catalasemic, and not essentially acatalasemic, as the duck. The same difficulty of lysis of rooster erythrocytes was observed as is referred to above in connection with duck blood.

Summary. The dog is shown to have very low blood catalase activity levels, ranging from an immeasurably low blood catalase to a level which in other species would be considered hypocatalasemic. Although some tissue levels of catalase activity are lower than, say, those of the normal mouse, the dog and the duck are not acatalatic.

We wish to thank Dr. W. P. Norris for making his beagle colony freely available to us, Dr. C. M. Poole for providing the dog blood samples, and Dr. T. E. Fritz for providing the dog necropsy tissue samples.

1. Takahara, S., Lancet **263**, 1101 (1952).

2. Aebi, H., Heiniger, J. P., Bütler, R., and Hässig, A., Experimentia **17**, 466 (1961).

3. Radev, T., Bull. Inst. Comp. Pathol. Domestic Animals **6**, 135 (1958).

4. Feinstein, R. N., Howard, J. B., Braun, J. T., and Seaholm, J. E., Genetics **53**, 923 (1966).

5. Nakamura, H., Yoshiya, M., Kaziro, K., and Kikuchi, G., Proc. Japan Acad. **28**, 59 (1952).

6. Allison, A. C., ap Rees, W., and Burn, G. P., Nature **180**, 649 (1957).

7. Feinstein, R. N., Sacher, G. A., Howard, J. B., and Braun J. T., Arch. Biochem. Biophys, in press.

8. Feinstein, R. N., J. Biol. Chem. **180**, 1197 (1949).

9. Feinstein, R. N., Braun, J. T., and Howard, J. B., Arch. Biochem. Biophys. **120**, 165 (1967).

10. Feinstein, R. N. and Dainko, J. L., Cancer Res. **19**, 612 (1959).

Received Dec. 4, 1967. P.S.E.B.M., 1968, Vol. 127.

The Immune Response of Bursaless Birds as Influenced by Antibiotics and Age*† (32868)

BRUCE GLICK (Introduced by R. A. Good)

Poultry Science Department, Mississippi State University, State College, Mississippi 39762

Bursectomy of young chicks (1,2) or chemical bursectomy during embryonic development (3,4) result in an impairment of the immune response to a variety of antigens. The chemically bursectomized chicks develop a wasting syndrome (5,6) not unlike that observed in thymectomized mice (7). Immunological incompetence in thymectomized mice appears to be a direct result of the thymus since wasting did not occur in thymectomized-germ free mice but the homograft response was impaired in these mice (8). The objective of this experiment was to improve the growth rate and reduce mortality of chemically bursectomized chicks with antibiotics and determine if there would be an accompanying improvement in the immune response of these birds. A portion of this paper has been presented in a preliminary report (9).

Materials and Methods. All chicks were from a strain of New Hampshire, an American breed, developed by our departmental geneticist, Professor L. J. Dreesen. The birds were reared in battery brooders each consisting of 5 vertical decks with a wire mesh floor or in floor pens supplied with wood shavings and infrared brooding lamps. Chemical bursectomy was attained by dipping eggs, incubated for 3 days, into ethyl alcohol containing more than 1.28 gm/100 ml testosterone propionate (10). The penicillin (P), 50 gm/ton, oxytetracyline (O), 200 gm/ton, and tylosin (T), 200 gm/ton, were combined and added to the basal ration of day-old birds reared in floor pens while penicillin, 100 gm/ton, and tylosin, 300 gm/ton, were fed separately to battery-reared birds. The composition of the basal diet has been described (11). The chemically bursectomized birds reared in floor pens were fed basal plus POT or basal beginning at day-old. Each treatment was replicated three times. A total of 93 chicks were started in

* Supported, in part, by PHS grant 03398, National Institutes of Allergy and Infectious Diseases. I am indebted to Sandra Whatley for laboratory assistance.

† Mississippi Agricultural Experiment Station journal article no. 1594.

TABLE I. Influence of POT[a] on Body Weight and Precipitin Production in Chemically Bursectomized Chicks.

	Diet	
	POT	Basal
Body weight (gm)		
Male, age in weeks		
3.5	393.04[c] ± 46.90 (41)[b]	299.61 ± 57.33 (21)
7.5	1244.00[c] ± 174.80 (31)	1065.61 ± 106.90 (21)
Female, age in weeks		
3.5	330.96[c] ± 66.70 (31)	264.05 ± 54.33 (35)
7.5	1084.21[c] ± 151.23 (27)	860.30 ± 178.70 (20)
Mortality to 7.5 weeks	35.80	56.38
Precipitin, interfacial test:		
No. birds positive/ total no. tested	1/18	1/18

[a] Penicillin, oxytetracycline, tylosin.
[b] No. of birds is given in parentheses.
[c] $p < .01$.

each of the 2 diets. Eighteen birds from each of the two different diets were immunized with 40 mg of bovine serum albumin (BSA)/kg of body weight at 6.5 weeks of age. At 7.5 weeks of age, the presence of precipitin was determined by the interfacial ring test. In the batteries, a Latin square design was employed allowing each treatment to be replicated on the basis of deck position. The battery-reared birds were between 4 and 6 weeks of age when they were immunized with BSA. The serum was analyzed for precipitin by a quantitative procedure (12,13). A microtiter procedure (Cook Engineering Co.) was used in determining the antibody response of chickens to a 1 ml i.v. injection of a 7% suspension of sheep red blood cells. Carbon particles (16 mg/100 gm of body wt.) were i.v. injected into 4-week-old birds to assess the influence tylosin had on phagocytosis. The procedure of Biozzi *et al.* (14) was followed as employed in our laboratory (15) with the slight modification that we used 3 ml of a 0.1% solution of sodium carbonate per blood sample.

Results. In a preliminary experiment with normal chickens, the μg of antibody (Ab) N/ml of serum produced by a single i.v. injection of BSA was the same for birds receiving POT, 135.6 μg of AbN/ml of serum, or basal, 132.8 μg AbN/ml of serum. The feeding of POT to chemically bursectomized birds significantly improved body weight and markedly reduced mortality, especially during the first 3.5 weeks of life (Table I). However, the improvement in growth rate and apparent health of the chemically bursectomized birds did not change their antibody response to BSA (Table I). The body weight of the males from an untreated group of chickens receiving the basal diet was 54 gm less than the body weight of chemically bursectomized males receiving POT while the body weight of untreated female chickens was 77 gm more than the females of the chemically bursectomized group receiving POT. All the untreated birds produced precipitin to BSA. These data were not included in the analysis since only one pen of untreated birds was available.

The addition of penicillin to the basal ration did not alter the immune response of untreated chickens challenged with BSA while tylosin significantly reduced the antibody response to BSA (Table II). However, the ability of tylosin fed birds to clear carbon particles was not significantly altered (Table II). The improvement in body weight with antibiotics lacked statistical significance; a fact we have generally found to be true with battery-reared birds. The addition of penicillin to the diet of chemically bursectomized birds has not altered their response to BSA.

The percentage of chemically bursectomized

TABLE II. Antibody Titer in Untreated Chicks Immunized with BSA and Fed Penicillin or Tylosin.

	No.	Diet		
		Basal	Penicillin	Tylosin
Test A[a]: μg of antibody N/ml of serum	12	112.22 ± 40.84	113.60 ± 45.00	77.41 ± 23.03
Body wt. (gm)	12	968.5	1020.6	1047.3
Test B: μg of antibody N/ml of serum	14	114.63[b] ± 64.28		57.33 ± 31.69
Body wt. (gm)		602.43		649.06
Phagocytic response:				
CO[c]		0.4825 ± 0.2730		0.4800 ± 0.1190
GPI[d]		23.53 ± 14.58		29.11 ± 5.92

[a] All means not underlined by the same line are significantly different at the 1% level, Duncan (19).

[b] $p < .01$.

[c] An extrapolated initial value based on optical density at 5, 10, and 15 min after the injection of carbon.

[d] Granulopectic index.

(CB) birds producing precipitin at 5 weeks of age and 6 months of age was 0 and 25, respectively (Table III). The 6-month-old CB chickens immunized with BSA were simultaneously immunized with SRBC and found to produce normal levels of agglutinin (Table III). In our laboratory, we have found that 4–6-weeks-old CB chickens either produce no detectable level of agglutinin to SRBC or a significantly reduced level.

Discussion. It was necessary to determine the antibody response of our untreated chicks fed antibiotics since antibiotics have been found to interfere with the production of antibody (16). The feeding of tylosin alone significantly reduced precipitin production while the feeding of penicillin, oxytetracycline, and tylosin; or penicillin alone did not interfere with antibody production. Therefore, the depression in precipitin production observed in CB birds fed POT or P alone could not be attributed to the antibiotics.

TABLE III. The Primary Antibody Response at Two Different Ages in Chemically Bursectomized (CB) and Control Birds.

	BSA		SRBC (Log_2)	
Age	CB	Control	CB	Control
5-week-old	0/20	12/14[a]		
6-month-old	3/12	11/12	7.50 ± 3.81	7.08 ± 1.63

[a] 12 positive of 14 birds tested; number producing antibody/total number tested.

The failure to improve precipitin response with antibiotics in CB birds even though the growth rate was significantly improved and mortality reduced strongly suggests that the immunological incompetence was a direct result of embryonic bursectomy and not secondary to growth rate or factors that increase mortality. Also, at the time of sexual maturity, the body weight of CB birds (3.50 kg) fed a basal ration had equaled that of the controls (3.63 kg) yet only a slight improvement was noted in the precipitin response of the CB birds (Table III). On the other hand, these same 6-month-old CB birds produced normal amounts of agglutinin to SRBC. Surgically bursectomized birds failed to produce hemagglutinin to human O erythrocytes on primary challenge at 6 months of age while all bursectomized birds produced hemagglutinin to a secondary challenge (17). Therefore, the ability of surgically or chemically bursectomized birds to make antibody depends on the type of antigen, age of bird, and number of antigen challenges. Assuming that the bursa

never functioned in the CB birds, one would be forced to conclude that other sites in the chicken are capable of conditioning or supplying immunocompetent cells. The delay in immune response observed in CB birds may be a reflection of the time necessary for these other sites to attain the level of development found in the bursa of neonatal chicks. The dependence of normal immune response on bursal development has been demonstrated by producing bursa regression in neonatal chicks which was followed by bursa regeneration and a delay in normal antibody production (18). Also, a cellular adaptation may have occurred in our CB birds. That is, certain cells in the chicken may have the potential for an immune commitment but only develop in the altered environment produced by bursectomy.

Summary. Feeding antibiotics to chemically bursectomized (CB) birds has demonstrated that immunological incompetence in CB birds is a direct result of embryonic bursectomy and not secondary to growth rate or factors that increase mortality. Mature CB birds appear to produce normal amounts of agglutinin to sheep red blood cells, but are still deficient precipitin producers. Apparently, the chicken is capable of adapting immunologically to the altered internal environment produced by embryonic bursectomy.

1. Glick, B., Chang, T. S., and Jaap, R. G., Poultry Sci. 35, 843 (1956).

2. Mueller, A. P., Wolfe, H. R., and Meyer, R. K., J. Immunol. 85, 172 (1960).

3. Glick, B., in "The Thymus in Immunobiology," Good, R. A. and Gabrielsen, A., eds., p. 343. Harper (Hoeber) New York 1964.

4. Warner, N. L. and Szenberg, A., Ann. Rev. Microbiol. 18, 253 (1964).

5. Glick, B. and Sadler, C. R., Poultry Sci. 40, 185 (1961).

6. Mueller, A. P., Wolfe, H. R., Meyer, R. K., Aspinal, R. L., J. Immunol. 88, 354 (1962).

7. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., and Papermaster, B. W., J. Exptl. Med. 116, 773 (1962).

8. McIntire, K. R., Sell, S., and Miller, J. F. A. P., Nature 204, 151 (1964).

9. Glick, B., Federation Proc. 25, 1397 (1966).

10. Wilson, J. and Glick, B., Poultry Sci. 45, 892 (1966).

11. Glick, B. and Dreesen, L. J., Poultry Sci. 46, 396 (1967).

12. McDuffie, F. C. and Kabat, E. A., J. Immunol. 77, 193 (1956).

13. May, D. and Glick, B., Poultry Sci. 43, 450 (1964).

14. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exptl. Pathol. 34, 441 (1953).

15. Glick, B., Sato, K., and Cohenour, F., J. Reticuloendothelial Soc. 1, 442 (1964).

16. Stevens, K. M., J. Immunol. 71, 119 (1953).

17. Jankovic, B. D. and Isakovic, K., Nature 211, 202 (1966).

18. Glick, B., J. Immunol. 98, 1076 (1967).

19. Duncan, D. B., Biometrics 11, 1 (1955).

Received Dec. 4, 1967. P.S.E.B.M., 1968, Vol. 127.

Obesity in Force-Fed, Hypophysectomized Rats Bearing Hypothalamic Lesions* (32869)

PAUL W. HAN[1] (Introduced by J. R. Brobeck)
Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Because of the prevalence of the belief that some persons gain weight and become obese even though they do not overeat, there has been continued search for some disturbance other than overeating among obese patients. Obesity produced surgically by bilateral, electrolytic lesions in the ventromedial nuclei of the hypothalamus of experimental animals has been one of the most extensively studied obesities. Adult rats bearing such lesions when fed *ad libitum* eat more food than their controls, and the extra food intake of the lesioned

* Supported by grant G-15930 of the National Science Foundation and by USPHS Grant 1 R01 AM11731-01.

[1] Scholar of the Pennsylvania Plan to Develop Scientists in Medical Research of the University of Pennsylvania.