine receptor antagonist haloperidol, indicating that both agents induce relaxation in this tissue by stimulation of dopamine receptors. This study confirms the presence of aortic β-adrenoceptors[14,15] and renal dopamine receptors[1,8] but supports the in vivo findings[13] that isoprenaline acts on dopamine receptors in the renal artery.

Effect of propranolol and haloperidol on the relaxant effects of dopamine and isoprenaline on renal artery and aorta

	Aorta	Renal artery
Dopamine	707 ± 63	575 ± 92
Dopamine + propranolol	1096 ± 147*	660 ± 65
Dopamine + propranolol + haloperidol	1112 ± 132*	802 ± 45*
Isoprenaline	8 ± 1	263 ± 28
Isoprenaline + propranolol	208 ± 17**	316 ± 35
Isoprenaline + propranolol + haloperidol	189 ± 41**	360 ± 31*

Mean EC_{50} (μM) values with standard errors for 10 determinations are shown. These values were taken from dose-response curves measuring % relaxation of prostaglandin-induced contractions and control values for each relaxant drug were measured in the absence of antagonist. The significance of differences produced by the presence of antagonists was tested using Student's t-test and 2 levels of significance are shown. * $p < 0.05$ and ** $p < 0.01$.

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Erythrocyte deformability improving action of β-pyridylcarbinol tartrate

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Summary. β-Pyridylcarbinol tartrate was found to prevent the hyperosmotically induced worsening of filterability of rat erythrocytes after in vivo treatments. The effect is attributed to the prevention of the morphological change of the erythrocytes from normal discocytes to echinocytes. The study was made using a scanning electron microscope.

β-Pyridylcarbinol tartrate (β-PC) and its active metabolite, nicotinic acid, are known to decrease the plasma level of low density lipoproteins (LDL) and to increase that of high density lipoproteins (HDL) not only in experimental animals but also in man[2,3]. Such effects of the drugs would be expected to improve erythrocyte deformability; this suggestion is based on the recent evidence that the shape of the erythrocyte may be regulated by both LDL and HDL[4,5]. Therefore, the effects of β-PC and nicotinic acid on both erythrocyte deformability and shape change were examined in the present study.

Materials and methods. Erythrocyte filterability as a measure of erythrocyte deformability was measured in rats, the erythrocytes of which are known to have a similar size and deformability to that of human erythrocytes[6].

In in vitro filterability experiments, packed erythrocytes[7] from male Wistar rats weighing 200–250 g were suspended in an isotonic buffer or a hypertonic buffer. The isotonic buffer contained 145 mM NaCl, 5 mM KCl, 6 mM glucose and 12 mM Tris-HCl buffer, pH 7.4. The concentration of NaCl was doubled in the hypertonic buffer. After incubation at 37 °C for 1 h in the presence or absence of a drug, a 1 ml aliquot was transferred to a glass pipette (⌀3 mm) attached to an stainless steel sieve of 3200 mesh (5 μm) (Fuji Seive, Tokyo) and the filtration time required for the passage of 0.6 ml of erythrocyte suspension was measured. Results were expressed as percentage increase in filtration time of erythrocyte suspension in the hypertonic buffer as compared with that in the isotonic buffer. In ex vivo filterability experiments, rats were orally pretreated with either saline or a drug which was administered in a single dose or repeatedly. 1 h after the (last) administration, the blood collected by cardiac puncture was immediately added to an equal volume of the isotonic buffer or a

Effects of in vitro and ex vivo treatments with β-pyridylcarbinol tartrate and nicotinic acid on erythrocyte filterability in rats

Drug	Dose or concentration	Increase in filtration time by hyperosmorality (%) Control	Drug treated	Statistical significance
β-Pyridylcarbinol tartrate	1×10^{-3} M	161±2 (n=3)	188±13 (n=3)	N.S.
	3×10^{-4} M	161±2 (3)	204±18 (3)	N.S.
	1×10^{-4} M	173±6 (3)	175±15 (3)	N.S.
Nicotinic acid	1×10^{-3} M	161±2 (3)	158± 4 (3)	N.S.
	3×10^{-4} M	161±2 (3)	181±13 (3)	N.S.
	1×10^{-4} M	163±2 (3)	182±14 (3)	N.S.
β-Pyridylcarbinol tartrate	300 mg/kg×1	170±5 (6)	154± 4 (10)	$p<0.05$
	100 mg/kg×1	162±2 (6)	153± 4 (10)	N.S.
	100 mg/kg×9	172±3 (6)	150± 3 (10)	$p<0.001$
Nicotinic acid	300 mg/kg×1	163±4 (6)	152± 4 (10)	N.S. ($p<0.10$)
	100 mg/kg×1	163±3 (6)	162± 5 (10)	N.S.
	100 mg/kg×9	165±3 (6)	161± 3 (10)	N.S.

Results are expressed as mean±SEM with the number of experiments in parentheses; N.S., not significant.

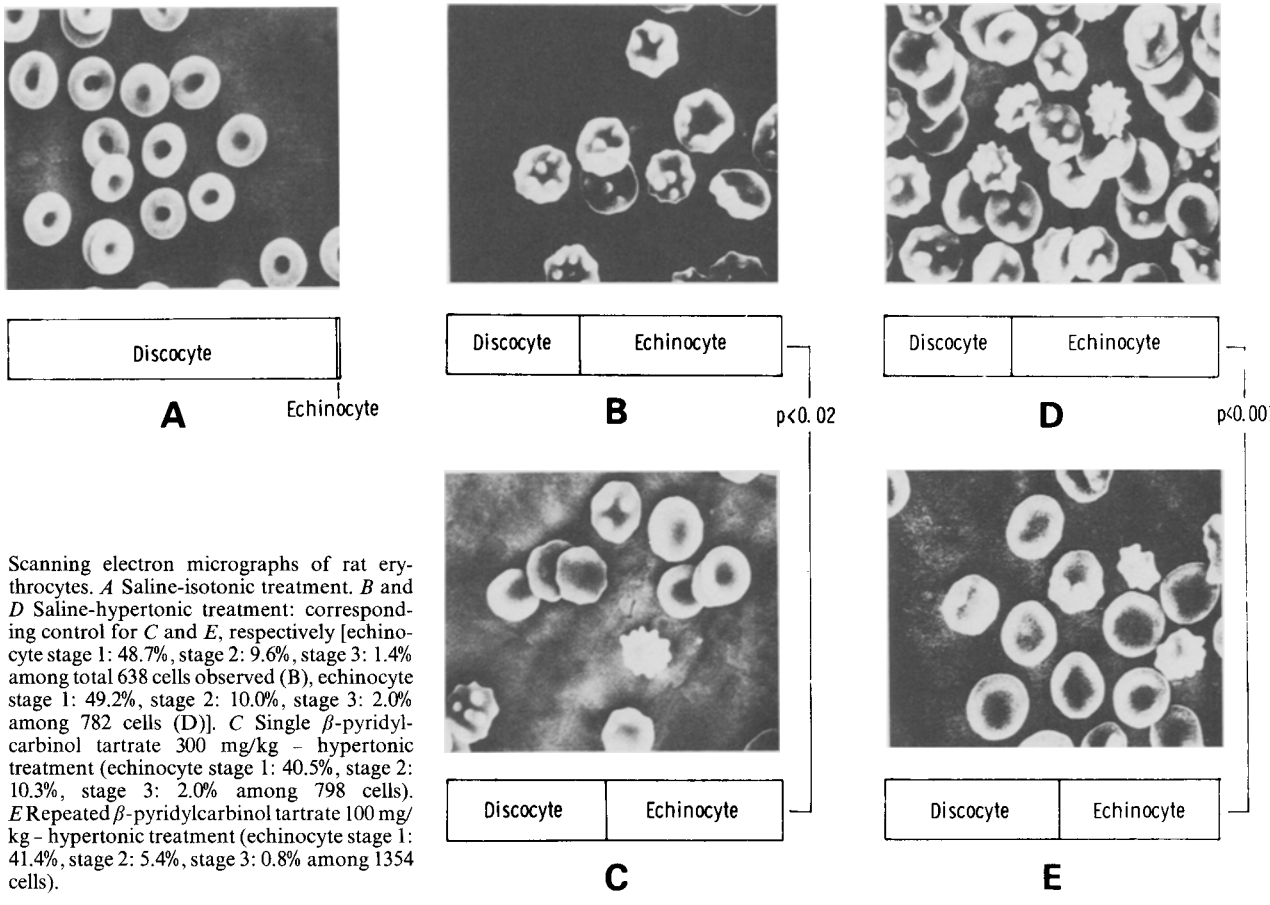
hypertonic buffer and gently mixed. The concentration of NaCl was quadrupled in the hypertonic buffer. The procedure thereafter was the same as above. Blood suspension, processed similarly to those in the ex vivo filterability experiments, were fixed in 1.0% glutaraldehyde in Na-phosphate buffer (pH 7.3) for 30 min at 4 °C[8]. After washing with the buffer, dehydration and coating, the erythrocyte shape was observed with a scanning electron microscope (Hitachi-Akashi, MSM-6, Tokyo) at magnification ×3000.

Results. In the in vitro experiments, as shown in the table, hyperosmolarity caused an increase in the filtration time of the erythrocyte suspension through a microsieve by 61–73% (control). Neither β-PC nor nicotinic acid prevented the increase even at 10^{-3} M.

In the ex vivo experiments (table), hyperosmolarity also caused the increase in the filtration time of the blood suspension through a microsieve by 62–72% (control). β-PC when given in a single dose of 300 mg/kg, but not at 100 mg/kg, significantly prevented the increase ($p<0.05$). The significant preventive effect of the drug ($p<0.001$) was also observed when given repeatedly at 100 mg/kg (twice daily for 4 days and once on the 5th day). On the contrary, nicotinic acid when examined at comparable doses was hardly active even at 300 mg/kg ($p<0.10$).

Morphological results are summarized in the figure, where the percentage of cell types in various samples is expressed as bar length under each electron micrograph. Echinocyte sub-stages are categorized according to Fujii et al.[9].

The normal discocyte shape was well preserved in the



Scanning electron micrographs of rat erythrocytes. *A* Saline-isotonic treatment. *B* and *D* Saline-hypertonic treatment: corresponding control for *C* and *E*, respectively [echinocyte stage 1: 48.7%, stage 2: 9.6%, stage 3: 1.4% among total 638 cells observed (B), echinocyte stage 1: 49.2%, stage 2: 10.0%, stage 3: 2.0% among 782 cells (D)]. *C* Single β-pyridylcarbinol tartrate 300 mg/kg – hypertonic treatment (echinocyte stage 1: 40.5%, stage 2: 10.3%, stage 3: 2.0% among 798 cells). *E* Repeated β-pyridylcarbinol tartrate 100 mg/kg – hypertonic treatment (echinocyte stage 1: 41.4%, stage 2: 5.4%, stage 3: 0.8% among 1354 cells).

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