Immunosuppressive Action of Heat in Chickens[1,2] (34493)

D. S. V. Subba Rao and Bruce Glick
(Introduced by R. A. Good)
Poultry Science Department, State College, Mississippi 39762

A paucity of information exists concerning the influence of temperature on the immune response. Demonstrations of stress-induced suppression of antibody synthesis have been rare (1, 2), and only one paper has appeared which correlated the primary antibody response with heat stress (3).

The objectives of this research were to determine (1) the number of heat exposures necessary to influence antibody production, (2) the stages of antibody production (preinduction or induction) influenced by heat, and (3) the phagocytic ability of heat-exposed birds.

Materials and Methods. The chickens were a strain of New Hampshire, an American breed, developed by our departmental geneticist, Prof. L. J. Dreesen at the Mississippi Agricultural Experiment Station. The birds were raised in battery brooders and fed a basal corn-soybean ration amply fortified with vitamins and minerals (4). All birds were between 4 and 5 weeks of age at the time of the heat treatment. The chickens were randomly assigned to the various trials without regard to sex.

The chickens were placed in a constant-temperature chamber (Electric Hotpack Co., New York) and exposed to heat (43–45°) for 30-min periods. Relative humidity was not controlled but adequate circulation, maintained by a blower system, provided fresh air.

In order to evaluate the influence of the number of heat exposures on antibody production, birds were immunized 24 or 12 hr after 1, 2, 3, or 4 heat periods. In the remaining experiments chickens were exposed to 30 min of heat each hour for 4 consecutive hours.

In an attempt to determine how early in the preinduction phase of antibody formation heat would suppress antibody production, birds were immunized 36, 48, 60, or 72 hr after the initial heat exposure. In another series of experiments, birds were immunized 3 hr after the initial heat period, at the time of initial heat exposure, and 12, 24, 36, 48, 60, or 72 hr after the initial heat exposure. All birds were bled 5, 7, and 10 days after a single iv injection of a 7% suspension (1 ml) of sheep red blood cells (SRBC). Serum hemagglutinin levels were determined by a microtiter procedure (5). All data were analyzed by the analysis of variance (6) and the multiple-range test of Duncan (7).

The *in vivo* phagocytosis of heat-exposed birds was studied by the method of Biozzi *et al.* (8), as employed in our laboratory (9), with the single modification that the blood samples (0.1-ml) were transferred to a tube containing 3 ml of a 0.1% solution of sodium carbonate. A highly significant difference was found for the mean granulopectic index (GPI) of chickens receiving 8(2.76 ± 0.75), 16 (13.5 ± 2.35), or 32(28.28 ± 6.28) mg of gelatinized carbon (Pelikan C11/1431a Gunther Wagner) per 100 g body weight. The concentration of carbon in our stock solution ranged between 130 and 160 mg per ml. Therefore, to limit the volume injected and still maintain a high GPI, we selected 16 mg carbon/100 g body weight for the heat experiments. Birds were heat-treated four times and their carbon clearance measured 24 or 12 hr after the initial heat exposure. The data were statistically analyzed by the method of least squares (10). Liver and

[1] Supported, in part, by PHS Grant AI 03398, National Institute of Allergy and Infectious Diseases.
[2] Journal Article No. 1785, Mississippi Agricultural Experiment Station.

TABLE I. Hemagglutinin Levels of Chickens Treated with 30 Minutes of Heat (43–45°) Beginning at —24 hr for 1, 2, 3, or 4 Times in as Many Consecutive Hours.

	Mean hemagglutinin levels (log_2)				
	Trial 1 (mean of 40 birds)		Trial 2 (mean of 20 birds)		
Treatments (hr)	Day 7	Day 10	Day 5	Day 7	Day 10
—24, —23, —22, —21	$4.03^{e} \pm 0.73$	$2.90^{c} \pm 0.78$	$1.15^{d} \pm 0.75$	$3.35^{e} \pm 0.75$	$2.40^{c} \pm 0.60$
—24, —23, —22	$4.68^{d} \pm 0.80$	$3.38^{c} \pm 0.74$	$2.85^{c} \pm 1.76$	$4.50^{d} \pm 1.32$	$3.05^{c} \pm 0.89$
—24, —23	$5.30^{c} \pm 0.88$	$4.28^{b} \pm 0.72$	$3.85^{b} \pm 1.27$	$5.65^{c} \pm 1.04$	$4.10^{b} \pm 0.91$
—24	$5.82^{b} \pm 1.01$	$4.53^{b} \pm 0.88$	$4.05^{b} \pm 1.23$	$6.75^{b} \pm 1.25$	$4.10^{b} \pm 1.02$
Control	$7.68^{a} \pm 1.49$	$5.78^{a} \pm 1.10$	$5.25^{a} \pm 1.59$	$8.50^{a} \pm 0.61$	$5.65^{a} \pm 0.93$

Note: Means are included with their standard deviations; negative signs identify the treatments before antigen injection; means within a column and without a common superscript are significantly different, $p < .05$ (7).

spleen weights were held constant in the analysis. Partial regressions of GPI on liver and spleen weights were included in the model.

Results. The hemagglutinin titers of the chickens exposed 1, 2, 3, or 4 times to heat 24 hr before antigen injection were significantly lower than the controls (Table I). There was a progressively lower titer as the number of heat periods was increased. Exposing birds to heat 12 hr prior to antigen administration produced results similar to those reported when birds were heat-treated 24 hr prior to antigen administration. The agglutinin titer (log_2) was not depressed when the birds were given 30 min of heat treatment every hour for 4 consecutive hours starting at 72(7.83 ± 1.09), 60(7.43 ± 1.14), 48(7.63 ± 1.16), or 36(7.53 ± 1.33) hr prior to antigen administration. The control titer was 7.47 ± 1.01. Peak antibody titers were significantly reduced in birds receiving heat treatment beginning 3 hr before SRBC injection, at the time of SRBC injection, or 12 or 24 hr after SRVC injection while heat treatment initiated later in the induction period failed to significantly influence hemagglutinin levels (Table II). Depressed agglutinin levels of chickens which received water during heat stresses and higher agglutinin levels of birds exposed to unplugged heat chamber (11) suggest that lack of water or handling of birds did not contribute to the lower antibody titer in the heat-exposed birds. The GPI of birds heat-treated 12(13.80 ± 2.20) or 24 hr (14.55 ± 2.30) before carbon injection was not significantly different from the control (14.20 ± 2.50). There were 20 birds per mean.

Discussion. Heat treatment within a 24-hr period before or after SRBC injection will significantly reduce the chicken's ability to produce hemagglutinin. Therefore, both the preinduction and early induction phases of antibody formation are sensitive to heat.

If heat is a stressor agent (12), the immunosuppressive action of heat may be a result of an increased release of corticosteroids which are known to suppress antibody production in the chicken (11, 13). An analysis of blood and adrenals for glucocorticoids before and after heat stress would be necessary to determine if heat affects a significant change in adrenal corticoids. Adrenalectomized birds would be an ideal model for these studies.

Thaxton *et al.* (3) reported that heat exposures or ACTH injections during the preinduction phase will significantly reduce antibody production to SRBC while only ACTH caused a significant reduction in adrenal cholesterol. Therefore, it is possible that heat has a direct and not an indirect effect on immunobiological tissue. It is unlikely that the disruption of the bursa of Fabricius *per se* by heat would explain the reduced immune response since by 3–5 weeks of age the bursa has performed its role in antibody-mediated immunity (14). Heat may affect

TABLE II. The Effect of Preheat and Postheat Treatment on Future Antibody Production in Chickens.

Treatment (hr)	Mean hemagglutinin levels (mean of 20 birds)		
	Day 5	Day 7	Day 10
−3, −2, −1, 0	$2.10^{b} \pm 0.91$	$2.20^{e} \pm 0.77$	$2.00^{d} \pm 0.46$
0, +1, +2, +3	$2.10^{b} \pm 0.55$	$2.90^{d} \pm 0.72$	$2.35^{cd} \pm 0.59$
+12, +13, +14, +15	$2.75^{a} \pm 0.78$	$5.60^{c} \pm 1.10$	$2.95^{b} \pm 0.76$
+24, +25, +26, +27	$3.05^{a} \pm 0.76$	$6.20^{b} \pm 1.15$	$2.85^{bc} \pm 0.88$
+36, +37, +38, +39	$3.35^{a} \pm 0.49$	$6.60^{ab} \pm 0.75$	$2.65^{bc} \pm 0.67$
+48, +49, +50, +51	$2.95^{a} \pm 0.76$	$6.60^{ab} \pm 0.54$	$2.90^{b} \pm 0.64$
+60, +61, +62, +63	$2.75^{a} \pm 0.64$	$6.85^{a} \pm 0.81$	$2.75^{bc} \pm 0.64$
+72, +73, +74, +75	$2.85^{a} \pm 0.75$	$6.50^{ab} \pm 0.61$	$2.60^{bc} \pm 0.88$
Control	$3.25^{a} \pm 0.72$	$7.05^{a} \pm 1.00$	$3.60^{a} \pm 0.75$

Note: Means are included with their standard deviations; negative signs identify treatments before antigen injection while positive signs identify treatments given after antigen injection; means within a column and without a common superscript are significantly different, $p < .05$ (7).

directly a peripheral lymphoid tissue like the spleen. The ability of the spleens from heat-stressed birds to produce antibody could be determined by the plaque technique of Jerne (15). Damage to the immunoglobulin system could be evaluated by immunoelectrophoresis and disc electrophoresis (16).

Phagocytic cells participate in the initial uptake and intracellular digestion of particulate antigenic material. The processed antigen is made available to the antibody-forming cells (17). If the process of phagocytosis and intracellular solubilization is reduced as a result of heat treatments, little or no processed antigen would be available to react with the antibody-forming cells and antibody production would be correspondingly reduced. The reticuloendothelial (RE) activity was not impaired by heat treatments. Therefore, the reduced antibody production in heat-stressed chickens cannot be ascribed to a lack of phagocytic activity. However, the heat treatments of chickens may have reduced the ability of the phagocytic cells to process the engulfed antigen. In this way, heat would be influencing a phase of the afferent limb of the immune response.

Summary. The influence of heat (43–45°) on hemagglutinin production and phagocytosis was evaluated in a strain of New Hampshire chickens. Production of antibody to sheep red blood cells (SRBC) was significantly reduced during the preinduction phase of antibody formation when birds were exposed to 1, 2, 3, or 4 consecutive hours of 30 min of heat within 24 hr of the administration of SRBC. Heat failed to influence hemagglutinin levels when administered 36 hr before the injection of SRBC. Birds exposed to 30 min of heat during the initial 24 hr of the induction phase of antibody formation exhibited a significant reduction in their hemagglutinin titers. Heat treatment did not impair the *in vivo* clearance of carbon particles.

1. Vessey, S. H., Proc. Soc. Exptl. Biol. Med. **115**, 252 (1964).

2. Solomon, G. F., Int. Arch. Allergy Appl. Immunol. **35**, 97 (1969).

3. Thaxton, P., Sadler, C. R., and Glick, B., Poultry Sci. **48**, 264 (1968).

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

5. Sever, J. L., J. Immunol. **88**, 320 (1962).

6. Snedecor, G. W., "Statistical Methods." Iowa State Univ. Press, Ames, Iowa (1952).

7. Duncan, D. B., Biometrics **11**, 1 (1955).

8. Biozzi, G., Benacerraf, B., and Halpern, B. N., Brit. J. Exp. Pathol. **34**, 441 (1953).

9. Glick, B., Sato, K., and Cohenour, F., J. Reticuloendothel. Soc. **1**, 442 (1964).

10. Harvey, W. R., Agr. Res. Service Bull. **20**, 8 (1960).

11. Subba Rao, D.S.V., M.S. Thesis. Mississippi State University (1969).

12. Chancellor, L. R. and Glick, B., Am. J. Physiol. **198**, 1346 (1960).

13. Glick, B., J. Immunol. **98**, 1076 (1967).

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

15. Jerne, N. K. and Henry, C., *in* "Cell-Bound Antibodies" (B. Amos and H. Kaprowsky, eds.), Wistar Institute Press. Philadelphia (1963).

16. Glick, B., Poultry Sci. **47**, 807 (1968).

17. Campbell, D. H. and Garvey, J. S., Lab. Invest. **10**, 1126 (1961).

Received Sept. 16, 1969. P.S.E.B.M., 1970, Vol. 133.