

TABLE I.

Colloidal solution	Substance tested	C. O. P. $\pm$ S.E. mm Hg
Dog serum	—	24.8 $\pm$.8
"	80 mg% Toluidine blue	22.9 $\pm$ 1.1
Dog plasma*	—	23.7 $\pm$ 1.6
"	5 mg% Protamine	25.5 $\pm$ 1.0
"	20 mg% Neutral red	22.7 $\pm$ 1.2
6% serum albumin	—	24.5 $\pm$.5
"	10 mm% Polybrene	24.3 $\pm$.7
"	10 mg% Protamine	24.1 $\pm$.9
"	80 mg% Neutral red	25.5†

* Prepared by adding 20 ml of buffered 2% ethylene diaminetetraacetate to 180 ml whole blood and then centrifuging.

† Average of only 2 measurements, all other values are averages of 4 or more measurements.

ma colloid osmotic pressure when added in concentrations slightly above those expected

in clinical use. Therefore, an effect on the colloid osmotic pressure seems highly unlikely as part of the mechanism in production of pulmonary edema by basic dyes and polybasic macromolecular substances.

1. Visscher, M. B., Absolon, K., Ballin, H., *Univ. of Minn. Med. Bull.*, 1962, v33, 181.

2. Visscher, M. B., *J. Lancet*, 1962, v82, 43.

3. Sela, M., Katchalski, E., *Advances in Protein Chem.*, 1959, v14, 391.

4. Meehan, J. P., Hyman, C., Sasnow, M., *Measurement of Colloid Osmotic Pressure with an Unbounded Strain Gage Transducer*, Statham Laboratories, Instrument Notes #15, Univ. of Southern Calif. School of Med., May 1950.

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A Dietary Factor Influencing Lymphoid Tumor of the Chicken.* (27701)

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It is now recognized that a virus of a lymphoid tumor(1) when given in low doses to day-old chickens, will cause a significant number of them to develop lymphoid tumors (lymphocytoma or lymphomatosis) after a long latent period(2). If a large dose of virus is given to day-old chickens, they develop erythroblastosis after a short incubation period(3). The resistance of the chicken is another factor subject to genetic influence(4) and immunity of the host stimulated by prior exposure to viable tumor cells(5). Probably many birds harbor the virus but do not develop the disease. Such birds may shed the virus and infect very young chickens(6). The effect of certain mineral and vitamin levels as nutritional factors was found without significant effect on fowl paralysis(7). Vit. E deficiency was implicated by the reports of

some workers(8); other workers(9,10) could not support this contention. A decrease in incidence of neoplastic disease was noted by Waters(11) when the diet of non-inoculated chickens in genetic experiments was changed from one containing cod-liver oil to one containing a synthetic form of vit. D. Three experiments have been done which indicate that one particular lot of cod-liver oil in the diet of chickens increased the incidence of lymphoid tumors in experimentally exposed birds. Another lot of cod-liver oil did not have this property. Experimentally induced erythroblastosis was not affected by cod-liver oil in the diet. These results are briefly presented here.

Materials and methods. The basal diet had the following percentage composition: ground yellow corn 52.4285, wheat standard middlings 5, wheat bran 5, soybean oil meal (solvent) 25, alfalfa meal 5, meat scrap 5, steamed bone meal 1, calcium carbonate 1, iodized salt 0.5, manganese sulphate (Mn SO 4 • H₂O) 0.025, riboflavin supplement (4 g riboflavin per lb.) 0.01, vitamin B₁₂ supple-

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† Now at U.S.D.A. Regional Poultry Disease Laboratory, East Lansing, Mich.

ment (6 mg. vit. B₁₂ per lb.) 0.01, vit. D supplement (1500 units vit. D per gram) 0.0265. Three percent of different cod-liver oils was added to the diets of test chickens. Four kinds of cod-liver oil were used in Experiment 1. Two of these were "off-grade," somewhat rancid oils that would not have been used for commercial feeds. Cod-liver oil from 2 different sources (sources A and B) were used in Exp. 2 and 3.[‡]

The cod-liver oil A was a medicinal grade and cod-liver oil B was a feeding grade and assurance was given by the manufacturer that they contained no oils other than cod-liver oil.

The chickens of Exp. 1 were from 2 stocks; one was Line 15 I stock from the USDA Regional Poultry Laboratory at East Lansing, Mich. and the other was a cross of Univ. of Wisconsin stocks. Line 15 I test chickens were used in Exp. 2 and 3.

Inocula containing the infective agent of fowl leukosis material were prepared by the Regional Poultry Disease Laboratory, East Lansing, Mich. from RPL 12 tumor. The RPL 12 tumor was derived after several passages with cell-free material in chickens(2,3) from a transplantable lymphoid tumor(1). The inocula were 5% neoplastic liver tissue in Simms' salt solution which had been centrifuged and filtered through a Selas 0.2 filter. Inoculum L-29 was used in Exp. 1, L-30 in Exp. 2 and L-31 in Exp. 3. The intraperitoneal dosage in Exp. 1 was adjusted so as to induce erythroblastosis. Chickens of Exp. 3 received a large intraperitoneal dose calculated to induce lymphoid tumors. The exposure for chickens of Exp. 2 (half on the basal diet and half on the basal diet plus cod-liver oil) was provided by contact with chickens of the same age which had been inoculated with the leukosis agent and which was subsequently shed by them. Another group of chickens in Exp. 2 (half on the basal diet and half on the cod-liver oil diet) was exposed by contact with chickens of the same age coming from a stock with a record of high

incidence of lymphocytoma.

Inoculations were made and exposures begun when the chickens were one day of age. The chickens were started on their respective diets at the time of hatching and fed for 105 days in Exp. 1, 497 days in Exp. 2 and 350 days in Exp. 3 at which times the feeding experiments were terminated.

All birds that died were examined for neoplasia and the type of tumor determined by microscopic examination of histological sections.

Results. In Exp. 1, 20 to 31% of the chickens developed erythroblastosis. The inoculated chickens of Exp. 2 developed erythroblastosis (68%), osteopetrosis (17%), lymphocytoma (5%) and other tumors (8%) thus providing good opportunity for contact exposure of the critical test groups. The non-inoculated chickens providing contact exposure for the other portion of Exp. 2 developed erythroblastosis (3%), lymphocytoma (6%), and other tumors (16%) and also provided a good source for contact exposure of the critical test groups.

The results are summarized in Table I. The number of chickens indicated for each group represent the sum of (a) survivors at end of experiment, (b) those which died with lymphocytoma, (c) erythroblastosis or (d) other tumors. Chickens which died from inter-current non-neoplastic disease during the experiment were not considered in evaluation of the results.

Lymphocytoma occurred in a sufficient number of chickens in Exp. 2 and 3 to permit comparison of the different groups (Table I). Those birds receiving cod-liver oil of source A in their diet had a distinctly higher incidence of lymphocytoma than the control birds on the basal diet alone. One group of chickens fed source A cod-liver oil in Exp. 2 had an incidence of lymphocytoma of 30.4% (the exposure of this group was contact with Line 15 stock inoculated with RPL 12 material) and another group similarly fed had an incidence of 40.9% (the contact exposure for this group was with birds having a history of high incidence of naturally occurring lymphocytoma). The incidence of lymphocytoma in the 2 control groups on basal diet in Exp. 2

[‡] Arista Oil Products Corp., New York, and Silmo Chemical Corp., Vineland, N. J. were very helpful in obtaining the cod-liver oils. Appreciation of their assistance is gratefully acknowledged.

TABLE I. Incidence of Neoplasia in Chickens Exposed to the Leukosis Agent and Fed a Basal Diet Plus 3% Cod-liver Oils. Results are given in % of number of birds, and figure in () is actual number of cases.

Exp	Type of cod-liver oil in diet	No. of birds	Weeks duration	Lympho-cytoma	Erythro-blastosis	Other tumors	Neurolymph-omatosis
1	Medicinal	48	15	2.1 (1)	25.0 (12)	6.2 (3)	
	Poultry	45	15	.0 (0)	31.1 (14)	6.6 (3)	
	D "off-grade"	45	15	.0 (0)	31.1 (14)	4.4 (2)	
	E "off-grade"	49	15	6.1 (3)	20.3 (10)	6.1 (3)	
	None	48	15	2.1 (1)	20.8 (10)	6.2 (3)	
2	Source A	69	71	30.4 (21)	5.6 (4)	.0 (0)	
	None	80	71	20.0 (16)	5.0 (4)	1.2 (1)	
	Source A	61	71	40.9 (25)	13.1 (8)	.0 (0)	
	None	46	71	19.5 (9)	26.1 (12)	6.5 (3)	
3	Source A	85	50	54.1 (46)	2.4 (2)	1.2 (1)	15.3 (13)*
	Source B	78	50	38.5 (30)	.0 (0)	2.5 (2)	11.5 (9)†
	None	80	50	37.5 (30)	6.2 (5)	2.5 (2)	12.5 (10)‡

* 7 cases also recorded as lymphocytoma, 1 as erythroblastosis and 1 as other tumor.

† 5 cases also recorded as lymphocytoma.

‡ 9 cases also recorded as lymphocytoma and 1 as other tumor.

was 20 and 19.5% respectively. Chickens on the basal diet in Exp. 3 had an incidence of 37.5% lymphocytoma; those receiving source B cod-liver oil, an incidence of 38.5% and those receiving source A cod-liver oil 54.1%.

Time of death from lymphocytoma in Exp. 2 and 3 ranged from 100 to nearly 500 days with the majority of deaths occurring between the ages of 200 to 300 days (Table II).

One group of chickens in Exp. 1 receiving cod-liver oil designated "E" had an incidence of 6.1% lymphocytoma (3 cases), and the controls 2.1% (1 case).

Erythroblastosis occurred at the anticipated level in the chickens of Exp. 1, and the incidence varied from 20.3 to 31.1% among the birds receiving cod-liver oil in the diet as compared to 20.8% in the chickens on the basal diet. Age at death from erythroblastosis

ranged from 6 to 15 weeks in Exp. 1.

The age at death from erythroblastosis ranged from 22 to 150 days in the inoculated chickens and from 100 to 500 days in the birds exposed by contact in Exp. 2. Deaths from erythroblastosis began at 100 days in Exp. 3 also and continued to 300 days. The incidence of erythroblastosis was prominent in only 2 comparable groups of Exp. 2 (31.1 and 26.1%). In these groups the mortality was evenly spread from 100 to 500 days. The incidence of other tumors was relatively low in all groups except those of Exp. 1 where they ranged from 4.4 to 6.6%. There was no age period of marked frequency.

Neurolymphomatosis occurred in all 3 groups of chickens in Exp. 3. These were slightly more common during the last 150 days of the experiment. Several chickens were diagnosed as having both lymphocytoma

TABLE II. Occurrence of Lymphocytoma and Number of Birds at Risk at Different Periods of Experiment. Figures in () represent % incidence of lymphocytoma based on number of birds at beginning of the respective time period.

Exp	Type of cod-liver oil in diet	Period of experiment					
		0-200 days		201-350 days		351-500 days	
		No. of birds	Lympho-cytoma	No. of birds	Lympho-cytoma	No. of birds	Lympho-cytoma
2	Source A	69	2 (3)	67	17 (25)	48	2 (4)
	None	80	1 (1)	77	12 (16)	65	3 (5)
	Source A	61	2 (3)	58	20 (34)	36	3 (8)
	None	46	— (0)	43	7 (16)	33	2 (6)
3	Source A	85	7 (8)	76	39 (51)		
	" B	78	10 (13)	66	20 (30)		
	None	80	6 (8)	70	24 (34)		

and neurolymphomatosis (Table I).

Discussion. The only consistent result with respect to lymphocytoma was the increased incidence of those birds that had cod-liver oil of source A in their diet. This result occurred in essentially replicate trials in Exp. 2 and again in Exp. 3. The difference in incidence was not great in any of the comparable test groups. The increased incidence of erythroblastosis in 2 test groups of Exp. 2 might cause concern since erythroblastosis is sometimes believed to occur at an early age. A high early incidence would therefore reduce the number of chickens at risk from lymphocytoma. In these 2 test groups as well as the other pair in Exp. 2, the mortality rate from erythroblastosis was actually slightly higher during the last 150 days of the experiment which was after the peak incidence of lymphocytoma. Since the results of the 3 trials showed a higher incidence of lymphocytoma in chickens fed cod-liver oil A the data were pooled to calculate a summary adjusted chi square test for significance(12). A chi square of 11.29 was found indicating a statistically high degree of significance. Cod-liver oil B from another source did not demonstrate this effect in Exp. 3. The 6% incidence of lymphocytoma in the group receiving cod-liver oil "E" in Exp. 1 is of questionable importance because of the small number of cases.

There was no evidence of a consistent influence of cod-liver oil in the diet on incidence of erythroblastosis or tumors other than lymphocytoma.

The mechanism by which cod-liver oil in the diet may influence the development of lymphocytoma is unknown. This influence was possessed by only one cod-liver oil and may be unrelated to anything usually found in cod-liver oils. Since erythroblastosis and lymphocytomas of these experiments were induced with what is presumed to be the same viral agent, there may be a metabolic difference between the lymphoid cell of the lymphocytoma and the erythroblast of erythroblastosis.

The occurrence of neurolymphomatosis in chickens of Exp. 3 which received RPL inoculum preparation L-31 was different than the result obtained with preparation L-30 in Exp. 2, or preparation L-29 in Exp. 1. This may be due to a factor peculiar to inoculum L-31 or it may be due to the different dosage levels used. The dosage of L-29 in Exp. 1 was larger for the purpose of producing erythroblastosis and the experiment was of shorter duration. If the factor producing neurolymphomatosis was present in inoculum L-30 it was not transmitted from the inoculated birds to the birds exposed by contact to them. Burmester(13) is of the opinion that neurolymphomatosis and visceral lymphomatosis (lymphocytoma) are caused by different viruses.

Summary. One particular lot of cod-liver oil increased the incidence of lymphocytoma (lymphomatosis) in chickens exposed to an infective leukosis agent by contact with infected chickens as well as in chickens given the infective agent by intraperitoneal injection. Another lot of cod-liver oil did not have this effect. There was no evidence of influence of cod-liver oil in the diet on the incidence of erythroblastosis, or tumors other than lymphocytoma.

1. Olson, C., *Cancer Res.*, 1941, v1, 384.
2. Burmester, B. R., *ibid.*, 1947, v7, 786.
3. Burmester, B. R., Gentry, R. F., *Poultry Sci.*, 1956, v35, 17.
4. Waters, N. F., *Poultry Sci.*, 1945, v24, 259.
5. Olson, C., *Am. J. Vet. Res.*, 1945, v6, 103.
6. Burmester, B. R., *Poultry Sci.*, 1956, v35, 1089.
7. Wilcke, H. L., Patterson, F. D., Henderson, E. W., Murray, C., *ibid.*, 1933, v12, 226.
8. Butler, W. J., Warren, D. M., *J. Am. Vet. Med. Assn.*, 1938, v92, 204.
9. Taylor, L. W., DeOme, K. B., *ibid.*, 1939, v95, 73.
10. Jungherr, E., *Poultry Sci.*, 1940, v19, 94.
11. Waters, N. F., personal communication.
12. Steel, R. G. D., Torrie, J. H., *Principles and procedures of statistics*, McGraw-Hill Book Co. Inc., New York, 1960, p371.
13. Burmester, B. R., Gross, M. A., Walter, W. G., Fontes, A. K., *J. Nat. Cancer Inst.*, 1959, v22, 103.

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