In the pilot experiments for the present study, of 7 fasted rabbits given 600 r of X-ray and injected with labeled platelets after thrombocytopenia had developed, 2 showed a normal survival of labeled platelets, 3 animals died before platelet radioactivity had disappeared from the circulation, and 2 showed an early disappearance of platelet radioactivity coincidental with hemorrhage. When 3 larger rabbits, not fasted, were used, 2 doses of 750 r 2 weeks apart were required to produce persistent thrombocytopenia below 100,000/mm^3. None of these developed the intestinal necrosis syndrome, or generalized bleeding and injected labeled platelets survived normally for 5, 6, and 7 days. These studies, while incomplete, do not suggest any circulating anti-platelet factors resulting from radiation.

There is some evidence that irradiation in large doses has a damaging effect on circulating erythrocytes(2,10), in addition to the effect on erythropoiesis. The normal platelet survival reported here does not support such a contention with respect to platelets, at least with the doses of X-ray employed. No satisfactory explanation is available for the observed post-irradiation rise in circulating platelet radioactivity. It was present in 9 of 13 irradiated animals, and only 3 of 12 controls, but was not statistically significant. It was not accompanied by any change in the peripheral platelet count.

Conclusions. The life span of circulating platelets labeled *in vitro* with sodium chromate^{51} was normal in rabbits exposed to 750 r of X-ray subsequent to injection of the labeled platelets.

1. Lawrence, J. S., Dowdy, A. H., and Valentine, W. N., *Radiology,* 1948, v51, 400.

2. Jacobson, L. O., Marks, B. K., and Lorenz, E., *ibid.,* 1949, v51, 371.

3. Cronkite, E. P., Jacobs, G. J., Brecher, G., and Dillard, G., *Am. J. Roentgenol.,* 1952, v7, 796.

4. Morgan, M. C., Keating, R. P., and Reisner, E. H., Jr., *J. Lab. and Clin. Med.,* 1955, v46, 521.

5. Odell, T. T., Gamble, F. N., and Furth, J., *Fed. Proc.,* 1953, v12, 398.

6. Dillard, G., Brecher, G., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 796.

7. Lawrence, J. S., and Valentine, W. N., *Blood,* 1947, v2, 40.

8. Hirsch, E. O., and Gardner, F. H., *J. Lab. and Clin. Med.,* 1952, v39, 556.

9. Cronkite, E. P., and Brecher, G., *Tr. Fifth Macy Conf. on Blood Clotting,* Josiah Macy Fd'n., 1952, 171.

10. Levin, W. C., and Krise, G. M., Sixth Internat. Cong. of Hematol., Boston, Mass., 1956, abst. in program, p230.

Received June 7, 1957. P.S.E.B.M., 1957, v96.

A Modified Live Newcastle Disease Virus Vaccine.* (23408)

R. A. BANKOWSKI (Introduced by S. A. Peoples)
School of Veterinary Medicine, University of California, Davis

Use of viable, virulent virus vaccines can increase incidence of respiratory disease in poultry, perpetuate disease in birds or produce a serious respiratory disease complex when superimposed in an already diseased flock. Failure to induce adequate immunity with inactivated Newcastle disease virus vaccine makes desirable an attenuated nonspreading virus strain; for vaccine production the strain should be propagable in nonchicken tissue to avoid contamination of poultry with egg-borne infections. This report describes modification of Newcastle disease (ND) virus and preliminary results of immunization of chickens.

Materials and methods. Modification of the virus. The California strain 11914 of Newcastle disease virus was modified by serial passage through tissue cultures composed of minced chicken embryo tissues suspended in Simm-Sanders medium containing bovine ultrafiltrate. Virus of passage 47 to 55 when

* Study supported in part by grant-in-aid from Swift & Co., Chicago, Ill.

administered by the air borne route to chickens in an enclosed chamber induced an excellent immunity, but on occasion caused symptoms or a mortality which reached 6.6% (1,2). Also parenterally administered virus killed 48-hour-old chicks. Investigations toward a solution of the respiratory disease complex reopened the search for a more desirable vaccine in 1954. Beginning with 47 passage, a series of 16 passages was continued using the same technic but alternating the passages through cultures containing bovine serum ultrafiltrate and cultures prepared without the ultrafiltrate. The series was continued for 12 passages using Sabin's technic in which the incubation period was reduced to 24 hours and the inoculum increased to at least 20% (3). A complete loss of virulence following parenteral inoculation into young chicks took place between the 58th and 75th passages. Intramuscular inoculation of 130 12-day-old chicks with the 71st passage virus was innocuous and larger amounts of the cultures were prepared for more extensive trials. Virus propagated in chicken embryo tissue was designated as line TC. To eliminate the use of chicken tissue, the 77th serial passage was propagated in quantities for study in monolayer tissue cultures of HeLa cells(4). The latter was designated as line TCH. *Titration of vaccine virus in embryos.* The inoculum employed (line TC or TCH) for vaccination consisted of the supernatant fluid of the culture which was frozen and stored in ampoules at −20°C. Aliquots of the infectious material were titrated in 8- or 9-day-old chicken embryonated eggs. Before use, the thawed suspension was diluted to the desired concentration with phosphate-buffered saline (pH 7.2).

Challenge virus. All chickens vaccinated with the TC or TCH modified virus were challenged intramuscularly at various intervals thereafter with strain GB Newcastle disease virus. The challenge for each bird consisted of 0.2 ml of infectious allantoic and amniotic fluids of chicken embryos diluted to contain 200,000 chicken embryo lethal doses (ELD_{100}). *Experimental chickens.* All chickens were obtained at one day of age and placed in a separate isolation building until used. Chicks from ND-susceptible dams were obtained from the University Poultry Husbandry flock which is not vaccinated and is under constant surveillance for disease. The commercially hatched chicks were obtained from breeding flocks with known vaccination histories. Hemagglutination inhibition (HI) and neutralization (SN) tests were conducted to determine the presence and degree of passive immunity in the chicks before each experiment(5).

TABLE I. Serological and Immune Response of 30-Day-Old Chickens Vaccinated Intramuscularly with 0.25 ml of Line TC Newcastle Disease Virus of the 76th Tissue Culture Passage.

Vaccine titer ELD_{100}	HI titers and challenge tests following vaccination				
	3 wk	7 wk		12 wk	
	HI*	HI*	Challenge†	HI*	Challenge†
10^6	7.0	3.3	0/3	1.6	0/3
10^4	8.0	2.8	0/3	1.2	0/3
10^2	4.0	1.7	0/3	1.0	2/3
None	1.0	1.0	3/3	1.0	3/3

* Expressed as geometric mean titer.
† No. susceptible/No. challenged.

Results. Immunogenicity of the virus. To determine optimum dosage of virus to induce an adequate immunity, 3 groups of 30-day-old chicks were inoculated intramuscularly with 10^6, 10^4 and 10^2 ELD_{100} doses respectively of the 76 passage of line TC. It will be observed in Table I that 10^4 and 10^6 ELD_{100} of virus produced a high degree of immunity which was effectively maintained for at least 12 weeks. Reducing quantity of virus resulted in a weaker immunological response and a shorter immunity. The results of 4 experiments demonstrating the avirulence of the strain TC and TCH for young chicks and the immunogenic properties of the virus are shown in Table II. In no instances were symptoms of the disease or any systemic reaction observed during the experiments. It is readily seen that one dose of .25 ml of line TC virus administered intramuscularly into 5-day-old chicks from susceptible dams resulted in a substantial HI antibody response, and all birds were solidly immune to an intramuscular challenge of virulent ND virus 12 weeks later. Progeny from immune dams in which HI and SN antibodies were demonstrated prior to vaccination acquired an irregular and

TABLE II. Immunological Response of Young Chicks of Susceptible or Immune Dams to Vaccination with Strains TC or TCH Newcastle Disease Virus.

Exp. No.	Vaccine: Dosage* and age given	Vaccine: Virus and passage	Flock source of chicks	No. of birds	3 wk: HI‡	3 wk: Chal.†	Weeks following vaccination† 5 wk: HI	5 wk: Chal.	8 wk: HI	8 wk: Chal.	12 wk: HI	12 wk: Chal.
1	.25 ml IM at 5 days	TC 76th	Susceptible	23			8.6	0/14			4.7	0/ 9
2	*Idem*	" "	Commercial	38			1.03	10/11	1.07	3/10	1.08	7/17
3	.25 ml IM at 3 wk	" "	"	30			3.0	0/14	4.0	0/16		
4A	.2 ml IM at 6 wk	TCH 95th	Susceptible	20	3.5	0/10					1.10	0/10
4B	*Idem*	TC 76th	"	20	3.3	0/10					1.0	0/10

* Dosage: Adjusted to contain 10^4 embryo lethal doses/0.1 ml of suspension.
† At least 20% of animals in each experiment were held as controls. All were completely susceptible through the trials.
‡ See Table I (footnotes).

less effective immunity within the group. The influence of passive immunity on vaccination of young chicks with the modified virus was corroborated in Exp. 3. Chickens obtained from a commercial flock but held in isolation for 21 days before intramuscular vaccination gave a good serological response and showed a solid immunity to intramuscular challenge 8 weeks later. To determine the immune response produced in chickens by the modified ND virus propagated in HeLa cells (line TCH), the 95 passage of the latter was compared with the 76 passage of virus grown in chicken embryo tissues (line TC). Two groups, each containing 20 chicks, were vaccinated intramuscularly with 0.2 ml of each of the lines of virus. Exp. 4 of Table II illustrates that comparable serological responses were observed in the birds given the 2 lines of the modified virus and a solid immunity to intramuscular challenge was observed in all of the chicks 12 weeks after vaccination. In contrast, an equal number of nonvaccinated challenge controls suffered 100% mortality. *Duration of immunity.* Following the immunological response observed in young chicks, which lasted for a period that would be practical for broiler producers, the experiments were continued with the object of adapting the procedure for immunizing laying hens. The results are summarized in Tables III and IV. No respiratory or nervous symptoms of ND were observed in any of the birds receiving the virus. Birds vaccinated at 11, 20 or 5 weeks of age (Exp. 1B, 1C in Table III and 2 and 3 in Table IV) for the first time responded with a good antibody response and a solid immunity evident on challenge 4 and 8 weeks later. Administration of the second dose of 0.25 ml of the vaccine 9 weeks later (Exp. 1A, Table III) resulted in a marked anamnestic response in which the HI serum antibodies attained titers greater than those following a single injection into birds of varied ages and sources. The immunity following the second dose, expressed as a high antibody response and refractivity of the birds to intramuscular challenge, was still demonstrable 33 weeks after vaccination. This was the last time the birds were tested.

TABLE III. Duration of Immunity following Vaccination with Strain TC Newcastle Disease Virus.

Exp.	Dosage* and age given	No. of birds	Weeks following first vaccination: 4 wk HI†	4 wk Chal.†	8 wk HI	8 wk Chal.	24 wk HI	24 wk Chal.	27 wk HI	27 wk Chal.	33 wk HI	33 wk Chal.
1A	.25 ml IM at 11 wk .25 " " " 20 "	85	3.0	0/6	2.8	0/ 6	6.5 or >		3.7	0/10	4.0	0/10
B	.25 " " " 11 "	15					1.4		1.4	1/13		
C	.25 " " " 20 "	35	2.6	0/6	2.1	0/ 8	2.1	0/8				
D	None penmate controls	60	1.0	6/6	1.0	15/15			1.0	8/ 8	1.0	10/10

* See Table II (footnote *). † See Table I (footnotes).

The remaining chickens will be challenged at intervals to determine duration of immunity, and the complete study will be published elsewhere. There was no evidence of virus transmission from the vaccinated to susceptible-in-contact birds over a period of 33 weeks. Since no adverse effects were observed following exposure of approximately 900 chickens to the virus in the laboratory trials, the vaccine was administered to chickens on a commercial chicken ranch. In addition to the number of chickens reported in this paper, at least 20,000 chickens were vaccinated at 5 weeks of age without showing any symptoms of the disease. Of these, 12,000 were revaccinated when 12 to 14 weeks of age. Table IV gives the HI antibody titers of a representative number of birds following exposure to virus of 2 groups of 3750 chicks each. Revaccination with a reduced dosage of 0.2 ml (Table IV) did not result in as great a rise in HI antibody response; however, data from other experiments indicate that following vaccination a solid immunity to an intramuscular challenge dose of virulent virus can be demonstrated in the absence of serum HI antibodies. Nevertheless, more studies relative to dosage and the interim between inoculations are necessary to obtain the optimum immune response. It is also interesting to note that in each experiment the 150 birds left unvaccinated as pen contacts in each group (Exp. 2 and 3) did not show evidence of transmission of the virus from the vaccinated birds. Challenge data for these groups are not available at this time because of the limited number of controls and the desire to preserve for use at a later date. The avirulence of the modified virus (line

TABLE IV. Response of Chickens in Field to Vaccination with Modified Line TC Newcastle Disease Virus.

Exp.	Dosage* and age given	No. birds	HI response at weeks following first vaccination: 4 wk HI†	8 wk HI	12 wk HI	Remarks
2	.2 ml IM at 5 wk .2 " " " 12 "	3750	3.7	1.5	2.8	First dose given when symptoms to infectious bronchitis vaccination were acute and severe.
	None pen contact controls	150	1.0	1.0	1.0	
3	.2 ml IM at 5 wk .2 " " " 14 "	3750	3.7	2.3	3.5 or >	
	None pen contact controls	150	1.0	1.0	1.0	
4	.2 ml IM at 12 wk	2500		3.0		Vac'inat'd during acute stages of chronic respiratory disease.
	None ranch controls	2500		1.0		

* See Table II (footnote *). † See Table I (footnote *).

TC) was also demonstrated when administered to birds experiencing an acute outbreak of another respiratory disease. In Exp. 4, 2500 12-week-old pullets were each injected with 0.20 ml of the vaccine and compared with an equal number of nonvaccinated birds under the same managemental conditions. Over a period of 12 days, no difference was observed in symptomatology, weight gains, or feed consumption between the two groups. Failure of symptoms to become aggravated was also observed in Exp. 2, in which the vaccination was conducted during an acute respiratory phase of infectious bronchitis induced by vaccination for the disease 10 days previously.

Summary. 1) Newcastle disease virus was modified by serial passage of the California 11914 strain through tissue cultures using minced chicken embryos suspended in Simm-Sanders medium. 2) The modified virus was administered intramuscularly to over 20,000 chickens of all ages without producing any nervous or respiratory symptoms, but produced an immunity as demonstrated serologically and by intramuscular challenge with a virulent strain of ND virus. One dose of the vaccine given to susceptible chicks at 5 days of age or over induced an immunity lasting at least 12 weeks. Two doses from 7 to 9 weeks apart have resulted in a high degree of immunity which was still evident 33 weeks later. 3) When administered to 2 flocks of chickens, one in acute stage of infectious bronchitis and the other with chronic respiratory disease, the virus apparently did not increase the severity of the respiratory disease condition. 4) Repeated trials showed that vaccinated chickens did not transmit the infection to unvaccinated susceptible birds within the same pen during 33 weeks. 5) The modified virus can be propagated on HeLa cells to produce a vaccine for poultry, thus avoiding the introduction of egg-borne infections to poultry flocks through vaccines produced in chicken embryonated eggs.

The author wishes to express his appreciation for the fine technical assistance of E. E. Stuart, R. Corstvet and J. Fabricant.

1. Bankowski, R. A., and Boynton, W. H., *Vet. Med.*, 1948, v43, 305.

2. ———, *ibid.*, 1950, v45, 322.

3. Sabin, A. B., *Dynamics of Virus and Rickettsial Infections*. 1954, Blakiston Co., Inc., New York, p. 290.

4. Bankowski, R. A., and Hyde, J., *Vet. Res.*, 1957, v18.

5. Beach, J. R., *JAVMA*, 1948, v112, 85.

Received June 10, 1957. P.S.E.B.M., 1957, v96.

Some Physical and Chemical Properties of Antibody Against Complement.* (23409)

W. S. JETER AND ALBERT P. MCKEE

Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City

We(1) have previously described an antibody against complement. Most of the experiments then reported were concerned with activity of anticomplement as an inhibitor in the immune hemolytic system. Mention was made, however, of experiments to determine more of the physical and chemical properties of this antibody. The purpose of this paper is to describe in more detail studies on quantitative antibody protein, antibody expressed as carbon-14, Tiselius electrophoretic analyses, and fractionation of anticomplement-containing sera.

Methods. Antibody was prepared in rabbits to complement of the guinea pig, human being, monkey, and mouse by the method previously described(1). Briefly, the immunizing procedure consisted of giving animals several subcutaneous injections of different immune precipitates to which complement had

* Supported in part by grants from Office of Naval Research and College of Medicine Fund, State University of Iowa.