and in several instances, both at 300 mm and at 250 mm, the respiratory movements of the untreated rats were too irregular to be counted with accuracy. This latter irregularity consisted of periodic breathing of the Cheyne-Stokes type, the periods of complete apnea being very long. The differences in respiratory rates of the contrasting groups, both at 300 mm and at 250 mm, are highly significant when analyzed by Fisher's t test.

Summary. Rats treated with nicotinic acid or its amide were found to survive until they reached a barometric pressure of 75 mm Hg (equivalent altitude, 53,800 feet); untreated controls died at an average pressure of 93 mm Hg (49,275 feet). Statistically, the differences are probably significant.

The tachypnea developing at low barometric pressure is much less in treated than in untreated rats. Preliminary injection of 1 mg nicotinic acid was followed by an increase in respiratory rate of only 22% at 300 mm Hg as opposed to approximately 80% in untreated controls. Differences of comparable magnitude were noted at pressures of 300 and 250 mm Hg following the administration of nicotinamide. Statistical analyses indicate that these differences are highly significant.

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Effect of 3,3'-Methylene-Bis-4-Hydroxycoumarin (Dicumarol) on Embryonic Chick Liver and Spleen.*

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Dicumarol is being used today in the control and possible prevention of thrombo-embolic phenomena. Its mode of action is as yet not well understood. Some workers have presumed that it works by inhibiting the utilization of vitamin K by the liver cell in the synthesis of prothrombin. Others think that it may act as a toxic agent on the liver cells, especially when used in large amounts.[1] This study was initiated to observe the effect of dicumarol on isolated sections of liver and spleen of the 14 day chick embryo.

Method. 14 day chick embryos were removed from their shells and placed in petri dishes containing Tyrode's solution. The liver and spleen were isolated and small portions were placed in individual petri dishes containing various concentrations of dicumarol in a 10% rabbit serum-Tyrode solution. Since the volume of the medium used was small, the pieces of tissue were sectioned in the petri dishes containing the dicumarol in the given concentrations. This was done in order to prevent dilution of the dicumarol serum-Tyrode medium. These pieces of liver and spleen were then cut into approximately one cubic millimeter sections with a sharp scalpel and placed on 20 x 20 mm No. 2 cover slips in a small drop of media containing the disodium salt of dicumarol in 10% rabbit serum and Tyrode's solution. These cultures were placed on deep-welled micro culture slides in a lying-drop position, sealed with paraffin and incubated at 38° C. The disodium salt was prepared by adding the crystalline material to .345 N sodium hydroxide. The same amount of base was added to the media containing the different amounts of dicumarol. The pH of the media ranged from 7.74 to 7.88. Little or no proliferation of cells was noted at 24 hours. This was especially true in the liver cultures. Therefore, the period of observation was begun at 48 hours. The longest period of observation was 72 hours. According to Nordman, before 14 days an extensive proliferation of

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1 Rose, C. L., Harris, P. N., and Chen, K. K., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 228.

TABLE I.
Effect of Dicumarol on Growth of Embryonic Chick Liver and Spleen in Tissue Culture.

Dilution	Period of observation (hr)	Liver		Spleen	
		No. of cultures	Proliferating cells	No. of cultures	Proliferating cells
Control	48	11	+	8	+
1/10,000		5	+	3	+
1/50,000		4	+	1	+
1/100,000		5	+	3	+
Control	72	8	+	3	+
1/10,000		5	0	3	+
1/50,000		4	+	1	+
1/100,000		5	+	3	+

fibroblasts is not observed in the embryonic liver.[2] The appearance and growth pattern of these cells indicates that they are proliferating endothelial cells. The proliferating cells of the spleen cultures were predominantly blood elements. Those seen in the media were immature and mature polymorphonuclear leucocytes and nucleated red cells.

At the end of these periods of observation the cover slip containing the liver tissue was removed and placed in a small stendor dish containing Bouin's solution. After one hour in Bouin's solution the small pieces of tissue, which remained very firmly fixed to the cover slips, were washed in tap water for one hour, in a saturated aqueous solution of lithium carbonate for fifteen minutes, in tap water again for 15 minutes, and in 3 changes of distilled water. They were then stained in Harris's haematoxylin, dehydrated, and mounted in clarite on micro slides.

A number of the spleen cultures were fixed in the above manner. Some, however, were allowed to dry and were stained with Wright's solution.

Results. All the control cultures of liver show proliferation of endothelial-like cells at 48 and at 72 hours. In concentrations of dicumarol of 1/50,000 and 1/100,000, the cultures contained living cells at the end of 48 as well as 72 hours as evidenced by cell structure and staining reactions after fixation. Those cultures containing dicumarol in concentrations of 1/10,000 showed proliferating cells at the end of 48 hours, but in all of these cultures incubated for 72 hours the cells died and disintegrated. Spleen cultures of 14 day chick embryos showed cellular proliferation of blood elements in media containing dicumarol in concentrations of 1/50,000 and 1/10,000 at 48 and 72 hours.

Summary. The effect of the disodium salt of dicumarol was observed on 14 day embryonic chick liver and spleen cultured in a 10% rabbit serum and Tyrode solution. Concentrations of dicumarol of 1/100,000 and 1/50,000 did not appear to inhibit the proliferation of endothelial cells at 48 and 72 hours when compared with control cultures. However, with a concentration of 1/10,000 cellular proliferation was present at 48 hours, but at 72 hours these cells had been killed and lysed. In contrast, spleen cultures of 14 day chick embryos showed cellular proliferation in media containing dicumarol in concentrations of 1/50,000 and 1/10,000 at both 48 and 72 hours.

[2] Nordman, M., *Arch. f. exp. Zellforsch.*, 1929, **8**, 371.