response to vigorous prodding was not significantly changed. 4. Normal control monkeys under the acute influence of chlorpromazine did not exhibit clinical convulsions and remained refractory to treatment with intramuscular Metrazol (32 mg/kg) and to vigorous prodding stimulation.

1. Kopeloff, L. M., Chusid, J. G., and Kopeloff, N., *Neurology*, 1954, v4, 218.

2. Chusid, J. G., Kopeloff, L. M., and Kopeloff, N., *J. Appl. Physiol.*, 1953, v6, 139.

3. Das, N. N., Dasgupta, S. R., and Werner, G., *Bull. Calcutta School Tropical Med.*, 1954, v1, 5.

4. Gibbs, F. A., and Gibbs, E. L., *Atlas of Electroencephalography*, Addison-Wesley Press, Cambridge, Mass., 1952, v2, 19.

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In vitro and *in vivo* Neutralization of the Virus of Visceral Lymphomatosis. (22011)

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It has been reported(1) that the progeny of hens which had received a series of injections of the virus of visceral lymphomatosis were much more resistant to challenge with this virus than were the progeny produced by the same hens before the injections were made. The suggestion was made that the basis for this increased resistance was the transfer of antibodies from the dam to the egg and thence to the chick. This is known to occur in other diseases of chickens.

Tests reported here provide evidence that the tumor inciting properties of the virus of visceral lymphomatosis are neutralized by specific antiserum, that serum neutralizing antibodies in chickens are produced by the injection of materials containing the live virus, and that the increased resistance of the progeny is directly related to the increase in specific serum antibodies in dams.

Materials and methods. Antisera for *in vitro* and *in vivo* neutralization tests were obtained from hens that had received a series of injections of preparations containing the virus of visceral lymphomatosis. A description of these preparations and the sequence of injections have been given(1). Normal serum for both tests was collected 6 days before the hyperimmunizing injections were begun. Immune serum for the *in vitro* test was collected from one of the hens 4 days after the last of the first series of injections. Two months later a second series of injections was given intramuscularly. The material injected was live virus preparation similar to that used in the first series. Immune serum for the *in vivo* test was collected 6 weeks after the last of the second series of injections. There was an interval of about 6 months between the first injection and the final collection of immune serum. To provide a sufficient volume of serum for the *in vivo* test the serums from 9 hens were pooled. All serums were obtained from blood collected by heart puncture. The blood was transferred to large centrifuge tubes and refrigerated over night. After centrifugation the serum was transferred to glass vials and sealed. The tubes were stored at –30°C. The source of virus used in both tests was lymphomatous livers of birds in the sixteenth serial passage of strain RPL 12(2). The chicks were from an inbred line of S.C. White Leghorns maintained in isolation for the past 13 years. The incidence of lymphomatosis in isolation has been very low; however, when naturally or artificially exposed, the stock has manifested susceptibility to the disease. *In vitro* neutralization tests were made with constant volumes and dilutions of serums mixed with 4 serial 100-fold dilutions of the virus preparation. Dilutions were made with Simms' salt solution containing 1.5% bovine albumin. Each inoculating dose of 0.2 ml contained filtrate obtained from log –3, –5, –7, and –9 g of lymphomatous liver. All serums were heated to 56°C for 30 minutes,

TABLE I. *In Vitro* Neutralization of Virus of Visceral Lymphomatosis by Specific Antiserum.

Serum	Log dose of virus	No. inoculated Total	Corrected	% visceral lymphomatosis 112	140	168	201 days	Latent period (days)
Normal	−3	42	40.7	61.5	66.4	69.8	71.3	81.0
	−5	40	35	17.1	31.4	37.2	60.0	138.2
	−7	37	36.1	8.3	24.9	33.3	36.0	129.5
	−9	41	39.4	0	0	2.5	12.7	191.8
Immune	−3	41	35.2	11.3	22.7	56.9	76.8	148.0
	−5	42	40.1	2.5	2.5	5.0	25.0	172.5
	−7	41	39	0	2.6	5.1	5.1	149.0
	−9	40	39	0	0	0	0	—
"	0	38	38	0	0	0	0	—
Diluent	0	38	38	0	0	0	0	—

24 hours before use. One ml of serum was added to each 9 ml of virus preparation. The virus preparation and the virus-serum mixtures were maintained at melting ice temperatures at all times. The interval between mixing and inoculation of the various mixtures was 2-3 hours. The inoculations were made with a 1 ml tuberculin syringe into day-old chicks by the intraperitoneal route. For *in vivo* neutralization tests, chicks were injected with pooled serum—on the first day with 0.5 ml intraperitoneally, on the second day with 1 ml subcutaneously, and on the third day with 0.5 ml subcutaneously. On the third day the chicks received by the intraperitoneal route 0.2 ml of the virus preparation of a dilution equivalent to log −3, −5, or −7 g of tumor. All chickens were maintained for a period of 201 days under experimental conditions similar to those previously described(3,4).

Results. Data obtained from the *in vitro* neutralization test are summarized in Table I. The percentage of mortality from visceral lymphomatosis was based on the number per lot as corrected(3) for deaths due to other causes. The incidence of tumors at progressive ages is given in the table, since it has been shown(4) that differences in response to different dosages tends to decrease with an increase in the holding period. With one exception, the percentage of visceral lymphomatosis for each age interval and for different virus dosage lots was lower in chickens that had received the inoculum containing immune serum than in those that had received mixtures containing the normal serum. These quantal response data clearly indicate that at the dose of log −9 practically all, if not all, of the virus was neutralized and at the doses of log −7 and −5 at least 99% of the virus was neutralized. There was no essential difference in the incidence of tumors between the immune and normal serum at the log −3 dosage level and longest holding period, but at this highest virus dosage, partial neutralization is indicated by a marked delay in mortality. If the latent period is used as the basis for virus activity, it can be estimated that the addition of immune serum to the virus at a log dose of −3 had an effect equivalent to a dilution of the virus of at least 1:1000, or that neutralization of 99.9% of the virus occurred. This estimate was based on dose-latent period curves of 4 studies reported(4) and a fifth recently completed.

Data on the influence of injecting normal and immune serum into susceptible chicks prior to their being inoculated with various doses of virus are summarized in Table II. The mortality from visceral lymphomatosis to 201 days of age among chicks that received the normal serum injections was about the same as among chicks that did not receive any serum. The response in all of the three different dosage lots was less than expected on the basis of other inoculation results(4) with the same virus preparation. However, the responses were much greater than obtained with any of the 3 dosage lots in chicks injected with immune serum. Thus, none of the chickens that had received a total of 2 ml of immune serum during the first 3 days of life, and subsequently were inoculated with virus at a dosage of log −5 and log −7, and only 3 of 24 chickens similarly treated and challenged with the maximum dose of log −3, developed vis-

TABLE II. *In Vivo* Neutralization of the Virus of Visceral Lymphomatosis by Specific Antiserum.

Serum	Log dose of virus	No./lot		% visceral lymphomatosis at 201 days	Latent period (days)
		Total	Corrected		
0	−3	22	20.9	47.8	92.7
	−5	24	22.1	13.6	144.8
	−7	23	22.2	13.6	171.3
Normal	−3	23	20.7	63.8	83.1
	−5	23	21.4	14.0	157.1
	−7	24	23.4	14.0	144.3
Immune	−3	24	23.5	12.7	188.0
	−5	24	23.3	0	—
	−7	23	22.9	0	—
Non-inoc. controls		39	38.2	0	—

ceral lymphomatosis. Furthermore, these 3 died late in the experimental period, resulting in a mean latent period greater than that of any other lot.

Estimates of neutralization based on the latent period data of the *in vivo* experiment, are not nearly as reliable as those based on the *in vitro* experiments, because of the small number of birds that died; however, the differences are of such magnitude that they add considerable weight to the quantal response data.

Results from the 2 experiments described demonstrated the presence of an antibody which neutralizes the oncogenic activity of the virus of visceral lymphomatosis. This antibody was found to be present in serum of chickens after they had received a series of injections of filtered extract of lymphomatous liver containing the active virus, and was essentially absent in serum of the same chicks collected just prior to the injection of the virus. These chickens were from a flock that had been reared in isolation for many years and were relatively free from infection with any known disease agent.

There have been many reports [see review of Olson(5), and the reports of Kabat and Furth(6), Furth and Kabat(7)] of indications of immunity and of serum antibodies in experimental work with the leukemic forms of avian leukosis. Recently, Eckert *et al.*(8) working with the virus of myeloblastosis were regularly able to provoke the formation of specific neutralizing and precipitating antibodies in normal growing chickens not previously exposed. This was done by repeatedly administering virus concentrates inactivated by formalin and then injecting highly infectious plasma. Lee(9) reported the development in ducks and turkeys of neutralizing antibodies effective against myeloblastosis and neurolymphomatosis. There is indirect evidence to indicate that the various forms of avian leukosis are caused by different etiologic agents(10-12).

Summary. Evidence is presented for the production of antibodies which neutralize the oncogenic activity of the virus of visceral lymphomatosis, by hens injected with virulent virus. Serum from such hens injected into chicks has produced a passive immunity.

1. Burmester, B. R., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 153.

2. Burmester, B. R., and Cottral, G. E., *Cancer Res.*, 1947, v7, 669.

3. Burmester, B. R., and Gentry, R. F., 91st Ann. Meet. Am. Vet. Med. Assn. Proc., 1954, pp311-316.

4. Burmester, B. R., *Poultry Sci.*, in press.

5. Olson, Carl Jr., *Mass. Agr. Exp. Sta. Bull.* 370.

6. Kabat, E. A., and Furth, J., *J. Exp. Med.*, 1941, v74, 257.

7. Furth, J., and Kabat, E. A., *ibid.*, 1941, v74, 247.

8. Eckert, E. A., Sharp, D. G., Beard, Dorothy, Green, I., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 181.

9. Lee, C. D., *Am. J. Vet. Res.*, 1942, v3, 336.

10. Beard, J. W., Sharp, D. G., and Eckert, E. A., in press.

11. Burmester, B. R., Gentry, R. F., and Waters, N. F., *Poultry Sci.*, 1955, v34, 609.

12. Waters, N. F., *ibid.*, 1954, v33, 365.

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