TABLE I. Average Semen Quality of 14 Men, at Weekly Intervals during 3 Month Period of Griseofulvin Intake.

	Semen specimen #	Ejaculate vol (cc)	Sperm count/cc —in millions—	Total sperm count	% normal forms
Control	1	3.42	228	780	79
"	2	2.84	185	527	78
	3	3.20	100	320	84
	4	3.30	175	577	84
	5	3.42	180	615	82
	6	2.91	134	389	81
	7	3.10	111	341	79
	8	3.10	117	363	83
	9	2.50	128	320	80
	10	3.48	130	452	81
	11	3.18	130	413	80
	12	2.74	123	337	79
	13	3.38	152	513	81

Semen specimens #3-13 produced at weekly intervals after intake of griseofulvin (2 g daily) was begun.

data on semen quality (Table I) the testicular biopsies did not show significant changes. Only 8 of the 14 individuals who had been on the griseofulvin for 3 months consented to the second biopsy. Attention was given to tubal size, patterns of spermatogenic activity, sperm production, condition of the peritublar connective tissue and number and functional appearance of the Leydig cells. In all these respects, the histologic condition of the testes appeared to be unaffected by treatment with griseofulvin. Both before and after the period of treatment the histologic picture of the biopsies obtained from the men in this series was, in each instance, within the recognized range of normality.

Summary and conclusions. Daily administration of 2 g of griseofulvin to 14 normal men for 3 months did not produce significant changes in semen quality or in histology of the testes of 8 of the individuals.

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Antibody Production in Neonatal Chickens Following Injection of Adult Cells Mixed with Antigen *in vitro*.* (25213)

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Neonatal animals commonly exhibit an impaired immune response. They succumb easily to infection by pathogenic agents and do not respond, or react only poorly, to stimulation with non-living bacterial or protein antigens. Inability to produce circulating antibody following antigenic challenge appears to be a consistent feature among different species. Interspecies, interstrain, and individual variations, however, are characteristic of interactions between neonatal animal and transferred, viable lymphoid cells stimulated *in vivo* or *in vitro* with antigen(1-4). The variable reactivity of the chimera, *i.e.* the neonate containing transferred, stimulated, adult homologous lymphoid cells has been interpreted as representing a homograft reaction by Sterzl, Trnka, and co-workers(3,5), and the inadequacy or immaturity of the neonatal environment by Dixon and Weigle(1,8) and by Nossal(4). Our studies which confirm those of Trnka and Riha(2,3) demonstrate that antibody produc-

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tion can be initiated in neonatal chickens by injecting adult spleen cells which have been stimulated *in vitro* with a bacterial antigen. Variation in response by individual recipient chicks, however, indicates that in this experimental model, utilizing non-inbred chickens, many factors may determine formation of antibody or lack thereof in the recipient host. Important considerations are the technical manipulations and supportive and/or repressive influences contributed by both graft and host, which may interact to different degrees.

Methods. White leghorn hens (Ghostley strain) were used as *donors for spleen cells.* Donor hens were anesthetized with sodium pentothal and bled by cardiac puncture. Immediately thereafter the abdomen was opened and the spleen removed aseptically. The excised spleen was then rinsed 2 times with Hank's solution containing penicillin (100 units/ml), streptomycin (100 μg/ml) and Nystatin (50 μg/ml)—RBSS, cut into 6 or 7 large fragments, and rinsed again with RBSS. Fragments were transferred to a sterile test tube covered with 2 ml of Eagle's medium containing antibiotics and heparin (2 units/ml), and homogenized by a loosely fitting pestle of a Potter-Elvehjem tissue grinder until a smooth uniform suspension was obtained. Microscopic examination of Wright-stained smears of suspensions at this stage of preparation revealed nearly all intact single cells with a few clumps. A viable cell count was made by using appropriate dilutions of a cell suspension in Hank's solution containing 0.5% Trypan Blue and recording the number of unstained white cells in a hemocytometer. Cells from one donor spleen were then divided into equal aliquots. One of the aliquots was injected intraperitoneally into one of a pair of recipient chicks as a control and the other aliquot was mixed with antigen prior to injection into the remaining member of the pair. A standardized suspension of *Brucella abortus* antigen (U.S. Dept. of Agriculture standardized diagnostic antigen, kindly supplied by Dr. Robert Lindorfer, School of Veterinary Medicine, Univ. of Minnesota) was made by counting cells in a Petrof-Hauser Counting Chamber under dark field illumination. A turbidity curve was plotted from dilutions of the standard suspension and the number of cells in any diluted suspension could be obtained from the curve after reading the absorbancy at 600 mμ. Dilutions of antigen were prepared to give a ratio of 2 bacterial cells to one viable spleen cell, and the resulting mixture was mixed by repeated aspiration and expulsions from a pipette. *Recipient chicks* of the same strain were obtained 12-36 hours after hatching and given injections as described in the experimental protocol below. *Six days after cell transfer the chicks were bled* by cardiac puncture and autopsies were performed on the animals from each experimental group. All blood for testing was allowed to stand at room temperature for one hour and overnight in the cold. After loosening the clot, the sample was centrifuged for 15 minutes and the serum removed. For *determination of agglutinin titers,* an initial 1:10 dilution of serum was made in phenolized, buffered saline (0.5% phenol in 0.01 M phosphate buffer containing 0.8 g NaCl/100 ml, pH 7.4) and thereafter serial 2-fold dilutions were made by adding 0.2 ml of the successive serum dilutions to tubes containing 0.2 ml of phenolized, buffered saline. To each tube, 0.2 ml of antigen (1:100 dilution of the original stock as supplied by Dept. of Agriculture) was added and incubation was carried out at 37°C with intermittent shaking for 24 hours. Immediately after removal from the water bath, tubes were examined in diffuse light with the aid of a concave, magnifying, reflector mirror. Titers were recorded as the last tube demonstrating agglutination after slight shaking.

Results. As controls agglutinin titers were measured in serum from the donor hen, normal chicks, chicks receiving Eagle's solution only, *Brucella abortus* antigen only, heated hen spleen cells (60°C for one hour) plus antigen, heated cells only, and viable cells only (Table I). In every case, control sera gave no agglutination ($<1:10$). All chicks were housed similarly; members of each group were distributed in each cage. Normal chicks were distributed among experimentals in order to detect any possible unfavorable factors in diet and housing. Mortality rates of chicks receiving spleen cells, antigen, or both may re-

TABLE I. Experimental Design for Study of Neonatal Chickens (12-36 Hours Post-Hatch) Receiving Transferred Homologous Adult Hen Spleen Cells by Intraperitoneal Injection.

Inoculum*	No. of recipient chicks	Mortality rate†
Viable cells + *B. abortus* antigen	35	9/35
Viable cells only	35	6/35
Heated cells (60°C for 1 hr) + *B. abortus* antigen	5	0/5
Heated cells only	5	1/5
B. abortus antigen only	20	7/20
Normal chicks‡	15	1/15
Eagle's only	4	0/4

* Each adult donor spleen was divided into 2 doses after counting the cells. Thus, chicks from each group receiving cells are part of a pair, one of whom received cells, the other, cells plus antigen.

† Mortality rate refers to chicks dying before 6th day after cell transfer.

‡ Normal chicks received neither cells nor antigen.

flect the toxicity of cells and or of components of the antigen.

Antibody response in chicks receiving cells stimulated in vitro with antigen. Chicks receiving cells plus antigen responded in a variable manner (Table II). That the agglutinin titer was not directly related to the number of spleen cells can be seen from the table.

Gross pathologic changes in the chicks receiving viable cells plus antigen appeared to correlate well with antibody production. The most striking alterations consisted of splenomegaly, ectopic growths of splenic cells over the serosa of the intestine, hyperplasia of the visceral and parietal peritoneum, and occasional white, nodular overgrowths and capsular thickening near the lower border of the liver. Yellow gelatinous effusions in the subcutaneous tissue overlying the site of injection and occasionally over the entire peritoneal cavity were commonly noted. It should be emphasized that these gross pathologic changes were also found in chicks receiving cells only. Nevertheless, chicks which evidenced little or no pathologic change after receiving cells plus antigen did not produce any antibody which could be detected within the limits of the agglutination technic.

TABLE II. Agglutinin Titers in Chicks Receiving Intraperitoneal Injections of Adult Homologous Hen Spleen Cells Mixed with *B. abortus* Antigen *In Vitro*.

No. of cells $\times 10^8$	Reciprocal of titer	No. of cells $\times 10^8$	Reciprocal of titer
1.1	320	13.0	80
2.3	1280	13.0	2560
3.8	"	14.0	640
4.7	320	15.0	20
7.0	1280	15.0	320
8.0	<10	24.0	640
8.0	"	24.0	"
8.0	80	"	10
8.0	320	"	320
10.0	160	"	"
11.0	2560	"	640
12.0	"		

* Cells were not counted.

Antibody response in chicks receiving graded dilutions of cells and antigen. In an effort to define the limiting number of adult spleen cells necessary for transferring the antibody producing capacity in this system, decreasing 10-fold dilutions of cells were injected with antigen into neonatal chickens. From these experiments (Table III) it is apparent that higher titers can be obtained routinely by the intraperitoneal route only if more than 10^8 viable cells are introduced. Pathologic changes comparable to those described above were observed only in those chicks receiving 10^8-10^9 cells.

TABLE III. Agglutinin Titers in Chicks Receiving Intraperitoneal Injections of 10-Fold Dilutions of Adult Homologous Hen Spleen Cells Mixed with *B. abortus* Antigen.*

No. of adult spleen cells transferred	Reciprocal of agglutination titers†
10^9	40, 80, <10, D‡, D, <10, 1280, <10, <10, 160
10^8	<10, 320, 20, 160, <10, D, 320, <10, D, D
10^7	All <10
10^6	*Idem*
10^5	"

* Antigen was adjusted to maintain a ratio of 2 bacterial cells to one spleen cell.

† Titers represent individual chicks.

‡ D refers to chicks dying before 6th day after cell transfer when all remaining chicks were sacrificed.

Agglutinin titers of chicks receiving a constant number of in vitro stimulated adult spleen cells from the same donor. The variations in individual response noted in previous experiments utilizing different concentrations

TABLE IV. Variability of Response by Recipient Chicks to a Constant Dose of 10^9 Cells.*

Hen donor	Reciprocal of titer†
A	640, <10, <10, D, D‡
B	1280, 2560, D
C	<10, <10
D	<10, 320, 320, 640, D, <10
E	160, <10, D

* Each recipient received 10^9 cells from a single donor's spleen. Cells were stimulated *in vitro* with 2 bacterial cells/viable spleen cell.

† Titers refer to individual chicks.

‡ D refers to chicks dying before 6th day after cell transfer.

of transferred cells, prompted a study of the response by different chicks to a constant dose of cells and antigen from a single donor spleen. Variation among the individual recipients was found (Table IV). As in other experiments, the gross pathologic changes correlated with the antibody response. Absence of splenomegaly and associated alterations were consistently associated with little or no formation of antibody.

Discussion. In the experiments described above, transfer of spleen cells from normal, non-stimulated adult donor hens, mixed with antigen *in vitro,* resulted in antibody production by neonatal recipient chicks. Our results confirm the observations of Trnka and Riha(2,3) and attest further to the possibilities of using this experimental model to gain further information about the cellular mechanisms of antibody production, and associated aspects of transplantation immunity. In addition, antibody formation against added *Brucella abortus* antigen was noted only in recipient chicks which developed a characteristic pathologic picture, consisting primarily of splenomegaly. That this pathologic picture developed also in chicks receiving cells only indicates that antibody formation in the recipient chick may be correlated with a successful "take" by the transferred cells rather than by stimulation of the neonatal immunologic mechanisms or non-specific conditioning of the neonatal environment. When resistance in the recipient can be overcome by the transferred cells, the "graft versus host" reaction described by Simonsen(6,7) may develop. If this occurs, the transferred cells may react to both the antigenic stimulus provided by the host and any added antigens. Conversely, absence of antibody formation, splenomegaly, and associated changes may indicate either that successful transfer may not have been accomplished through simple mechanical failure or that the neonatal host may have overcome the immunological activity of the transferred cells by some form of resistance or lack of supportive nutritional factors. Experiments designed to clarify the function of transferred cells in formation of antibody by the neonatal recipients are in progress. Preliminary attempts to transfer the capacity to produce antibodies in response to added antigens through successive generations of chicks have been made. Results of these experiments, which are similar to Simonsen's transfer of the spleen enlarging factor(6), appear to support the observations outlined above and indicate that the pathologic alterations associated with antibody production are transmissible (unpublished).

In these studies no attempt has been made to compare quantitatively the response of newly hatched chicks and that of adult animals. Consequently, these data do not bear on the relative adequacy of the environment provided for transferred cells by the neonate and adult(8). However in these studies substantial antibody production has been achieved in newly hatched chicks. We feel that further study of this model may be useful in elucidating the mechanisms of antibody synthesis as well as the nature of the immunological deficit in immature animals.

Summary. Antibody production to *Brucella abortus* antigen was initiated in neonatal chicks by transfer of adult homologous hen spleen cells mixed with antigen *in vitro.* The studies indicate a wide variation in response and that a pathologic picture consisting primarily of splenomegaly is regularly associated with good agglutinin titers in the recipient.

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