PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 107–111 (1977)

Developmental Changes of Immunoglobulins in Rats Treated Neonatally with Hydrocortisone[1] (39614)

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Immunodeficiency states can develop through a variety of mechanisms. Genetically determined immunodeficient mice have been described and investigated extensively. The Ames dwarf mouse exhibits lymphopenia, involution of thymic and peripheral lymph tissue, and a wasting syndrome. The serological response to sheep erythrocytes (RBC) is delayed and decreased in the dwarf, as is the graft vs host response. However, marked deficiencies in resting serum immunoglobulins are absent. These findings have been interpreted as an immunodeficiency state restricted to the thymic system (1). Nude mice exhibit a different immunodeficiency syndrome, though they also have wasting syndrome and decreased serological response to challenge with sheep RBC. IgM levels are normal in comparison to controls, while IgG and IgA are low (2).

Deficiencies in the immune system have been produced by administering adrenal cortical hormones. The subsequent thymic involution first was described by Dougherty and White (3), and since has been confirmed by others. Recently, Greengard and Machovich (4) have found decreased levels of thymic thymidine kinase and increased levels of L-glutamyl-transferase and glutamine synthetase. Levels of six other enzymes were unaltered. These studies may provide a biochemical basis for the effects of glucocorticoids on thymic size. Halpern *et al.* (5) report that daily injections of cortisone, beginning at Day 5, impair survival and are associated with decreased antibody response to typhoid bacilli and with lower gamma-globulin levels by 21 days of age. In rats, a single injection of 1 mg of cortisol acetate within 24 hr of birth is correlated with decreased passive hemagglutination titers of *Salmonella typhosa* and decreased serological response to sheep RBC in 60 to 70-day-old rats (6).

The immunological change produced by neonatal injection of glucocortocoids suggests that a model of immunodeficiency which may resemble one of the clinically described diseases in man could be produced by this method. Thus, further study of the immunological consequences of neonatal glucocorticoid injection seems appropriate. The present study attempts to characterize them further.

Methods and materials. Pregnant Sprague–Dawley rats from our own colony were used. Within 24 hr after birth, pups were injected sc with 20 μl of cortisol acetate (Upjohn, Cortef acetate, 50 mg/ml). We refer to these rats as corticoid runts. Controls were injected sc with an equal volume of 0.9% NaCl. Litters were culled to a maximum of 10 animals. After treatment, rats were returned to their mothers until sacrifice or weaning at 24 days of age. Animals were maintained at 25°; Purina rat chow and water were provided *ad libitum*.

Rats were decapitated at 3, 6, 11, 15, 20, 30, and 60–75 days of age. Blood was collected and centrifuged, and the serum was removed. Serum from very young rats was pooled so that each sample contained approximately 0.5 ml. Samples were frozen at 0°.

Globulins were isolated from pooled rat serum (7). The isolated material contained 6.58 mg/1 ml as determined by the method of Lowry (8). Rabbit anti-rat globulin was obtained from Gibco (Grand Island, New York). Rat IgG and rabbit anti-rat IgG were purchased from the Pentex Division of Miles Laboratories (Kankakee, Ill.). The

[1] Supported by USPHS Grants No. HD8575 and No. AM17594.

vendor states that the rabbit anti-rat IgG is immunospecific, and we have verified this by immunoelectrophoresis against rat serum.

Serum levels of globulin and IgG were measured by agar-gel diffusion against anti-rat globulin and anti-IgG on immunodiffusion plates (Pattern C, Hyland Laboratories, Costa Mesa, Calif.) with serial dilutions of standards run daily. One-third of the samples were run in duplicate. The endpoint of the dilutions for IgG and globulin was designated as equivalent to one unit and serum concentrations were calculated accordingly. Statistical comparisons were made by Student's *t* test.

To investigate immunoglobulin absorption from the intestinal tract, five samples of 100 μg of IgG were dissolved in 100 μl of phosphate buffer, pH 7.4, and iodinated with 60 μCi of ^{125}I (Amersham Searle), as described by Yalow and Berson (9). Each sample was purified on a Sephadex G-50 column, 1.2 by 5 cm, connected to an LKB fraction collector recorder. Protein was monitored by ultraviolet absorption. Fractions containing protein were pooled. The free ^{125}I in the purified preparation was estimated from the radioactivity remaining in solution after deproteinization as compared to the total radioactivity of the sample. To that end, 100 μl of each sample was directly dissolved in 1 ml of soluene in a counting vial. A second 100 μl of each sample was treated with 150 μl of 10% trichloroacetic acid, centrifuged, and 100 μl of the protein-free supernatant was similarly added to a vial, followed by 1 ml of soluene. Ten milliliters of Bray's solution was added to each vial and radioactivity was determined in a liquid scintillation counter. Since free iodine in the five samples averaged only 13.3% ± 4.1 (standard error), the samples were not further purified, but were pooled, lyophilized, and dissolved in 0.9% NaCl.

At 9 AM the following day, the mothers were removed from one litter of controls and one litter of corticoid runts. All pups were 13 days old. One hour later, 0.2 ml of [^{125}I]IgG was intubated into each pup. Two control rats were intubated with saline only. Three hours later, animals were decapitated, the blood collected, and the serum removed. One-hundred or two-hundred microliters of serum was solubilized with 10 vol of soluene, 10 ml of a soluene-based scintillation cocktail was added, and the samples, together with a vial containing 100 μl of the [^{125}I]IgG, were counted. Quench corrections were made by recounting the vials after addition of 0.1 ml of a mixture of 150 μl of [^{125}I]IgG and 1.6 ml of soluene.

To determine the fraction of ^{125}I bound to the rat serum proteins, the serum from all rats intubated with radioactive IgG was pooled and 0.3 ml was taken to estimate total radioactivity. Unlabeled albumin was added to a second 0.3-ml aliquot to act as carrier, protein was precipitated with 1 ml of 10% trichloroacetic acid, the sample was mixed and centrifuged, and 0.5 ml of the supernatant was used to determine unbound ^{125}I.

To investigate antibody synthesis, rats were sensitized by intraperitoneal and intradermal injections of 10% sheep red blood cells (SRBC) on Days 14 and 18. The animals were exsanguinated 1 week after the last injections and their spleens were removed. Hemolytic direct plaques were measured as described by Levy and Harris (10). Hemagglutination titers were measured in microtiter plates using mercaptoethanol pretreatment to identify the relative amounts of IgG and IgM by the method of Braley-Mullen (11).

Survival rates were evaluated by counting the number of rats remaining in five litters of corticoid runts and three liters of controls. Litters were checked every 3 to 4 days. The percentage of the total surviving at a given age is shown in Fig. 2.

Results. Developmental changes in total globulins and IgG in corticoid runts and controls are shown in Fig. 1. Control levels of globulins rise steadily in the suckling rat and reach a peak at 11 days. Levels remain high until after weaning. Although levels are lower at 30 days than at 20 days, they are not as low as in newborn rats. Between 30 and 75 days, levels rise gradually. Serum globulins in newborn corticoid runts rise very slowly during development, so that at 11 days they are significantly lower than levels in controls and remain down until 30 days. Thereafter no significant difference is found between the two groups.

Serum IgG levels show a similar trend. In

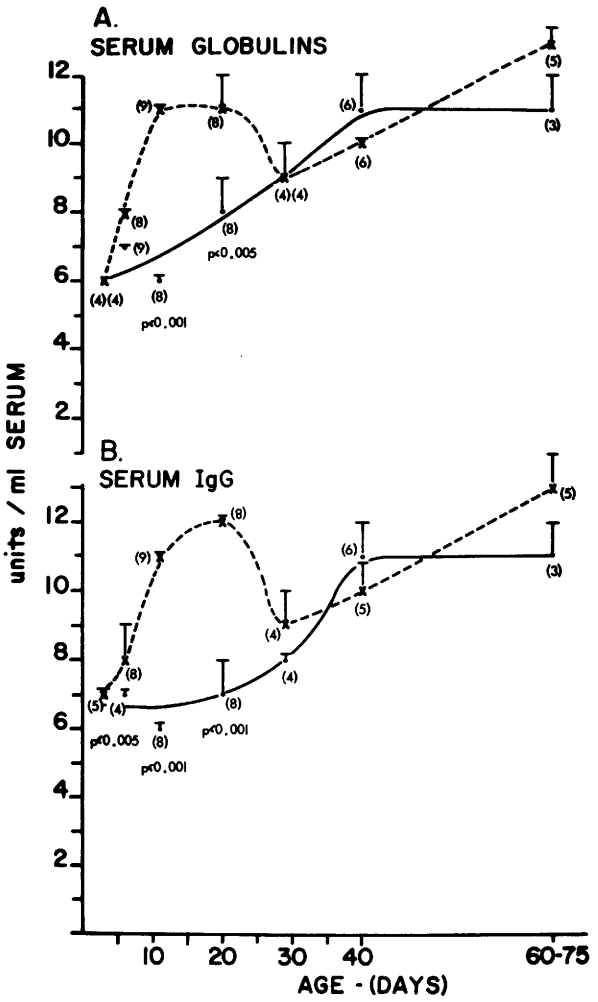

FIG. 1. Developmental changes in concentration of (A) serum globulins, and (B) IgG levels in controls (×- - -×) and in corticoid runts (●——●). Mean ± SE is shown at each age. Sample size is in parentheses.

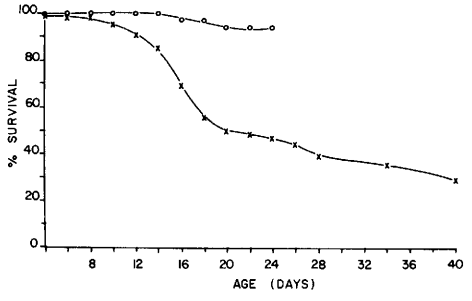

FIG. 2. Comparison of survival rates in controls (○——○) and in corticoid runts (×- - -×).

controls they rise rapidly until the animals are 20 days old. After weaning, they fall precipitously and then gradually rise. By contrast, serum IgG levels in runts remain low throughout the suckling period. At 6 days, they are significantly lower than in controls and remain so until after weaning, when they begin to rise slowly. Levels peak at 40 days and do not rise further.

Absorption of IgG from the gastrointestinal tract is expressed in counts per minute per 100 μl of serum per 0.1 ml of [^{125}I]IgG. At 13 days of age, the mean ± standard error in four untreated rats is 527 ± 138; in three corticoid runts, it is 593 ± 29 ($P > 0.7$). In rats intubated with saline levels were 57 and 67. Free iodine levels in the intubation mixture of [^{125}I]IgG were 13.3%. The free iodine in the pooled sera of intubated rats was 22%.

The immunological response to t-depend-

TABLE I. EFFECT OF NEONATAL HYDROCORTISONE TREATMENT ON HEMAGGLUTINATION TITERS TO SRBC AT 25 DAYS OF AGE.

Group	Log titer	
	IgG + IgM	IgG
Controls	1.2861 ± 0.0586 (11)[a]	0.7661 ± 0.0624 (11)
Corticoid runts	0.7525 ± 0.1028 (10)[b]	0.3311 ± 0.1304 (10)[b]

[a] Mean ± SE. Sample size is in parentheses.
[b] Significantly less than control titer ($P < 0.01$).

TABLE II. EEFFECT OF NEONATAL HYDROCORTISONE TREATMENT ON PLAQUE FORMATION AT 25 DAYS OF AGE.

Group	Plaques/spleen	Plaques per 4×10^6 lymphocytes
Controls	1062 ± 253 (11)[a]	27 ± 4 (22)
Corticoid runts	108 ± 51 (10)[b]	2.8 ± 0.9 (20)[b]

[a] Mean ± SE. Sample size in parentheses.
[b] Significantly less than control titer ($P < 0.005$).

ent SRBC (Tables I and II) demonstrates a severe depression in the corticoid runts' ability to produce antibodies. The hemagglutination assay (Table I) shows that the control rats had a titer of 1:16 (IgG + IgM) and 1:6 of IgG alone while the steroid-treated animals had a titer of 1:6 (IgG + IgM) and 1:2 of IgG alone. This depression in antibody formation also is reflected in the plaques either on the basis of number of lymphocytes studied or on the basis of spleen weight (Table II). Essentially, in either case, the corticoid runts only had 10% of the plaques seen in the controls. The runts were somewhat smaller, but proportionally they had a greater spleen to body weight ratio of 6.27 ± 0.35 (mean ± SE) than controls (4.62 ± 0.44). Units are in milligrams per gram of body weight.

Survival rates of both controls and corticoid runts are seen in Fig. 2. By 24 days of age, 94% of controls were alive, but less than 50% of the runts had survived; in fact, 94% survived only until Day 10. Between Days 12 and 18, survival of corticoid runts fell rapidly from 90 to 55%. Thereafter the drop was less dramatic, although by 40 days only 30% of the runts were alive. Growth is severely retarded in the runts, as evidence by persistently lower body weight as described previously (12). Generally, they appear less active than controls and more prone to diarrhea and eye infections.

Discussion. At least part of the decreased survival of runts may be due to their limited immunological capacity, which is most dramatic during the first 30 days (Fig. 1). Indeed, the time at which corticoid runt mortality is most marked (from 12 to 18 days of age) correlates with the onset of their most severe immunological impairment.

Two possible mechanisms for this impairment exist in the suckling rat. First, the acquisition of passive immunity from maternal milk might be affected by neonatal hydrocortisone treatment. However, we found no significant difference in absorption of IgG from the gastrointestinal tract of 13-day-old controls and corticoid runts. Halliday (13) reports that the effects of cortisone acetate on absorption of hyperimmune rat serum from the gastrointestinal tract of 10- to 24-day-old rats depend on the time of hormone administration. If the hormone is given at the same time as the serum, antibody absorption is *increased.* If the hormone is administered 3 days before intubation of the hyperimmune serum, antibody absorption is *reduced.* Our results suggest that neonatal administration of adrenal steroids does not alter immunoglobulin absorption from the intestinal tract of 13-day-old rats. Thus, the persistently low levels of serum globulins and IgG in corticoid runts cannot be attributed to long-term alterations in absorption of maternal immunoglobulins. A second alternative is decreased synthesis and secretion of endogenous antibody in corticoid runts. Schapiro and Hupport (6) describe such impairment in the

adult. Our findings demonstrate that this deficit is present prior to weaning. Hence, neonatal corticoid treatment produces immediate effects on immunological capacity which persist into adulthood.

Halpern *et al.* (5) also report decreased survival and failure of antibody production in young rats treated daily with cortisone. However, the simplicity of the single injection we used in the present study in producing chronic defects is very appealing.

Summary. Serum globulin and IgG levels were measured by radial immunodiffusion from 3 to 75 days after birth in rats neonatally treated with 1 mg of cortisol acetate. In controls, globulins rose steadily during suckling, peaked at 11 days, and remained high until weaning, when they dropped considerably. Thereafter, they rose steadily until 75 days. In treated animals, levels increased slowly during development and were significantly lower than controls until 30 days. Serum IgG followed a similar pattern. The most severe immunological deficit in runts began on Day 12. The most marked mortality in treated rats also began at this age and in part may be due to the severe immunological impairment. No difference was detected in absorption of IgG from the gastrointestinal tract of the two groups. However, dramatic deficits were demonstrated in the ability of treated rats to synthesize antibody when sensitized with a t-dependent antigen. Thus, the difference in immunological competence is due to endogenous deficits produced by a single injection of cortisol acetate at birth.

We gratefully acknowledge the technical assistance of Willie Mills, Peggy Cohen, and David Chia.

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Received December 2, 1975. P.S.E.B.M. 1977, Vol. 154.