extraordinarily resistant, a prothrombin activating enzyme is an improbable contaminant of the heterogeneous protamine because activity resists 100°C heat and DFP treatment.

The ability of polymyxin to activate prothrombin by the nonenzymic process may be a toxic mechanism of this drug as well as of some other antibiotics. Interestingly, by simply adding heparin to the antibiotics, Higginbotham(9) diminished the lethal effect in mice of polymyxin, neomycin, viomycin, and dihydrostreptomycin while not affecting neomycin antibiotic properties.

If a model for physiologic processes, the mechanism of nonenzymic prothrombin activation may *initiate* blood coagulation at bleeding sites(10). Similarities between this reaction and the action of the combined calcium ion, tissue thromboplastin, and accessory factors include insensitivity to and progression in DFP, whereas autocatalytic activation is inhibited by small amounts of DFP (1,11). If not a physiologic process, nonenzymic prothrombin activation still remains a potential *nonspecific* contributor to *in vitro* coagulation experiments, particularly those involving purified protein reactants in high concentration. Extreme care should be exercised in controlling, ruling out, or including this reaction when interpreting coagulation experiments.

Summary. Synthetic polyORN, polymyxin B, and protamine activate horse prothrombin by processes resembling the nonenzymic activation with polyLYS. Neomycin and streptomycin may function to a slight degree in the same mechanism. Nonenzymic prothrombin activation with polyLYS is inhibited by heparin, polyGLU, and polyASP. The capacity of protamine preparations to activate prothrombin is stable to heat, DFP, and dialysis. Prothrombin consumption in prothrombin-protamine-DFP mixtures is concomitant with thrombin formation in DFP-free systems. Nonenzymic prothrombin activation may be associated with some drug toxicities as well as with physiologic hemostasis.

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Cholesterol in Vitamin B_{12}-Deficient Chick Embryo. (26865)

LOUISE J. DANIEL, SHIRLEY COHEN AND DAVID W. YESAIR*
Department of Biochemistry, Cornell University, Ithaca, N. Y.

Vitamin B_{12} has been implicated in cholesterol metabolism in various species. The blood cholesterol level was found to be abnormally low in pernicious anemia patients in relapse(1,2,3). After effective liver therapy, owing presumably to the action of the Vit. B_{12} present, cholesterol level increased. Forbes and Patterson(4) showed that the livers of Vit. B_{12}-deficient rats had higher levels of cholesterol than the control livers. Hsu

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TABLE I. Cholesterol Content of Developing Chick Embryos from Vitamin B_{12}-Deficient and Normal Eggs.*

	Tissue	Days of incubation	No. samples†	Avg dry wt (g)	Cholesterol Free	Cholesterol Ester	Total (mg/tissue)‡
Normal	Egg§	0	16	14.42	256	14	270
	Embryo	12	—‖	—	—	—	—
		15	7 (21)	1.84	16.3	7.4	23.7
		18	7 (21)	4.21	34.7	16.4	51.1
		21	7 (19)	6.74	39.9	48.6	88.5
	Yolk sac¶	12	4 (20)	14.73	268	7	275
		15	7 (21)	11.96	213	24	237
		18	7 (21)	9.33	151	33	184
		21	7 (19)	4.88	89	30	119
Vit. B_{12}-deficient	Egg	0	16	14.27	258	7	265
	Embryo	12	—‖	—	—	—	—
		15	7 (22)	1.65	16.7	6.6	23.3
		18	7 (20)	3.55	30.4	16.7	47.1
		21	6 (16)	5.86	37.1	51.6	88.7
	Yolk sac	12	4 (20)	13.89	250	3	253
		15	7 (22)	11.87	190	45	235
		18	7 (20)	9.70	137	59	196
		21	6 (16)	6.41	87	51	138

* Liver and 0.02-0.1 ml blood were removed from each embryo for other analyses, cholesterol was not determined on these samples. Cholesterol contents of livers from normal 21-day-old embryos were: free, 2.8 mg; esterified, 27 mg; and total, 29.8 mg/liver.

† Individual eggs were assayed and composite samples of embryos and yolk sacs. Numbers in parentheses represent total No. of embryos and yolk sacs.

‡ All values are on a dry wt basis.

§ 8 eggs were collected after 5 wk on experimental diets, and 8 eggs after 9-10 wk on diets. There was no difference in results obtained, so values have been averaged.

‖ Insufficient tissue left for analyses.

¶ Sample consists of egg contents except the embryo.

and Chow(5) reported increased amounts of cholesterol in sera of B_{12}-deficient adult rats and chicks, and in adrenals of deficient young rats and chicks. In view of these conflicting data, it was thought appropriate to report the results obtained on B_{12}-deficient chick embryos which were produced for an extensive study of the effect of a Vit. B_{12} deficiency on lipid metabolism in the developing embryo (6,7,8).

Procedure. Vit. B_{12}-deficient and control embryos and yolk sacs† were obtained by methods previously described(8). The lyophilized samples were extracted with acetone-ethanol (1:1) and total and free cholesterol determined by the method of Sperry and Webb(9) using aliquots of the extract.

Results. Table I shows cholesterol contents, free, esterified and total, in the chick embryo and yolk sac as the embryo develops. There is no statistical difference between amount of cholesterol in the Vit. B_{12}-deficient tissues and that in the corresponding control tissues. Since the deficient embryo was smaller than the control embryo at 18 and 21 days of age, concentration of cholesterol on a per gram basis is slightly higher in the deficient tissue. The deficient eggs were definitely low in B_{12}‡ averaging 65 mμg/egg *vs.* 800 mμg for the control egg.

Almost all of the cholesterol in the unincubated egg is free cholesterol, as others have shown(10-13). As incubation proceeds beyond the 12th day, the free cholesterol in the yolk decreased markedly and the level of esterified cholesterol increased somewhat. In the embryo an increase in both free and esterified cholesterol occurred from the 15th day on.

At 21 days there was a 15-25% decrease in total cholesterol in the egg in both control and deficient tissue. The "loss" seemed to be in the ester fraction of the embryo. At 21

† All contents of the egg except the embryo.

‡ Determined by *Ochromonas malhamensis* assay, see reference(8).

days the cholesterol esters are reported to be much increased in the embryo(13). The reason for this discrepancy is not apparent. However, the livers had been removed from all of the embryos, and since a large portion of the esterified cholesterol occurs in the liver, and livers of the 21-day-old embryos were much larger than the others, some cholesterol may have been unaccounted for. This seems not to be the case in samples other than at 21 days. The majority of these values obtained agree well with those in the literature(13).

Summary. The cholesterol content (free and esterified) of the chicken egg during incubation did not differ from that of its Vit. B_{12}-deficient counterpart. Under the conditions of this experiment where a marked B_{12} deficiency existed, Vit. B_{12} has no effect on cholesterol status of the developing chick embryo.

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Brain 5-Hydroxytryptamine in Ozone-Exposed Rats.* (26866)

RALPH G. SKILLEN, CLINTON H. THIENES, JOSEPH CANGELOSI AND LORRENE STRAIN
Institute of Medical Research, Huntington Memorial Hospital, Pasadena, Calif.

A previous communication(1) discussed the elevation of lung 5-hydroxytryptamine (5-HT) content in rats subjected to acute ozone exposure. In studying potential mechanisms of this increase, the possibility that non-pulmonary tissues might be sources of lung 5-HT was considered, and brain 5-HT levels of ozone-exposed rats were determined. The following describes the results.

Materials and methods. Male albino Wistar rats (225-275 g) were exposed for 4 hours to ozone, 6 parts per million (p.p.m.) by volume in air, as previously described(1). Control animals were handled similarly but exposed to air alone. Control and exposed groups consisted of 22 animals each. After exposure the animals were anesthetized with pentobarbital sodium. Brain stems were severed at the foramen magnum, the brains removed, weighed, and assayed for 5-HT spectrophotofluorometrically in an Aminco-Bowman spectrophotofluorometer by the method of Bogdanski, *et al.*(2). Brain protein was determined by the method of Lowry, *et al.* (3).

Results. Exposure of rats to 6 p.p.m. ozone by volume for 4 hours resulted in a significant decrease in brain 5-HT from a mean control value of 0.63 $\pm$ 0.14 μg/g to a mean of 0.39 $\pm$ 0.10 for the ozone-exposed group. In view of the observations by Bourdillon *et al.*(4) that inhalation of 5% CO_2 increased Na^{24} entry into the cerebrospinal fluid, the

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