

produced by the two types of cells was roughly equivalent. Macrophages in culture retained their phagocytic properties and were susceptible to the virus after uptake of carbon black. On the other hand, infected cells failed to phagocytize foreign carbon particles.

1. Smith, M. G., *Progr. Med. Virol.* 2, 171 (1959).
2. Gresser, I. and Lang, D. J., *Progr. Med. Virol.* 8, 62 (1966).
3. Enders, J. F. and Peebles, T. C., *Proc. Soc. Exptl. Biol. Med.* 86, 277 (1954).
4. Brodsky, I. and Rowe, W. P., *Proc. Soc. Exptl. Biol. Med.* 99, 654 (1958).
5. Henson, D. and Pinkerton, H., *Proc. Soc. Exptl. Biol. Med.* 114, 130 (1963).
6. McGavran, M. H. and Smith, M. G., *Exptl. Mol. Pathol.* 4, 1 (1965).
7. Ruebner, B. H., Hisaw, T., Slusser, R., Osborn, J., and Medearis, D. N., *Am. J. Pathol.* 48, 971 (1966).
8. Cohn, F. A. and Benson, B., *J. Exptl. Med.* 121, 153 (1965).
9. Glasgow, L. A. and Habel, K., *J. Exptl. Med.* 117, 149 (1963).
10. Glasgow, L. A. and Habel, K., *J. Exptl. Med.* 115, 503 (1962).
11. Osborn, J. E. and Medearis, D. N., *Proc. Soc. Exptl. Biol. Med.* 121, 819 (1966).
12. Bang, F. B. and Warwick, A., *Proc. Natl. Acad. Sci. U.S.* 46, 1065 (1960).
13. Theis, G. and Koprowski, H., *Federation Proc.* 20, 265 (1961).
14. Henson, D., Smith, R. D., Gehrke, J., and Neapolitan, C., *Am. J. Pathol.* 51, 1001 (1967).

Received July 11, 1968. P.S.E.B.M., 1968, Vol. 129.

Passive Cutaneous Anaphylactic-Like Reactions in Young Chicks* (33400)

ANNA M. CONWAY, PIERSON J. VAN ALTEN, AND ARTHUR A. HIRATA

Department of Anatomy, University of Detroit School of Dentistry, Detroit, Michigan 48207;

Department of Anatomy, University of Illinois, Chicago, Illinois 60680;

and Department of Molecular Biology, Abbott Laboratories, North Chicago, Illinois 60064

It has been reported that chickens, unlike guinea pigs and other mammals, fail to undergo anaphylactic reactions (1-4). Although several investigators have reported on immediate hypersensitivity reactions resembling systemic anaphylaxis (5-11), corroborative evidence for the anaphylactic reaction has been lacking, e.g., sensitization by passive antibody.

In the present study using chicken antiserum, we showed that a passive cutaneous anaphylactic-like (PCA-like) reaction could be elicited only in the skin of newly hatched and young chicks and not in the skin of the adult chicken.

Materials and Methods. Chicken antbovine gamma globulin sera (anti-BGG) were obtained from adult white leghorn chickens

after two injections of the antigen, 50 days apart; serum samples were collected 8 days after the last injection. Chicken antirabbit gamma globulin sera (anti-RGG) were obtained from hyperimmunized chickens which were bled 11 days after the final injection. Of 7 precipitating antisera tested (5 anti-BGG and 2 anti-RGG), 6 gave PCA-like reactions in young chicks; one anti-BGG serum was negative. For comparing the reactivities of the young and the adult chickens, one anti-BGG and one anti-RGG serum were selected for testing. These sera contained 43 and 12 μ g of precipitin/10 μ l, respectively.

Results. Consistent positive reactions were obtained in the young chicks when 10 μ l of anti-BGG or anti-RGG serum were injected intracutaneously and 0.6 mg of the specific antigen per ml of blood volume was injected 2 hr later intravenously. Each 0.6 mg of antigen was dissolved with 0.02 ml of saline and 0.04 ml of 0.4% solution of Evans blue.

* This work was supported in part by grants from the Graduate Research Board, University of Illinois, The National Science Foundation (GB 2874), U.S. Public Health Service (AI 8289).

TABLE I. PCA-Like Susceptibility of Young and Adult Chickens.

Group	Age range (days)	Body wt. range (g)	Antigen	Wing web injection	
				Anti-BGG	Anti-RGG
I	0-8	40-56*	BGG	8/11 ^b	0/11
			RGG	0/24	19/24
II	120-210	1300-2400	BGG	0/17	0/17
			RGG	0/24	1/24

* Group I was injected with between 2.5 mg of antigen in 0.25 ml of saline to 3.3 mg of antigen in 0.33 ml of saline. Group II was injected with between 80 mg of antigen in 8.0 ml to 146 mg of antigen in 14.6 ml of saline.

^b Positive reactions over the total challenged.

The blood volume of the chicken was calculated as approximately 10% of the body weight for either sex (12). The intensity of the reactions were the same when antisera were diluted 5-fold, but further dilutions resulted in diminished responses.

In all experiments, 10 μ l of the chicken anti-BGG antiserum was injected intracutaneously into the right wing web and a similar amount of the chicken anti-RGG antiserum into the left wing web. Two hr following these injections either the bovine gamma globulin (BGG) or rabbit gamma globulin (RGG) in combination with the Evans blue dye was injected either intravenously or intracardially. Following this injection, within 20 min, blueing in the wing web appeared on the side which had previously been injected with the homologous antiserum. In contrast, wing webs which had been injected with the control heterologous antiserum never showed any blueing.

The results presented in Table I clearly show that a PCA-like reaction was elicited in the wing web of young chicks, i.e., those ranging from day of hatching to 8 days of age. In contrast, virtually no reaction was observed in chickens 4 months of age or older. It is also evident that the reactions occurring in the wing web are highly specific. In approximately 48 hr following a positive PCA-like reaction the blueing faded from the injection site. For several days following this, the wing webs were observed for signs of erythema, edema, vascular occlusion, hemorrhage, or necrosis. At no time were signs of such local inflammation observed in any of

the experimental chicks following the positive PCA-like reaction. Further clarification of this reaction was sought by treating a group of the young chicks with an antihistamine prior to challenging with the antigen. Two hr after the intracutaneous injection of antiserum into the wing web, and 40 min previous to the antigen-Evans blue injection, 8 chicks of 6 days of age were injected intraperitoneally with 2.5 mg of chlorcyclizine hydrochloride in 0.5 ml of saline. A similar group was injected with saline for control purposes. All chicks injected with the drug failed to develop a PCA-like reaction whereas 7 of 8 receiving the saline showed blueing of the wing web.

Discussion. Based upon these observations we conclude that the positive reactions in the skin of the newly hatched and young chick are PCA. We have also considered the possibility that the reaction might be a reverse Arthus' phenomenon. Such a conclusion, however, is very doubtful considering that positive skin reactions occurred within 20 min following antigen injection, that there was no gross evidence of local inflammation following the skin reaction, and that antihistamine given prior to the challenge antigen injection prevented the reaction in the wing web.

The most simple explanation for the differential susceptibility of the newly hatched and the adult chicken would be that antibody can sensitize the skin of the newly hatched but not the skin of the adult. With the growth of the animal, the characteristics of the skin might change so that antibody no

longer adsorbs on the skin. On the other hand, the intrinsic characteristics of the skin could remain constant but with increased antibody production accompanying growth, the skin may become preempted with antibodies (13), thus rendering the skin nonreactive with passive antibody.

Summary. Passive cutaneous anaphylactic-like (PCA-like) reaction could be elicited in the skin of newly hatched and young chickens with chicken antiserum. The reaction occurred within 20 min following antigen injection, reacted specifically with the antiserum, and the reaction could be prevented with antihistamine. The present findings indicate that differential susceptibilities to anaphylactic reaction exists depending on the developmental stage of the chicken.

1. Uhlenhuth, P. and Haendel, Z., *Z. Immunitätsforsch.* 3, 284 (1909).

2. Friedberger, E. and Hartoch, O., *Z. Immuni-*

taetsforsch. 3, 581 (1909).

3. Ovary, Z., *Immunology* 3, 19 (1960).

4. Hirata, A. A. and Campbell, D. H., *Proc. Soc. Exptl. Biol. Med.* 107, 68 (1961).

5. Makinodan, T., Wolfe, H. R., Goodman, M. and Ruth, R., *J. Immunol.* 68, 219 (1952).

6. Engelhardt, G. and Lendle, L., *Arch. Exptl. Pathol. Pharmacol.* 225, 402 (1955).

7. Lecomte, J. and Beaumariage, M. L., *Intern. Arch. Allergy Appl. Immunol.* 13, 145 (1958).

8. Van Alten, P. J. and Schechtman, A. M., *J. Exptl. Zool.* 153, 187 (1963).

9. Van Alten, P. J., *Nature* 204, 797 (1964).

10. Conway, A. M. and Van Alten, P. J., *Am. Zool.* 6, 307 (1966).

11. Lecomte, J. and Beaumariage, M. L., *Compt. Rend. Soc. Biol.* 151, 1452 (1957).

12. Newell, G. W. and Shaffner, C. S., *Poultry Sci.* 29, 78 (1950).

13. Austen, K. F., Block, K. J., Baker, A. R., and Arnason, B. G., *Proc. Soc. Exptl. Biol. Med.* 120, 542 (1965).

Received July 12, 1968. P.S.E.B.M., 1968, Vol. 129.

Neutralization of Mycobacteriophages by Sera of Patients with and without Sarcoidosis* (33401)

B. U. BOWMAN¹ (Introduced by Henry G. Cramblett)

*Ohio State University College of Medicine, Department of Medical Microbiology,
Columbus, Ohio 43210*

Mankiewicz (1, 2) reported that sera of patients without sarcoidosis and who harbored intestinal mycobacteriophages possessed phage neutralization activity against the carried phage or against mycobacteriophage D.W. In contrast, sera of patients with active sarcoid did not possess an appreciable amount of serum antibodies to their own intestinal mycobacteriophage or to phage D.W. Mankiewicz' inability to demonstrate anti-phage antibodies in sera of patients with sarcoidosis was supported by the work of Bönicke (3), however, his report failed to name the test phage.

Since the antigenicity of mycobacteriophages R1, D29, and Leo has recently been characterized (4), it was reasoned that applying the same standard bacteriophage neutralization techniques to sera of patients would greatly increase the possibility of demonstrating the presence of neutralizing antibody. Therefore, the present study was undertaken to determine if the sera of patients, both with and without sarcoidosis, contain antibodies to mycobacteriophages D29, Leo, or R1.

Methods. *Sera.* Sera were obtained from six patients with active sarcoidosis hospitalized in the Ohio State University Hospital. Sixty-four additional sera came from patients with biopsy-proven sarcoidosis hospitalized at the Eastern North Carolina Sanatorium, Wilson,

* Supported by a grant from the American Trudeau Society—National Tuberculosis Association.

¹ NIH Career Development Awardee.