ponents contribute to increased gluconeogenesis whereas proteins with slower turnover rates were not as effectively catabolized. Under these conditions lipid activity was found to be unchanged.

1. Wagle, S. R., Ashmore, J., J. Biol. Chem., 1963, v238, 17.
2. Long, C. N. H., Katzin, B., Fry, E. G., Endocrinology, 1940, v26, 309.
3. Thompson, R. C., J. Biol. Chem., 1952, v197, 81.
4. Thompson, R. C., Ballou, J. E., ibid., 1954, v206, 101.
5. Swick, R. W., ibid., 1958, v231, 751.
6. Stern, M., Wagle, S. R., Sweeney, M. J., Ashmore, J., J. Biol. Chem., 1963, v238, 12.
7. Wagle, S. R., Ashmore, J., ibid., 1961, v235, 2868.
8. Wagle, S. R., Arch. Biochem. Biophys., 1963, v103, 276.
9. Wagle, S. R., Ashmore, J., J. Biol. Chem., 1964, v239, 1289.
10. Shrago, B., Lardy, H. A., Nordlie, R. C., Foster, D., ibid., 1963, v238, 3188.
11. Wagle, S. R., Biochim. Biophys. Res. Comm. 1964, v14, 533.
12. Henning, H. V., Seiffert, I., Seubert, W., Biochem. Biophys. Acta, 1963, v77, 345.
13. Prinz, W., Seubert, W., Biochem. Biophys. Res. Comm., 1964, v16, 582.
14. Freedman, A. D., Kohn, L., Science, 1964, v145, 58.
15. Wagle, S. R., Biochim. Biophys. Acta, 1965, v97, 142.
16. Ray, P. D., Foster, D. O., Lardy, H. A., J. Biol. Chem., 1964, v239, 3396.
17. Segal, H. L., Lopez, C. G., Nature, 1963, v250, 143.

Received January 17, 1966. P.S.E.B.M., 1966, v121.

Inability of Neonatal Rabbit Thymus to Induce Antibody Producing Capacity in Bursectomized Chickens.* (31034)

G. ADOLPH ACKERMAN, MARYELLEN MCCARTY AND RONALD L. ST. PIERRE
(Introduced by C. A. Doan)
Department of Anatomy, The Ohio State University, Columbus, Ohio

The thymus has been shown to elaborate a humoral or hormone-like substance which is essential in the establishment of immunological reactivity in newborn mammals. Ablation of the thymus at a time before thymic development is complete results in an impairment of antibody production, prevents homograft rejection and causes hypoplasia of lymphoid tissue and lymphopenia. Implantation of thymic tissue enclosed in cell impermeable diffusion chambers restores the lymphoid tissue, the antibody capacity and homograft immunity in these animals(1-4). In birds, 2 separate organs are essential for the development of immunological reactivity. The thymus is involved primarily with delayed hypersensitivity and homograft immunity while the bursa of Fabricius is concerned with the antibody producing capabilities(5). The formation and development of the bursa of Fabricius can be completely prevented by the *in ovo* administration of testosterone(5-7). Birds hormonally bursectomized *in ovo* or surgically bursectomized at hatching are incapable of evoking an antibody response to most antigens(5,7-10). Implantation of bursal tissue enclosed in cell impermeable diffusion chambers acts to restore significant antibody producing capacities in bursectomized birds(9-11). Experimental evidence indicates that the bursa *per se* is not involved in the direct elaboration of antibody(9,12).

The mammalian thymus, avian bursa and probably the avian thymus function at least in part by the elaboration of a humoral substance which induces antibody formation and homograft responses in these animals. Since the avian thymus cannot induce the ability of bursaless birds to form circulating antibody, 2 independent humoral substances ap-

* This investigation was supported by research grant HE 04061-08 HEM from Nat. Inst. Health, Bethesda, Md. We are indebted to Dr. R. George Jaap for his help and cooperation.

pear to regulate immunological reactivity in the bird. In mammals, the thymus exerts both functions, either by the elaboration of a single substance or by the production of 2 independent substances. It was speculated that perhaps these humoral substances are not class, species, or tissue specific. Could the implantation of the thymus from a neonatal mammal restore antibody capacity in bursectomized birds? This investigation was designed to help answer this question. Neonatal animals were chosen for this study for the following reasons: 1) host birds could be bursectomized at hatching or *in ovo*, resulting in complete inhibition of their capacity to produce antibody; 2) the lymphocytic tissue of the young birds would be receptive to the action of a humoral substance as shown by the reversal of antibody producing capacity with bursal implants in bursectomized birds, and 3) the thymus of the mammalian donor would be actively elaborating the humoral substance as indicated by the action of thymic implants in thymectomized mammals of the same strain or species.

Materials and methods. A. *Bursectomy* Hormonal bursectomy was accomplished by the dipping technique detailed by Glick(13). Eggs obtained from the regional random-bred White Leghorn population were used. On the third day of incubation, a solution of 3% testosterone proprionate (Nutritional Biochemicals Co., Cleveland, Ohio) in 95% ethanol was prepared, cooled and maintained at exactly 40°F. Eggs at 99°F were dipped half way (tapered end down) into the testosterone solution for exactly 5 seconds. The eggs were returned immediately to the incubator. Injection of 0.1 ml (2.5 mg) of testosterone proprionate in sesame oil on the fifth day of incubation was employed as an alternate procedure. Surgical bursectomy performed on the day of hatching was done also to eliminate any possible residual reaction of testosterone.

B. *Transplantation of neonatal rabbit thymus into bursectomized chicks.* The thymus was removed aseptically from neonatal rabbits, cut into 4 pieces and placed in diffusion chambers constructed of two 13 mm plastic cellulose filters (Millipore Filter Corp., Bedford, Mass.) (0.45 μ porosity) on either side of a lucite ring. The sealed chambers were implanted subcutaneously into both hormonally and surgically bursectomized 7-day-old chicks. Three weekly injections of one ml of *Salmonella typhimurium* (standardized at 3×10^9 cells per ml) were given intramuscularly beginning at the time of the surgical procedure. One week after the third injection, blood for bacterial agglutination studies was obtained by cardiac puncture. Controls consisted of non-immunized, non-bursectomized chicks, immunized non-bursectomized chicks and immunized bursectomized chicks without thymic transplants. Each bursectomized animal was examined for residual bursal tissue at time of sacrifice.

C. *Bacterial agglutination procedures.* Agglutination tests were done by adding 0.25 ml of the standard antigen to 0.25 ml samples of serum in serial dilution from 1:2 to 1:512. Tubes were incubated at 45°C for 2 hours then refrigerated for 24 hours.

Results. Thirty-one control non-bursectomized chicks developed antibody titer to *S. typhimurium* ranging between 1:32 to 1:256 with a mean titer of 1:80. Twenty hormonally bursectomized and 9 surgically bursectomized chicks failed to develop significant antibody titers. Hormonally and surgically bursectomized chicks implanted with neonatal rabbit thymus in diffusion chambers also failed to develop significant antibody titers with the method employed. Table I indicates the antibody titers found in each group of birds including the non-challenged control group of chickens. The growth impairment of bursectomized birds was not prevented by rabbit thymus implants. Examination of the thymus implants at termination of the experiment indicated marked tissue necrosis and liquefaction.

Discussion. Since the antigen chosen produces an antibody response in normal chickens and in bursectomized chickens following implantation of neonatal bursal tissue, the most reasonable explanations for the inability of the neonatal rabbit thymus to induce antibody producing capacity in bursectomized chicks would be: 1) that the humoral substance of the mammalian thymus is species specific and unable to transcend the class bar-

TABLE I. Antibody Titers in Chicks Immunized with *S. typhimurium.*

Group	No. of birds	Mean titer	No. birds/antibody titer
Control, intact bursa	31	1:80	10/1:32; 11/1:64; 8/1:128; 2/1:256
Hormonal bursectomy	20	1: 1.3	15/0; 4/1:2; 1/1:4
Surgical bursectomy	9	1: 1.4	1/0; 2/1:2; 5/1:4; 1/1:8
Hormonal bursectomy, thymus implant	50	1: 2.9	16/0; 14/1:2; 15/1:4; 5/1:8
Surgical bursectomy, thymus implant	31	1: 3.6	5/0; 11/1:2; 9/1:4; 5/1:8; 1/1:16
Non-challenged, control, intact bursa	15	0	15/0

rier or 2) that the rabbit thymus does not elaborate a humoral substance involved specifically with induction of antibody responses in chickens. Prolonged tissue survival does not appear to be necessary since bursal implants in bursectomized birds survive for relatively short periods of time as based upon morphological evaluation of the recovered tissue implants(9,14). The action of the bursal tissue probably occurs during the first week of implantation; a similar situation would be expected with the thymic implant. Species specificity of the thymic humoral substance has been indicated by Dalmasso *et al*(15) and Yunis *et al*(16), in that rat thymus grafts survive for prolonged periods in neonatally thymectomized mice but do not restore immunological competence in the host. Preliminary studies in our laboratory involving the implantation of neonatal chicken bursal tissue in diffusion chambers into neonatally thymectomized mice challenged with 3 weekly injections of *S. typhimurium* have produced inconclusive results in regard to the restoration of antibody response in the host. Although the success of this experiment seems unlikely, it will be explored further with additional antigens and more sensitive serological techniques.

Summary. Implantation of neonatal rabbit thymus in either surgically or hormonally bursectomized chickens has failed to restore immunological competence as measured by their ability to produce antibodies to *S. typhimurium.*

1. Law, L. W., Trainin, N., Levey, R. H., Barth, W. F., Science, 1964, v143, 1049.

2. Levey, R. H., Trainin, N., Law, L. W., J. Nat. Cancer Inst., 1963, v31, 199.

3. Osoba, D., Miller, J. F. A. P., J. Exp. Med., 1964, v119, 177.

4. Wong, F. M., Taub, R. N., Sherman, J. D., Dameshek, W., Fed. Proc., 1964, v23, 189.

5. Warner, M. L., Burnet, F. M., Austr. J. Biol. Sci., 1961, v14, 580.

6. Ackerman, G. A., Knouff, R. A., Anat. Rec., 1963, v146, 23.

7. Mueller, A. P., Wolfe, H. R., Meyer, R. K., J. Immunol., 1960, v85, 172.

8. Glick, B., Chang, T. S., Jaap, R. G., Poul. Sci., 1956, v35, 224.

9. St. Pierre, R. L., Dissertation, Ohio State Univ., 1965.

10. St. Pierre, R. L., Ackerman, G. A., Science, 1965, v147, 1307.

11. Jankovic, B. D., Leskowitz, S., Proc. Soc. Exp. Biol. and Med., 1965, v118, 1164.

12. Dent, P. B., Good, R. A., Nature, 1965, v207, 491.

13. Glick, B., Endocrinology, 1961, v69, 984.

14. Isakovic, K., Jankovic, B., Papeskovic, L., Milosevic, D., Nature, 1963, v200, 273.

15. Dalmasso, A. P., Martinez, C., Sjodin, K., Good, R. A., J. Exp. Med., 1963, v118, 1089.

16. Yunis, E. J., Martinez, C., Good, R. A., Nature, 1964, v204, 664.

Received January 21, 1966. P.S.E.B.M., 1966, v121.