23. Temin, H. M., Rubin, H., *Virology*, 1958, v6, 669.

24. Dick, G. W. A., Smithburn, K. C., Haddow, A. J., *Brit. J. Exp. Pathol.*, 1948, v29, 547.

25. Smithburn, K. C., Hughes, T. P., Burke, A. W., Paul, J. H., *Am. J. Trop. Med.*, 1940, v20, 471.

26. Smithburn, K. C., Haddow, A. J., *J. Immunol.*, 1944, v49, 141.

Received June 20, 1960. P.S.E.B.M., 1960, v104.

Effect of Triparanol on Beta-Aminopropionitrile-Induced Dissecting Aneurysm and Blood Lipid Levels in the Turkey.* (25948)

P. E. WAIBEL, R. E. BURGER, R. A. BALL, J. H. SAUTTER, I. E. LIENER AND B. S. POMEROY

Depts. of Poultry Husbandry and Agric. Biochem., College of Agriculture, and Divisions of Vet. Pathology and Bacteriology, College of Veterinary Medicine, University of Minnesota, St. Paul

A probable cause of beta-aminopropionitrile (BAPN)-induced dissecting aneurysm in turkeys appears to involve defective connective tissue metabolism, since it has been shown(1, 2) that this is a known effect of the agent. However, the presence of fatty degeneration in the intimal layer of the aorta in the region of rupture in naturally occurring cases(3) of internal bleeding and the gross observation of yellow fatty areas in the lumen of the aorta in the area of rupture in BAPN-treated birds (4,5), raised the question of the importance of atherosclerotic lesions in development of the malady. The object of this study was to determine whether triparanol,† a drug having the property of lowering the ratio of blood cholesterol to phospholipid(6), could reduce the severity of BAPN-induced dissecting aneurysm in turkeys. The effects of triparanol on growth rate and plasma cholesterol and phospholipid levels are also reported here.

Methods. Turkey poults were housed during the first 3 weeks in electrically heated batteries with raised wire-mesh floors, then were moved to conventional growing batteries. Details showing breed, number of birds per group, and experimental duration are given in the tables. The diet is presented in Table I. Triparanol and beta-aminopropionitrile fumarate were added as one per cent premixes in ground corn. Vitamin mixture C-5 supplied the following per 5 g of mixture: biotin 0.2 mg, menadione sodium bisulfite—63% USP 2 mg, pyridoxine · HCl 2 mg, folic acid 2 mg, thiamine · HCl 2 mg, riboflavin 5 mg, calcium pantothenate 10 mg, niacin 40 mg, Vit. B_{12}—0.1% triturate in mannitol 30 mg, Cerelose 200 mg, and corn meal to 5 g. All birds succumbing during an experiment and many surviving birds were subjected to necropsy examination. Detailed pathological findings will be published later. Blood samples were drawn in a heparinized syringe as indicated in the tables. Plasma cholesterol and phospholipid were determined by the methods of Abell *et al.*(8) and Zilversmit and Davis(9), respectively.

Results. The depressing action of triparanol on turkey growth is shown in Fig. 1. The depression appears to be logarithmic in nature. The intercept of this straight line relationship with control growth was plotted at 10.6 ppm and 3.9 ppm triparanol for turkeys 5 and 3 weeks on experiment, respectively. It is felt that this is the approximate dosage range at which triparanol begins to depress growth rate in turkeys.

Triparanol did not protect against BAPN-induced aortic rupture (Table II). The hemorrhaging index, as defined by Waibel and Pomeroy(5), was at least as great when triparanol was added to the 0.05% level of

* Published as paper no. 4343, Scientific Journal Series at Minn. Agri. Expt. Station This investigation was supported in part by research grant from Nat. Heart Inst., P.H.S.

† Triparanol was formerly designated by code number MER-29. It is produced by Merrell Co., Cincinnati, O. Formula is 1-[(4-diethylaminoethoxy) phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol.

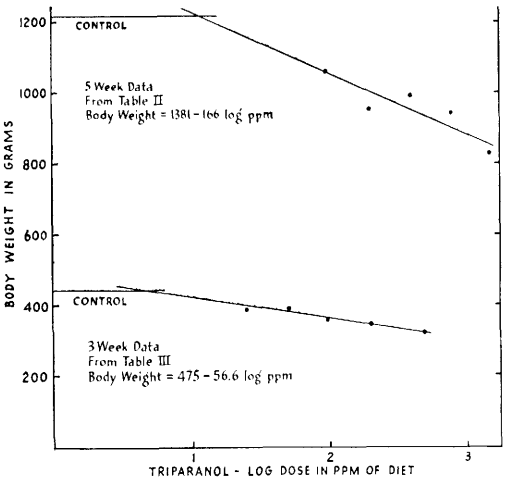

FIG. 1. Effect of graded levels of triparanol on growth of turkey poults.

BAPN. The larger the index, the more severe the expression of internal bleeding, based upon rapidity and degree of incidence. Feeding of triparanol resulted in generally reduced plasma cholesterol levels and increased plasma phospholipid levels, resulting in a markedly reduced cholesterol:phospholipid ratio.

A further experiment was conducted using levels of triparanol ranging from 25-500 ppm (Table III). Growth was depressed even at the lowest level of the drug (Fig. 1). The cholesterol:phospholipid ratio was depressed maximally at lowest level of the drug. However, there appears to be a trend showing decreasing levels of both cholesterol and phospholipid with increasing levels of the drug.

Discussion. According to the intercepts calculated in Fig. 1, it would be expected that triparanol at levels above approximately 10 ppm of diet (approximately 1.0 mg per kg body weight per day) would produce growth depression in young turkeys. Work with rats (10) indicated that 4.1 mg triparanol/kg body weight did not depress growth in a 30 day experiment. This suggests that the turkey is somewhat more sensitive than the rat to low levels of the drug.

Results with rats(10) indicate that triparanol decreased the ratio of blood plasma cholesterol to phospholipid by depressing the former more than the latter. In the present study, the ratio was similarly decreased and the cholesterol level was depressed. However, the phospholipid level was frequently higher in treated birds than in controls.

While growth was depressed even at the lower levels of triparanol, it is assumed that this depression did not influence the blood constituents, since studies utilizing the paired-feeding technic with rats(10) indicated that cholesterol and phospholipid changes were not dependent upon rate of growth.

In previous work from our laboratory(4,5), feeding of BAPN to turkeys until 10 or 21 weeks of age appeared to be responsible for the grossly-observable occurrence of yellow plaques in the area of the abdominal aorta where rupture occurs. In the present study, using turkeys 4 to 6 weeks of age, these lesions were not observable. Preliminary histological evidence with the younger birds indicated that fibrotic intimal plaques were present in the young turkeys, regardless of treatment. These lesions were negative to the fat stains Sudan IV and Oil Red O. Lindsay *et al.*(11) have reported that abdominal plaques in diethylstilbestrol-implanted cockerels were white in younger birds whereas they were yellow in older birds, indicating presence of fat. In view of the non-observance of yellow plaques in these younger birds, it was not possible to fulfill one of the objectives of

TABLE I. Composition of Diet.*

	%
Ground yellow corn	41.65
Soybean meal, dehulled (50% protein)	40.0
Fish meal (60% protein)	6.0
Alfalfa meal (dehydrated, 17% protein)	3.0
Dried whole whey	3.0
Dicalcium phosphate	2.25
Calcium carbonate	2.5
Salt, iodized	.5
Trace mineral mixture†	.1
Vit. mix C-5 (see experimental)	.5
Choline chloride (25%)	.1
Vit. A (5000 I.U./g)	1.1
D (3000 I.C.U./g)	.05
E acetate (44 I.U./g)	.05
DL-methionine	.1
Procaine penicillin G (4 g/lb)	.1

* Diet calculates to contain 28.7% protein and 811 productive energy calories (Fraps, 7) per lb.

† Delamix with 2% zinc, Limestone Products Corp. of America, Newton, N. J., provides diet with the following in mg/kg: Manganese 60, iodine 1.2, iron 20, copper 2.0, zinc 20, and cobalt 0.2.

TABLE II. Effect of Dietary Triparanol on Incidence of BAPN-Induced Aortic Rupture and on Plasma Cholesterol and Phospholipid Levels.*

(Exp. 1)

Level of triparanol (ppm)	At 3 wk				At 5 wk†				
	Wt, g	CHOL, mg %	P-LIP, mg %	C/P	Wt/REM, g	HI	CHOL, mg %	P-LIP, mg %	C/P
No BAPN-F									
0	673	126	266	.477	1211/8	0	129	256	.504
100	600	94	295	.353	1051/7	0	108	295	.386
200	579	108	283	.379	946/6	0	105	285	.369
400	559	88	266	.342	991/8	0	125	352	.354
800	628	103	278	.302	935/8	0	133	377	.352
1600	541	96	272	.352	814/8	0	135	373	.363
.050% BAPN-F									
0	521	127	211	.502	736/3	8.0			
100	600	107	245	.437	946/1	20.0			
200	583	110	270	.408	—/0	22.5			
400	525	107	240	.451	865/3	6.7			

* Experiment started at 7 days of age with 10 Broad Breasted Bronze males/group after pre-experimental period during which poults received control ration. Avg wt was 114 g. CHOL indicates blood plasma cholesterol level. P-LIP indicates blood plasma phospholipid level. Blood values represent avg of duplicate pooled samples of 3 poults each. C/P is cholesterol-phospholipid ratio.

† REM indicates remaining birds at 5 wk. Some were removed at 4 wk for tissue study. Otherwise, non-hemorrhaging mortality in experiment was negligible. HI = Hemorrhage index: 0 equals no aortic hemorrhage, higher numbers indicate increasing severity.

the present study; namely, the effect of triparanol on development of BAPN-induced yellow plaques.

Estrogens are noted for their effects on cholesterol:phospholipid ratio(12). As triparanol may be considered as a substituted stilbene, and therefore potentially estrogenic in action, the effect of triparanol on oviduct weight was studied. Samples of females fed triparanol at 200 ppm were sacrificed after 47 and 61 days on the drug. Mean oviduct weights, whether calculated from absolute values or percentages of body weight, were depressed. Testis weights on males similarly treated showed a weight depression, which was probably not due to estrogen-like inhibition of gonadotropic secretion but due to the general toxic effect of the drug on growth.

A striking observation in this work was that the liver of many triparanol-fed birds exhibited greenish discoloration. It is possible that the color is an accumulation of a decomposition product of triparanol.

Summary. Addition of 25-1600 ppm triparanol to the diet of turkey poults lowered the ratio of plasma cholesterol to phospholipid. Incidence of beta-aminopropionitrile-induced dissecting aneurysms was not lessened

TABLE III. Effect of Triparanol on Growth and Blood Lipids after 3 Weeks on Experiment.*

(Exp. 2)

Level of triparanol (ppm)	Avg body wt, g	Gain / Feed	Blood plasma		
			Cholesterol, mg %	Phospholipid, mg %	Ratio
0	441	.53	100	164	.62
25	388	.48	86	187	.47
50	382	.46	76	164	.47
100	356	.46	77	187	.42
200	346	.47	62	146	.44
500	320	.49	67	154	.47

* Newly hatched poults were placed on 4-day standardization period on control diet, prior to equalized segregation into experimental lots. Broad Breasted Bronze and Broad Breasted White turkeys were housed in separate pens. Each group contained 5 males and 5 females. Avg values of breed and sex are presented. Each plasma value is avg of 16 individual samples, acquired equally from breeds and sexes.

by triparanol. Degree of growth depression due to triparanol was proportional to logarithm of dose.

We express appreciation to Dr. T. R. Blohm of Merrell Co., Cincinnati, O., for supply of triparanol and to Dr. D. V. Frost, Abbott Labs., No. Chicago, Ill. for beta-aminopropionitrile fumarate.

1. Dasler, W., *Chicago Med. School Quart.,* 1957, v18, 1.
2. Dastur, D. K., Iyer, C. G. S., *Nutrition Rev.,* 1959, v17, 33.
3. Carnaghan, R. B. A., *Vet. Rec.,* 1955, v67, 568.
4. Waibel, P. E., Pomeroy, B. S., *Poultry Sci.,* 1958, v37, 934.
5. ———, *J. Nutrition,* 1959, v67, 275.
6. Blohm, T. R., Kariya, T., Laughlin, M. E., Palcpoli, F. P., *Fed. Proc.,* 1959, v18, 369.
7. Fraps, G. S., *Tex. Bull.,* 1946, 678.
8. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, R. E., *J. Biol. Chem.,* 1952, v195, 357.
9. Zilversmit, D. B., Davis, A. K., *J. Lab. Clin. Med.,* 1950, v35, 155.
10. Blohm, T. R., Kariya, T., Laughlin, M. W., *Arch. Biochem. Biophys.,* 1959, v85, 250.
11. Lindsay, S., Nichols, C. W., Chaikoff, I. L., *A. M. A. Arch. Path.,* 1955, v59, 173.
12. Tennent, D. M., Zanetti, M. E., Siegel, H., Kuron, G. W., Ott, W. H., *J. Nutrition,* 1959, v69, 283.

Received March 14, 1960. P.S.E.B.M., 1960, v104.

Prevention of Malignant Cell Growth and Spread by Tropical Pretreatment with Radioactive Chromic Phosphate.* (25949)

HORACE GOLDIE, MATTHEW WALKER, ROBERT E. AGEE AND MICHEL W. KILDARE
(With technical assistance of Esther White Turner)
Depts. of Experimental Oncology and Surgery, Meharry Medical College, Nashville, Tenn.

We have reported(1) that intraperitoneal pretreatment with radioactive Yttrium (Y^{90}) compounds prevented growth and implantation of malignant cells not only by acting directly on tumor cells, but also by increasing indirectly the resistance of the cavitary wall to their invasion. This line of research was continued in our recent experiments, using as the radioactive agent an isotope with a longer halflife and, as targets, tumor inocula in different locations. The results are reported below.

Material and methods. (A) *Mice and tumor strain.* Swiss Albino mice (Albino Farms, Red Bank, N. J.) and CFW mice (Carworth Farms, New City, N. Y.) of either sex, 24 to 27 g weight, were used. Krebs-2 tumor was maintained in serial transfers as "ascites tumor." (B) *Tumor cell inoculation and pretreatment with isotope.* Methods of inoculation of requisite number of tumor cells, of ascitic fluid withdrawal and of the work with isotopes have been described(2,3,4,9,10). *Radioactive chromic phosphate* in colloidal suspension was obtained from Abbott Labs, Oak Ridge, Tenn. This compound of a pure beta emitting phosphorus P^{32} is widely used as a substitute for colloidal radiogold (Au^{198}) which is a beta and gamma emitter(5,6,7,8, 11,12). (C) *Technic of injections.* (aa) *Intrapleurally.* Puncture of most areas of the thoracic wall will produce hemorrhages from the surfaces of the lung. However, it is quite safe to puncture the phrenico-costal sinuses (which are not occupied by the lung), in the area of the right thoracic wall at intersection of posterior axillary line and lowest intercostal space. This procedure was done. (bb) *Subcutaneously into scalp.* We have reported(4) sharply localized growth of scalp tumors in proportion to size of inoculum and their suitability for estimation of normal tissue invasion by tumor outgrowth. For either tumor inoculation or isotope injection a gauge 23 needle was introduced through the external ear into subcutaneous tissue of neck, scalp, nasal region and supraorbital region (see be-

* This work was supported by grants from Division of Biology and Med., U. S. Atomic Energy Commission, and from the Damon Runyon Fund.