acids. It would appear that the effect of epinephrine on mobilization of free fatty acids is not mediated by a decrease in carbohydrate availability, but possibly by increased rate of glyceride breakdown.

The authors wish to acknowledge the helpful criticism of Drs. Manfred L. Karnovsky and William B. Reddy.

1. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
2. Gordon, R. S., Jr., Cherkes, A., *ibid.*, 1956, v35, 206.
3. Laurell, S., *Scand. J. Clin. and Lab. Invest.*, 1956, v8, 81.
4. Bierman, E. L., Dole, V. P., Roberts, T. N., *Diabetes*, 1957, v6, 475.
5. Dole, V. P., Bierman, E. L., Roberts, T. N., *J. Clin. Invest.*, 1957, v36, 884.
6. Winegrad, A. I., Renold, A. E., *J. Biol. Chem.*, 1958, v233, 267.
7. ———, *ibid.*, 1958, v233, 273.
8. Cahill, G. F., Jr., Leboeuf, B., Renold, A. E., *ibid.*, 1959, v234, 2540.
9. Nelson, N., *ibid.*, 1944, v153, 375.
10. Reeves, R. E., *J. Am. Chem. Soc.*, 1941, v63, 1476.
11. MacFadyen, D. A., *J. Biol. Chem.*, 1945, v158, 107.
12. Buchanan, J. G., Dekker, C. A., Long, A. G., *J. Chem. Soc., London*, 1950, 3162.
13. Gordon, R. S., Jr., *J. Clin. Invest.*, 1957, v36, 810.
14. Cori, C. F., Cori, G. T., *J. Biol. Chem.*, 1928, v79, 321.
15. Walaas, O., Walaas, E., *ibid.*, 1950, v187, 769.
16. Cori, C. F., Cori, G. T., *ibid.*, 1931, v94, 581.
17. Crane, R. K., Sols, A., *ibid.*, 1953, v203, 273.
18. ———, *ibid.*, 1954, v210, 597.
19. Tuerkischer, E., Wertheimer, E., *J. Physiol.*, 1946, v104, 301.
20. Sutherland, E. W., Cori, C. F., *J. Biol. Chem.*, 1951, v188, 531.
21. Vaughan, M., Steinberg, D., Shafrir, E., *J. Clin. Invest.*, 1959, v38, 1051.
22. Sutherland, E. W., in *Phosphorus Metabolism*, W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, 1952, vII, 577.
23. Cahill, G. F., Jr., Leboeuf, B., Flinn, R. B., *Abstracts, Endocrine Soc.*, 41st Meeting, 1959, 62.
24. Shapiro, B., Chowers, I., Rose, G., *Biochim. Biophys. Acta*, 1957, v23, 115.

Received August 24, 1959. P.S.E.B.M., 1959, v102.

Antibody Response to Heterologous Protein in Rabbits of Varying Maturity.* (25307)

DONALD V. EITZMAN† AND RICHARD T. SMITH‡
Dept. of Pediatrics, University of Florida, School of Medicine, Gainesville, Fla.

Injection of a variety of heterologous proteins into the neonatal animal induces a state of immunological tolerance(1,2) which is of finite duration related to the amount injected at birth. The period of susceptibility to induction of tolerance to 100 mg bovine serum albumin in the rabbit has been found to end by 15-17 days of age(1). However, a state of immunological unresponsiveness similar in many respects to that induced at birth has been produced by repeated injections of very large amounts of heterologous proteins into adult rabbits(3). Calculations from available data do not entirely rule out the possibility that the difference in the adult and newborn response to heterologous protein may be a quantitative rather than a qualitative one. The 5-7 fold increase in body weight of rabbits during the first three weeks of age could conceivably reduce the concentration of injected antigen below a critical level. These experiments were designed to re-examine the immunological response of rabbits of varying maturity to weight-adjusted amounts of a relatively pure heterologous protein. The data add further evidence that a qualitative defect in immunological capacity exists in the neonatal rabbit.

Procedure and methods. New Zealand

* Supported by grants from Nat. Inst. of Arthritis and Metabolic Diseases, Am. Heart Assn., and Arthritis and Rheumatism Fn.

† USPHS Postdoctorate Fellow.

‡ Senior Investigator, Arthritis and Rheumatism Fn.

TABLE I. Effect of Weight Adjusted Amounts of BSA on Immune Response in Rabbits of Varying Maturity.*

Litter No.	Age at initial inj., days	Avg wt of animals at initial inj., g	Anti BSA produced after		Interpretation§	
			Initial inj.	Challenging inj.	Tolerance induced	Immune response
1	2	54	0/3†	‡	7/7 (100%)	0/7
2	8	141	0/4	0/4		
3	14	170	0/8	0/5	12/15 (80%)	3/15
4	14	221	0/4	2/4		
5	15	190	‡	1/6		
6	21	352	0/2	2/2		
7	21	220	1/6	2/6	4/15 (27%)	11/15
8	22	170	7/9	7/9		
9	28	426	4/5	4/4	0/12	12/12
10	30	524	8/8	8/8		

* Litters of rabbits were given 200 mg BSA/kg I.P. at ages indicated and bled serially for determination of presence of BSA or anti-BSA as precipitins in serum. At 10 wk of age the challenging inj., 20 mg BSA/kg was given I.V. followed by similar determinations on serial serum specimens.

† Denominator = No. in litter at time of bleeding. Numerator = No. with anti-BSA in serum.

‡ Not determined or not rechallenged.

§ Tolerance indicated by prolonged clearance of initial and challenging inj. *and* failure to produce detectable anti-BSA after antigen clearance. Immune response indicates production of detectable precipitating antibody.

white rabbits were used. Litters were kept with does from birth until weaning at 5 to 6 weeks of age. Procedures for identifying and handling neonatal rabbits have been previously described(1a). Blood samples were obtained for precipitin analysis by cardiac puncture. Crystalline Bovine Albumin (BSA) was obtained from Armour Labs. dissolved in 1% NaCl, sterilized by passage through Seitz filter, and analyzed for protein content by micro-Kjeldahl analysis. Rabbits were weighed and then given an injection calculated to total 200 mg BSA/kilo. Initial injections in each age group were given intraperitoneally. The amount was that known to produce tolerance lasting over 12 weeks in a majority of rabbits when given at birth(1a). Challenging injections of BSA were given intravenously through an ear vein in a dose of 20 mg/kg of body weight 10 weeks after initial injection. Following both initial and challenging injections, animals were bled at 5 day intervals and the serum assayed qualitatively for presence of BSA or of anti-BSA antibody by a capillary tube precipitation method(1a).

Results. Table I presents results in terms of production of precipitating antibody in serial bleedings following both first and second antigen injections. Litters 1, 2, and 3 had BSA present in their serum for 20 days or longer following initial injection and for 17 days or longer after the challenging injection (litters 2 and 3 only). These animals proved tolerant at the 10 week challenge as expected from results of previous studies. Of litters initially injected at 14 and 15 days of age, in only one litter (number 3) were all rabbits unresponsive at the challenge. In litters 4 and 5, 2 of 4 and 1 of 6 respectively, produced anti-BSA after the 10 week injection; whereas no antibody production was observed following neonatal injection.

Three litters were injected at 21 and 22 days of age. None of the rabbits in litter 6 but 1 of 6 in litter 7, and 7 of 9 in litter 8 had antibody in their serum following initial injection. Following the challenging injection, the majority in litters 6 and 8 produced antibody while ⅓ of litter 7 produced antibody.

Litters 9 and 10, who were initially injected on day 28 and 30 cleared antigen rapidly from their serum and 12 of 13 rabbits in both groups produced antibody following single exposure to antigen. The single rabbit in litter 9 which failed to produce antibody initially did not survive 10 weeks, and was not reinjected.

The experimental groups may conveniently be combined as shown in Table I, into 4 groups which responded to the antigen in a

similar fashion. Those animals which showed prolonged clearance time of antigen injected both in the neonatal period and at 10 weeks and which showed no precipitating antibody after either injection, are considered to be tolerant(1a). Those producing circulating antibody after either injection are considered immune. By this grouping it is shown clearly that the amount of antigen injected initially, even though adjusted in amount for rapid growth of animals, was adequate to induce tolerance regularly only during the first 2 weeks of life, as previously shown for a single quantity of antigen. A marked qualitative change in animals' response to this heterologous protein, therefore, must have occurred between 14th day of life when 80% of the litters became tolerant and 21st day when only 27% failed to produce antibodies.

Discussion. Rabbits older than 28 days of age consistently produced antibodies to a single injection of BSA, while rabbits under 14 days of age handled this protein with no evidence of immune response. Rabbits between these 2 age groups responded variably, but most injected at 14 days of age were unresponsive while most 21-day-old animals had a primary immune response with production of precipitating antibody. These data again demonstrate that the period during which unresponsiveness can be induced is of finite length, consistent with a qualitative deficiency in the immune mechanism. These data also suggest strongly that different mechanisms probably underlie the model of unresponsiveness which is induced in adults by large amounts of antigen(3) and the type tolerance induced during the neonatal period.

Summary. Neonatal rabbits respond to a single injection of BSA in a dose of 200 mg/kilo body weight with antibody production when given at 28 days of age or older and are unresponsive when this weight-adjusted amount of BSA is given at 8 days of age or younger. At 14 and 21 days of age the response is varied and the majority injected at 14 days of age are unresponsive while most formed antibody when injected at 21 days of age.

The authors acknowledge the technical assistance of Earle Calahan, Marjorie Wright, and Billievelyn Miller.

1. (a) Smith, R. T., Bridges, R. A., *J. Exp. Med.*, 1958, v108, 227. (b) Smith, R. T., *Czechoslovak Academy of Sciences,* 1959. (c) Humphrey, J., *ibid.*

2. (a) Hanan, R., Oyama, J., *J. Immun.*, 1954, v73, 49. (b) Dixon, F. J., Maurer, P. H., *J. Exp. Med.*, 1955, v101, 245. (c) Cinader, B., Dubert, J. M., *Brit. J. Exp. Path.*, 1955, v36, 515.

3. (a) Dixon, F. J., Maurer, P. H., *J. Exp. Med.*, 1955, v101, 233. (b) Johnson, A. G., Watson, D. W., Cromartie, W. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 421.

Received September 8, 1959. P.S.E.B.M., 1959, v102.

Inhibition of Cleavage and Development of the Sea-Urchin Egg by 2-Desoxy-D-Glucose.* (25308)

GERALD S. BERNSTEIN† AND ROBERT E. BLACK‡ (Introduced by Albert Tyler)
Division of Biology, California Inst. of Technology, Pasadena

An analogue of glucose, 2-desoxy-D-glucose (2DG), has been shown to be a competitive inhibitor of glucose metabolism in yeast(1,2) and rat tissues(3-6). It is also known to retard cell-division in chick heart fibroblasts cultured *in vitro*(7), and in tumors(8-10), and budding in yeasts(2). In view of above work, it appeared to us that it would be of interest to investigate the action of this inhibitor on respiration and development of fertilized eggs of sea-urchins. Both glycolytic and respiratory metabolism are required for cleavage of these eggs, since development is blocked by

* This investigation was supported by a research grant from Nat. Cancer Inst., N.I.H., P.H.S.

† Present address: Univ. of Southern California School of Medicine, Los Angeles.

‡ Postdoctoral Research Fellow of U. S. Public Health Service. Present address: Dept. of Biology, College of William and Mary, Williamsburg, Va.