

thrombin A."¹⁰ The presence of the factor in serum might explain Schofield's¹ and Rodrick's² therapeutic findings—findings difficult to understand if deterioration of prothrombin alone were present in cases of dicumarol or toxic sweet clover poisoning.

Summary. Evidence is presented that suggests the disappearance not only of prothrombin in dogs treated with dicumarol, but also of a factor important in the conversion of

prothrombin to thrombin. Like prothrombin, this factor is associated with the plasma pseudoglobulins, but unlike prothrombin it is plentiful in serum. Addition of plasma, serum or serum pseudoglobulin corrects the delayed prothrombin convertibility of early dicumarolization or of stored plasma. The discrepancy between the one-stage and 2-stage methods of estimating prothrombin seems to reside in this conversion factor.

¹⁰ Quick, A. J., *Am. J. Physiol.*, 1943, **140**, 212.

16263

Immunological Studies of Newcastle Virus.

REGINALD L. REAGAN, MARY G. LILLIE, JEAN E. HAUSER, AND A. L. BRUECKNER.

From the Maryland State Board of Agriculture, Live Stock Sanitary Service, University of Maryland, College Park, Maryland.

A California strain of Newcastle virus, No. 11,914, which was adapted to the Syrian hamster,¹ proved valuable as an immunizing agent in chickens against the virulent unmodified virus of this strain.² This successful immunization prompted continued experiments to study the relationship of the living modified virus as an immunizing agent, not only against the California strain but also against a Colorado strain, No. 8518, and a Connecticut strain, secured from the Bureau of Animal Industry, Washington, D.C.

Healthy 6-week-old New Hampshire Red chickens were used, and all birds were identified by wing bands. Serum from these chickens showed no neutralizing antibodies for Newcastle disease when tested by the chick embryo neutralization test prior to the start of the experiment. Three hundred fifty-two chickens were vaccinated by injecting 0.2 cc of a 10% suspension of virus-bearing hamster brain into the base of the wattle. Forty infected hamster brains of the eighty-

second subculture were used for preparation of the vaccine, with physiological saline as the diluent. One hundred thirty-four unvaccinated chickens were placed with the vaccinated birds as room contact controls. All of the chickens were checked carefully each day. Table I shows the observations during the 28-day period prior to challenge.

Three groups were then set up, each consisting of vaccinated birds, unvaccinated room control and normal susceptible birds of corresponding age. Group I was challenged with the virulent chick embryo propagated California strain, No. 11,914, subculture 18. Group II was challenged with the virulent chick embryo propagated Colorado strain, No. 8518, subculture 8. Group III was challenged with the virulent chick embryo propagated Connecticut strain, subculture 8. The results obtained after challenge are shown in

TABLE I.
Evaluation of Chicken Deaths Following Vaccination, Prior to Challenge.

	Vaccinated (352)	Room controls (134)
Coccidiosis	10	1
Newcastle	1	0
Smothered	1	2
Miscellaneous	15	2

¹ Reagan, Reginald, L., Lillie, Mary G., Poelma, Leo J., and Brueckner, A. L., *Am. J. Vet. Res.*, 1947, **8**, 136.

² Reagan, Reginald, L., Lillie, Mary, G., Poelma, Leo J., and Brueckner, A. L., to be published.

TABLE II.

Immunization of Chickens Against Newcastle Disease with Modified Newcastle Virus.

Route of vaccination: Base of wattle.

Date of vaccination: 9/5/47

Route of challenge: Base of wattle.

Date of challenge: 10/3/47

Challenge dose: 0.5 cc of given dilutions.

Challenge Virus (California Strain).

Group I	Dilutions								%	
	10-1		10-2		10-3		10-4			
	S*	D*	S	D	S	D	S	D	D	S
Vaccinated	18	2	15	5	17	3	17	3	16	84
Unvaccinated contact controls	2	8	7	3	4	6	3	7	60	40
Normal controls	3	7	5	5	6	4	3	7	57.5	42.5

* S—No noticeable symptoms.

D—Dead, also those showing Newcastle disease symptoms.

TABLE III.

Immunization of Chickens Against Newcastle Disease with Modified Newcastle Virus.

Route of vaccination: Base of wattle.

Date of vaccination: 9/5/47

Route of challenge: Base of wattle.

Date of challenge: 10/3/47

Challenge dose: 0.5 cc of given dilutions.

Challenge Virus (Colorado Strain).

Group II	Dilutions								%	
	10-1		10-2		10-3		10-4			
	S*	D*	S	D	S	D	S	D	D	S
Vaccinated	31	0	19	1	17	3	19	0	4.44	95.56
Unvaccinated contact controls	5	6	1	9	6	4	3	7	63.4	36.6
Normal controls	7	3	7	3	6	4	8	2	30.0	70.0

* S—No noticeable symptoms.

D—Dead, also those showing typical Newcastle disease symptoms.

TABLE IV.

Immunization of Chickens Against Newcastle Disease with Modified Newcastle Virus.

Route of vaccination: Base of wattle.

Date of vaccination: 9/5/47

Route of challenge: Base of wattle.

Date of challenge: 10/3/47

Challenge dose: 0.5 cc of given dilutions.

Challenge Virus (Connecticut Strain).

Group III	Dilutions								%	
	10-1		10-2		10-3		10-4			
	S*	D*	S	D	S	D	S	D	D	S
Vaccinated	13	1†	20	1	19	0	19	1	1.4	98.6
Unvaccinated contact controls	9	1	8	2	18	0	10	0	6.25	93.75
Normal controls	9	1	6	4	8	2	8	2	22.5	77.5

* S—No noticeable symptoms.

D—Dead, also those showing Newcastle disease symptoms.

† Crushed.

Tables II, III, and IV, respectively. The challenge was injected into the base of the wattle in various dilutions on the twenty-eighth day after vaccination. The results of these tests are also given in Tables II, III, and IV, respectively.

Table II shows that the living hamster-

adapted California strain provided good protection against the virulent egg-propagated California strain. The unvaccinated room controls and normal controls, challenged with the vaccinated chickens, showed approximately equal susceptibility.

In the case of the Colorado strain, No.

TABLE V.
Unchallenged Contact Control Chickens.

Exposure strain	Vaccinated		Unvaccinated	
	Survivors c/o NC symptoms	Newcastle symptoms	Survivors c/o NC symptoms	Newcastle symptoms
California	30	0	15	5
Colorado	30	0	17	3
Connecticut	22	0	20	0

8518, the challenge virus was not as virulent as the California strain, as shown in Table III. The calculated amount of protection appears low, although only 4% of the vaccinated group failed to withstand the challenge. The unvaccinated room controls challenged with the Colorado strain showed greater susceptibility than normal controls challenged similarly.

The challenge virus of the Connecticut strain was of slightly lower virulence than that of the Colorado strain, as shown in Table IV. Since only one per cent of the vaccinated group showed typical Newcastle symptoms after challenge, the amount of protection is significant. The unvaccinated room control group showed much less susceptibility than the normal control birds in this group.

A contact exposure experiment was also conducted. Vaccinated and normal chickens of corresponding ages were placed with the control virus-challenged birds of the 10^{-1} and 10^{-2} dilutions of the California, Colorado, and Connecticut groups respectively, as shown in Table V. The results of this exposure are shown in the same table. None of the vaccinated birds succumbed. The contact exposure to Connecticut virus was not sufficiently great to cause disease in the normal unvaccinated birds. Comparatively few unvaccinated contact birds developed Newcastle disease in the California and Colorado groups. All unvaccinated chickens for this contact

exposure were brought to the laboratory the day of challenge, at which time they were placed with their respective groups.

Conclusion. Hamster-adapted Newcastle virus of the 82nd subculture immunizes young susceptible chickens against virulent egg-adapted California strain No. 11,914 and against 2 less virulent strains, the Colorado strain No. 8518 and the Connecticut strain, subculture 8.

Summary. The pathogenicity of the 82nd subculture of the hamster-adapted California strain of Newcastle virus used as an immunizing agent was negligible in this experiment.

Through injection of the living hamster-adapted California strain No. 11,914, of Newcastle virus, young chickens were successfully immunized against the egg-adapted California, Colorado, and Connecticut strains. The modified Newcastle virus also produced exceptionally good protection in chickens to contact with chickens infected with all above-mentioned strains.

Susceptible birds, used as contact controls with birds injected with modified hamster-adapted Newcastle virus, failed to show clinical evidence of spread of this modified strain. There appeared to be some resistance to challenge injection in those birds challenged with the Connecticut strain, as compared with the normal unvaccinated control chickens of this group.