dimensions of the spacing between them.

Figures 1 and 2 illustrate a fortuitous observation made in the same section of material. Figure 1 is the more commonly observed arrangement of mitochondria, except that the mitochondria are unusually long and curved in at least part of the organelle. Figure 2 shows a most unusual concentric inlay of doughnut-shaped mitochondria. This apparent aberrant condition receives explanation if it is compared to the configuration in Fig. 1. The flattened mitochondria have assumed the shape of a series of nested cups. In Fig. 1 the "cups" have been sectioned from top to bottom, and in Fig. 2 they have been sectioned across their diameter.

The angle of section in relation to a particular set of membranes can be gaged by the sharpness of the profile (right angle) or the blurriness (less than right angle). With this in mind, it is obvious that the mitochondria have a relatively constant thickness in the dimension from one canalicular surface to the other. However, it is difficult to judge the longer dimension or diameter of any one pancake-shaped mitochondrion. There is probably considerable variation.

As in the case of the mosquito larvae (*1*) the spacing between the mitochondrial membranes and the canalicular membranes is relatively constant, as is also the dimension of the slot-like canalicular space. The rather unusual pancake-like dimensions of the mitochondria, together with the close tolerances of the membrane spacings, gives support to the idea that the structures constitute a cellular organelle that may participate in the metabolic transport of salt.

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Restoration of Gamma Globulin Production in Agammaglobulinemic Chickens

Abstract. *Chickens irradiated and bursectomized in the newly hatched period consistently develop agammaglobulinemia and form no circulating antibodies; if the birds are treated immediately after operations by intra-abdominal injection of unirradiated autologous bursa cells, immunoglobulin production, lymphoid germinal centers, and plasma cells are restored; however, the birds fail to produce antibody to specific antigenic challenge.*

Glick demonstrated that surgical removal of the bursa of Fabricius of young chickens prevented development of normal antibody-producing capability (*1*), a deficit later produced by treatment of the embryo with testosterone and other progestational hormones (*2, 3*). These immunologically deficient chickens generally have germinal lymphoid centers and plasma cells in the splenic and intestinal lymphoid tissues, but have reduced amounts of circulating immunoglobulins (*3–6*).

Experiments in this laboratory have led us to a definition of the immunoglobulin-production system as a system of cells which apparently originates from and is dependent on the bursa of Fabricius (*7*), and includes as well germinal center cells and plasma cells. Bursectomy and nearly lethal total-body irradiation at hatching prevents development of germinal centers and plasma cells and results in agammaglobulinemia and complete failure of antibody production. This group of morphologic and functional characteristics resembles the sex-linked recessive agammaglobulinemia described by Bruton (*8*).

Attempts to provide bursectomized-irradiated newly hatched chickens with the missing cell system involved administration of their own bursal cells in the experiments reported here. Germinal centers, plasma cells, and γ-globulin production were restored; but, unexpectedly, significant amounts of agglutinating antibodies to both bovine serum albumin (BSA) and whole *Brucella* organisms were not produced.

Newly hatched white Leghorn chickens were randomly divided into three experimental groups. Bursae were extirpated immediately from two groups. These groups and an unoperated control group were irradiated with 740 roentgens in air. Bursal-cell suspensions were prepared by mincing the extirpated bursa in tissue culture medium 199 containing penicillin (100 unit/ml) and streptomycin (100 μg/ml) and then further disrupting the cells in a loose-fitting glass homogenizer. The cell suspensions were allowed to settle in an upright hemagglutination tube (10 minutes in an ice bath). The supernatant containing mainly lymphoid cells (Fig. 1) was removed, concentrated by centrifugation (4°C) for 10 minutes at 1000 rev/min, and the cells were resuspended in 0.5 ml of the original media prior to intra-abdominal injection into chickens of the bursectomized-irradiated group.

Animals surviving at day 40 were injected intra-abdominally with 20 mg of crystallized BSA (Armour) in saline and 10^9 killed *Brucella abortus* organisms (U.S. Dept. of Agriculture). Nine days later the birds were bled and killed. The spleen and the cecal

Table 1. Presence of germinal centers and plasma cells in the spleen or cecum and circulating 19*S* and 7*S* γ-globulins in experimental chickens. (Results are expressed as the ratio of responses to total number.)

Germinal centers	Plasma cells	γ-Globulins	
		19*S*	7*S*
Control-irradiated			
6/6	6/6	6/6	6/6
Bursectomized-irradiated			
0/6	0/6	0/6	0/6
Bursectomized-irradiated injected with bursal cell suspension			
7/7	7/7	7/7	7/7

Table 2. Primary antibody responses to *Brucella abortus* and bovine serum albumin in experimental chickens.

B. abortus		BSA	
Response/ total	Mean titer ($\log_2$)	Response/ total	Mean titer ($\log_2$)
Control-irradiated			
6/6	7.2	6/6	6.2
Bursectomized-irradiated			
0/6	0	0/6	0
Bursectomized-irradiated, injected with bursal cell suspension			
0/7	0	0/7	0

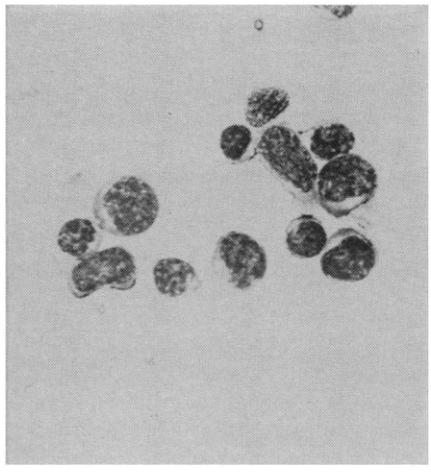

Fig. 1. Representative lymphoid cells of a suspension prepared from the bursa of Fabricius of a newly hatched chicken. Wright-Giemsa stain.

tonsils were fixed in alcohol and Formalin and stained with methyl green–pyronin and with hematoxylin and eosin, respectively. Antibody to BSA was assayed by a tube hemagglutination technique with BSA bisdiazotized with benzidine as a linkage to rabbit erythrocytes (*9*). *Brucella* antibody was measured by a standard-tube bacterial agglutination. The micro method of Scheidegger was used for immunoelectrophoresis of serums (*10*). The rabbit antiserum to chicken γ-globulin was prepared by absorption of rabbit antiserum to whole chicken serum (unfractionated) with lyophilized serum from agammaglobulinemic chickens.

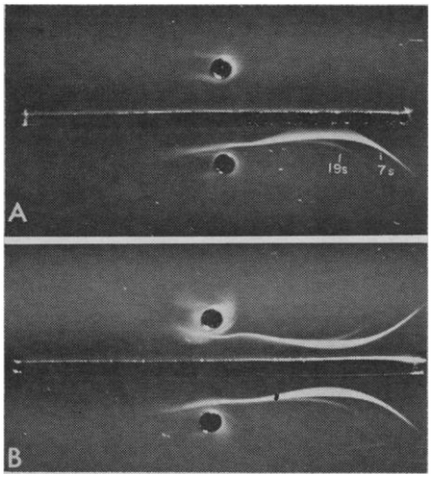

Fig. 2. Immunoelectrophoresis pattern of (*A*) the agammaglobulinemic serum of a typical bursectomized-irradiated chicken at 7 weeks of age, and (*B*) the normal pattern of γ-globulin in a bursectomized-irradiated chicken of the same age, injected with autologous bursal cells immediately after bursectomy and irradiation. In both instances the serum from the experimental bird is in the top well, serum from a control irradiated chicken in the bottom well, and rabbit antiserum to chicken γ-globulin in the trough.

Sections of spleen and cecal tonsils regularly revealed numerous plasma cells and frequent germinal centers in "reconstituted" birds. Germinal centers and plasma cells were completely absent in untreated bursectomized-irradiated birds (Table 1). Although accurate quantitative measurement of these morphologic structures is difficult, both seemed to be present in normal or near-normal numbers in the "reconstituted" birds. No differences could be detected between the lymphoid tissues of experimental birds and those irradiated only in the newly hatched period and stimulated with the same antigens at 40 days of age.

Immunoelectrophoresis revealed both 19*S* and 7*S* γ-globulin in the circulation of each of the "reconstituted" birds that had received the autologous cells. The bursectomized-irradiated chickens showed no detectable formation of either γ-globulin (Table 1). Figure 2 illustrates immunoelectrophoretic patterns of serums from birds of each experimental group.

Thus, administration of autologous bursal cells reconstituted the peripheral cellular apparatus of the immunoglobulin production system in agammaglobulinemic chickens. This reconstituted system of cells produced both heavy (19*S*) and light (7*S*) γ-globulins. However, as a test for antibody-production capacity challenge with BSA, a soluble and relatively "weak" antigen, and *Brucella* cells, a "strong" particulate bacterial antigen, at the 40th day of life resulted in no detected antibody production (Table 2).

Irradiation and bursectomy combined were necessary to prevent germinal center and plasma cell development because in chickens surgically or hormonally bursectomized, as well as in those chickens receiving irradiation alone or with thymectomy (*3, 4, 5, 7*), both germinal centers and plasma cells developed. These observations, and the similarity of the bursal and germinal center follicular structure and their constituent cell morphology, suggested that germinal centers and plasma cells might represent a direct cell lineage stemming from bursal lymphoid cells. The successful restoration in bursectomized-irradiated chickens of germinal centers and plasma cells by the intra-abdominal injection of their own unirradiated bursal lymphoid cells provides strong circumstantial support for this concept.

The augmentation of antibody-responding capacity in bursectomized chickens by bursal saline extracts (*11*) or transplantation of bursa tissue within cell-impenetrable diffusion chambers (*12*) suggests that a soluble factor is necessary for functional maturation of the immunoglobulin-production system. The simplest hypothesis that explains the persisting defect in "reconstituted" chickens is that adequate soluble factor was not available. This could be a consequence of loss of the hormone-producing cells in preparation of the suspension or the need for an intact bursal gland for hormone production.

The lack of normal response to antigenic stimulation and the presence of immunoglobulins in serum again raises the possibility that some proteins of the immunoglobulin class (size, mobility, and antigenicity as criteria) may not be antibodies (*5, 6, 13*). As an alternate explanation, the chickens may have been provided with clones of antibody-producing cells, representing only a fraction of the clones available to the intact bird. Antibodies produced to only a selected number of antigens would then account for the immunoglobulins observed. The irradiated-bursectomized chickens treated with bursal cells bring to mind the clinical syndrome proposed by Barandun (*14*) in which patients with antibody deficiency syndrome were thought to have normal amounts of immunoglobulin in their serums.

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Autoimmune Encephalomyelitis and Ocular Lesions in Monkeys Sensitized during the Neonatal Period

Abstract. *The neonatal rhesus monkey is susceptible to the induction of autoimmune encephalomyelitis. The disease has been produced regularly by injection of neonatal animals with guinea pig spinal cord antigen in complete Freund's adjuvant. The onset of the disease, as compared with onset in adults, is delayed and is most often heralded by intrinsic eye lesions, notably widespread retinal hemorrhages.*

Autoimmune encephalomyelitis has been produced with regularity in mice, rats, guinea pigs, rabbits, dogs, cats, chickens, and monkeys (*1*) by injection of homologous or heterologous brain or spinal cord tissue emulsified in Freund's adjuvant containing mycobacteria. Study of this experimental syndrome, notably in the transfer of autoimmune encephalomyelitis by lymph-node cells in rats and guinea pigs (*2*), has proved that the disease is autoimmune in nature. The newborn animal, however, is refractory to this disease, as has been shown in rats and guinea pigs (*3*). This lack of susceptibility or increase of resistance has been ascribed variously to neonatal tolerance, lack of myelin, lack of access to the target organ, or a deficit in the "development" of committed cells at some stage between sensitization and response. Our own recent findings have indicated that, while some newborn guinea pigs are indeed completely or partially refractory to the disease, others are markedly susceptible; susceptibility depends on various genetic factors. For example, sensitization of newborn, inbred guinea pigs (strain 13) with homologous or isologous spinal cord, emulsified in Freund's adjuvant, results in a delayed appearance of a chronic form of autoimmune encephalomyelitis, characterized by severe damage to the spinal cord, chronic wasting, and clinical and pathological findings qualitatively different from those of the acute form in the adult animal (*4*).

In view of the differences among guinea pigs of various ages with respect to the disease, it was considered important to determine whether the newborn primate was susceptible to the induction of this disease, and, if this proved to be the case, to determine what pattern the disease would take.

We used six laboratory-bred monkeys of the species *Macaca mulatta* (rhesus), whose dates of conception and gestational age were known. The babies were delivered vaginally or by caesarean section and were 2 to 16 days of age when inoculated. From birth and throughout the experiment, the monkeys were individually housed, fed milk formula by bottle, and provided with intensive nursing care when required by their clinical status. Detailed and continuous clinical, neurological, and laboratory observations were made throughout the course of their disease.

Guinea pig spinal cord from inbred, immunologically homogeneous, strain 13 animals (*5*) was emulsified with complete Freund's adjuvant and injected intradermally into four sites in the scapular region. Each animal received a single dose of 0.5 ml of emulsion containing 0.25 ml of 50-percent suspension of spinal cord in phenol water, 0.25 ml of Arlacel-Bayol mixture, and 1 mg of killed *Mycobacterium tuberculosis* H37Rv (*2, 6*).

Neurological disorders were observed in all the monkeys, appearing 33 to 74 days after injection (Table 1). These were sometimes preceded or accompanied by conjunctivitis, retinal abnormalities, or both. The neurological signs were extremely variable, but those most commonly seen were visual disturbances with impaired pupillary reflexes, nystagmus, strabismus, ataxia, and severe motor impairment. In three cases the onset of symptoms

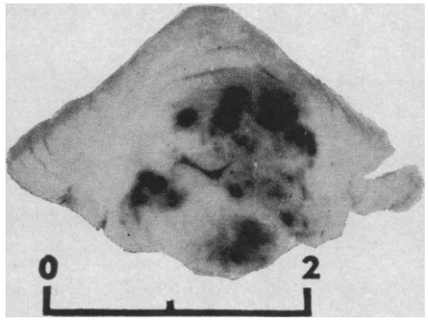

Fig. 1. Cerebellum and pons of an animal that died 1 day after symptoms appeared (58 days after injection). Note multiple large and small rounded areas of hemorrhagic infiltration and the distortion of landmarks. Scale in centimeters.

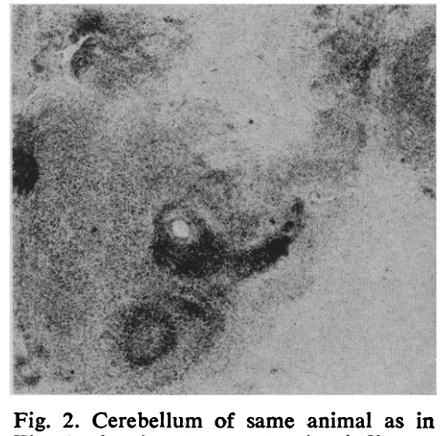

Fig. 2. Cerebellum of same animal as in Fig. 1, showing large, extensive infiltrates consisting of polymorphonuclear neutrophils, hemorrhage, and necrosis. Hematoxylin and eosin (× 23).