Table 1. Percentage increases in weight [means ± S.D. (g)] of ten adult *C. v. variegatus* given abundant food after prolonged fasting, with no food for 36 hours before weighing; S.D. appear in parentheses.

Days since first feeding	Increase (%)
5	47.6 (± 7.4)
10	89.9 (±26.0)

probably approximate only two or three large *Tenebrio* larvae per week, starved individuals readily consumed as many as six or eight in rapid succession; they were able to change their appearance from emaciated to well fed within 5 days of feeding. This change was accompanied by weight increases of about 50 percent (Table 1). During 10 days ten adults increased their weight by more than 80 percent. (No food was given for 36 hours prior to weighing so as to allow for digestion and defecation.) Fat was deposited throughout the body as well as in the tail. The volume of the tail increased on average 125 percent during the first 5 days. When after 11 days one female gecko had increased its weight from 4.27 to 7.89 g, it shed the complete tail in a fight; the tail weighed 1.15 g. Thus it is evident that in *Coleonyx* only a small part of the fat storage occurs in the tail. According to Klauber (*4*), *C. v. variegatus* is eaten extensively by nocturnal desert snakes, especially *Phyllorhynchus*, which, when not large enough to engulf the full-grown gecko, consumes its tail. Because of this high rate of tail autotomy, storage of sizable reserves of fat in areas other than the tail has survival value.

The loss of weight by ten adult specimens kept for 75 days, without food but with access to water under the temperature conditions described above for the colony, was recorded: the mean loss was 14.2 percent; standard deviation for the sample, 7.2.

On the basis of these data it appears that *C. v. variegatus* is remarkably adaptative to its environment in that it can consume, and convert into reserves, vast quantities of food within periods of a few days. During periods of food deprivation these reserves are used most economically, even at fairly high ambient temperatures. More specifically, specimens can consume enough food in 4 days following deprivation to enable them to fast for a period of 6 to 9 months, if the rate of weight loss recorded during the first 75 days is maintained throughout. Inasmuch as not all arid-area geckos are similarly adaptative, the situation recorded in *C. v. variegatus* must be seen as a special adaptation to its particular environment. [The Australian desert gecko *Lucasium damaeum*, for example, has no fat tail; captives quickly became emaciated when deprived of food for 2 to 3 weeks (*11*).]

It is noteworthy that in gekkonid genera the regrown tail can become much thicker than the original (*12*); such is true of *Coleonyx*.

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23 October 1967

Experimental Allergic Encephalomyelitis in Agammaglobulinemic Chickens

Abstract. *White Leghorn chickens were subjected to bursectomy and total body irradiation at hatching, thus rendering them agammaglobulinemic. At 4 weeks of age, these chickens and control chickens received lyophilized bovine spinal cord emulsified with complete Freund's adjuvant. Agammaglobulinemic chickens developed the lesions of allergic encephalomyelitis with the same frequency and intensity as the controls. Our findings support the contention that antibodies are not necessary for the development of experimental allergic encephalomyelitis.*

Experimental allergic encephalomyelitis (EAE) has been considered a manifestation of the tuberculin-type delayed hypersensitivity to central-nervous-system antigens (*1*). This conclusion is based on the following observations: (i) EAE can be passively transferred with sensitized lymphocytes, but not with specific antibody-containing serums (*2*); (ii) the onset of disease of the central nervous system (CNS) is temporally related to delayed hypersensitivity to CNS antigen injected intradermally; and (iii) the onset of EAE cannot be correlated with the appearance of circulating complement-fixing antibodies (*4*). As with delayed hypersensitivity, the role of humoral antibodies in the pathogenesis of EAE has been a source of controversy and confusion (*5*). Paterson and Harwin (*6*) have shown that complement-fixing antibody to brain antigen may protect animals against the development of EAE. On the other hand, Appel and Bornstein (*7*) demonstrated a complement-dependent factor in the serums of afflicted animals which produces demyelination in cell cultures of CNS tissue. By way of contrast, Janković and Išvaneski (*8*) found that chickens subjected to bursectomy at hatching, deficient in antibody responsiveness, developed EAE with the same frequency and intensity as controls did. In their study, chickens subjected to thymectomy at hatching, with a depressed, delayed reaction to allergy, were more resistant to the induction of EAE. They interpreted these findings as an indication that humoral antibody was not necessary for the production of EAE and that the pathological process was dependent on the developmental integrity of the thymus-dependent lymphoid system, that is, the small lymphocyte. Recently, these conclusions have been questioned because birds subjected to bursectomy in the newly hatched period can produce antibodies of the 19*S* class quite well, particularly if repeatedly immunized (*9*).

Cooper *et al.* (*10*) have since shown that newly hatched chicks subjected to both bursectomy and total body irradiation become agammaglobulinemic and do not produce antibodies even to repeated injections of antigen. Although these animals are agammaglobulinemic, they retain their capacity to manifest delayed skin sensitivity and to reject homografts of skin. We used this

experimental model to examine again the role of antibodies in the pathogenesis of EAE.

Newly hatched White Leghorn chicks were randomly sorted into four groups. The bursa was removed from one group, within 12 hours of hatching, and the thymus was removed from another group within the same time interval. These birds and an intact group received a total body x-irradiation of 650 roentgens (r) on the following day. A fourth group received neither operation nor x-rays. At 4 weeks of age, each surviving chick received 0.1 ml of an emulsion containing lyophilized bovine spinal cord (10 mg) in complete Freund's adjuvant (1 mg of *Mycobacterium tuberculosis* strain H37RV) by footpad injections.

Chicks were examined daily for clinical signs of neurological disease as judged by gait and righting reflexes. Because survival time for both the bursectomized and irradiated and the thymectomized and irradiated birds is markedly curtailed, it was considered necessary to terminate the period of clinical observation for all groups 3 weeks after antigen injection. Birds surviving 10 days or more after injection were included in the study. Those that survived for more than 10 days, but that appeared to be near death prior to 20 days after injection, were killed and included in the study. Two thymectomized and irradiated chicks were killed at 14 days, and one control and unirradiated chick was killed at 19 days.

Using goat antiserum specific for chicken immunoglobulins M and G (IgM and IgG), we analyzed serums from all experimental birds by immunoelectrophoresis. Of 53 chicks that were bursectomized and irradiated, 12 survived long enough for challenging with antigen. Of these, two birds died during the latent period of 10 days; their nervous systems showed no evidence of EAE. Ten survived the entire test period; of these, two had both IgM and IgG in their serums and consequently were excluded from the experimental group. Of the eight remaining bursectomized and irradiated chicks, immunoelectrophoresis of the serum showed that four were completely agammaglobulinemic. Serums from the other four chicks contained trace amounts of IgG but lacked detectable IgM. We did not find germinal centers or mature plasma cells in spleen sections (stained with methyl green pyronine) obtained from these eight birds. The trace amounts of IgG in four of the eight were therefore considered to be of maternal origin, and all eight were included in the experimental group.

At autopsy, the CNS was removed, and multiple sections were made of hemispheres, brainstem, cerebellum, and spinal cord. Histological sections were stained with hematoxylin-eosin, methyl green pyronine, and luxol fast blue. Grading of sections was based on the extent of the perivascular inflammatory reaction (Fig. 1). In all areas, grading of the intensity of the inflammatory change, done without knowledge of the experimental grouping of the birds, was based on the following system: 0, no evidence of inflammatory response; 1+, isolated vascular lesions; 2+, several isolated lesions; 3+, generalized vascular involvement; 4+, intensive generalized involvement. The sum of the values assessed from hemispheres, brainstem, cerebellum, and spinal cord was divided by four to obtain the average intensity of inflammatory involvement for each animal, and from these values the average for the group was derived.

Each of the bursectomized and irradiated chicks showed clinical evidence of disease through manifestation of ataxia and disturbances of righting reflexes (Table 1). Identical signs of CNS dysfunction were found in all animals in the control irradiated and unirradiated groups. However, in the thymectomized and irradiated chicks only four of nine showed neurological deficits. Histological findings were consistent with the clinical evaluation in that all of the agammaglobulinemic birds showed evidence of perivascular inflammatory change. Whereas all of the control irradiated and unirradiated chicks demonstrated histological changes equivalent to the bursectomized birds, we found perivascular lesions in only five of nine thymectomized and irradiated birds.

Table 1. Clinical and histological evaluation of EAE in experimental and control groups.

Clinical incidence	Histology		
	Incidence	Intensity	Plasma cells
	Control unirradiated		
10/10	10/10	1.82	9/10
	Control irradiated		
10/10	10/10	1.75	8/10
	Thymectomized irradiated		
4/9	5/9	1.17	3/5
	Bursectomized irradiated		
8/8	8/8	1.94	0/8

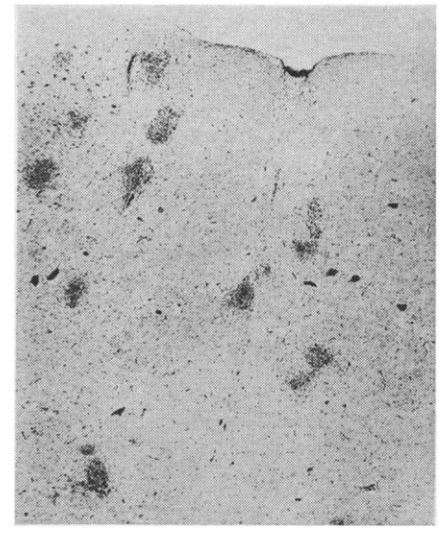

Fig. 1. Brain stem section of a control unirradiated chicken showing the characteristic perivascular inflammatory lesions of EAE. Cellular infiltration is predominantly mononuclear, including lymphocytes, histocytes, plasma cells, and macrophages. This section was graded 3+.

Plasma cells could be identified in the perivascular inflammatory lesions in the control groups (Fig. 2). However, these cells could not be identified in the lesions found in the agammaglobulinemic birds (Fig. 3).

Newly hatched chicks subjected to bursectomy and irradiation and thus rendered agammaglobulinemic are capable of developing the clinical and histological features of EAE. The lesions observed in this group were identical to those found in the control groups except for the absence of plasma cells. These findings support the thesis that humoral antibody is not necessary for

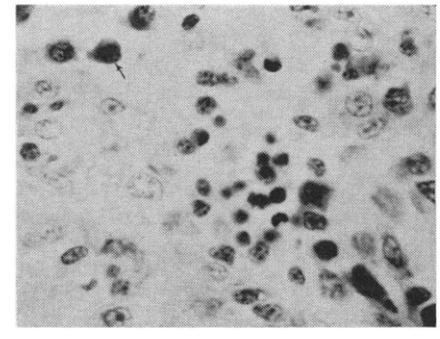

Fig. 2. High-powered photomicrograph of a perivascular lesion in the hemispheric white matter of a control irradiated chicken. Arrow, plasma cell.

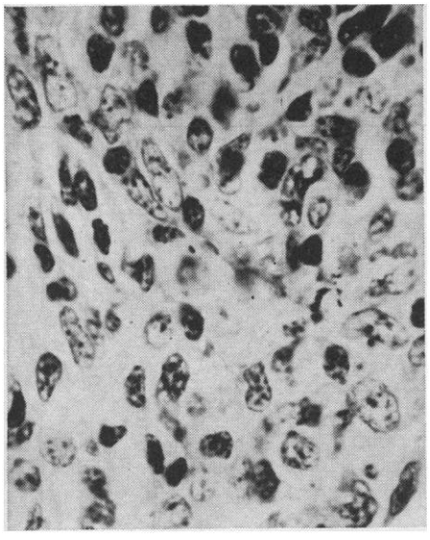

Fig. 3. High-powered photomicrograph of the mononuclear infiltrate about a small blood vessel in the cerebellum of an agammaglobulinemic chicken; this is similar to lesions in control animals except for the absence of plasma cells.

the development of EAE. In that the intensity of the disease was equivalent in the bursectomized and irradiated animals to that in both the control irradiated and unirradiated chicks, it could not be demonstrated that circulating antibody was protective. Complement-fixing antibodies to brain antigens were not measured. Our study confirms the findings of Janković that thymectomy in the newly hatched chick inhibits the ability of the animal to manifest EAE, and it adds support to the contention that EAE is a manifestation of delayed hypersensitivity.

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Immunological Time Scale for Hominid Evolution

Abstract. *Several workers have observed that there is an extremely close immunological resemblance between the serum albumins of apes and man. Our studies with the quantitative microcomplement fixation method confirm this observation. To explain the closeness of the resemblance, previous workers suggested that there has been a slowing down of albumin evolution since the time of divergence of apes and man. Recent evidence, however, indicates that the albumin molecule has evolved at a steady rate. Hence, we suggest that apes and man have a more recent common ancestry than is usually supposed. Our calculations lead to the suggestion that, if man and Old World monkeys last shared a common ancestor 30 million years ago, then man and African apes shared a common ancestor 5 million years ago, that is, in the Pliocene era.*

It is generally agreed that the African apes are our closest living relatives. However, the time of origin of a distinct hominid lineage has been a subject of controversy for over 100 years (*1*). The absence of an adequate fossil record has forced students of hominid evolution to evaluate the phylogenetic significance of anatomical and behavioral characteristics in the living primate species in order to attempt a solution to that controversy. The nature of the problem is such, however, that no definitive answer has yet been given. Current estimates range from a date in the late Pliocene (*2*) to one in the late Oligocene or early Miocene (*3*) for the origin of the hominids. This great range (4 million to 30 million years) effectively negates any meaningful discussion of the nature of our pre-Australopithecine ancestors, for the early dates bring us near to a primitive prosimian stock, while the late ones would suggest that a common ancestor for man and the African apes might well resemble a small chimpanzee.

One solution to this question lies in the measurement of the degree of genetic relationship which exists between man and his closest living relatives. As it has recently become clear that the structure of proteins closely reflects that of genes, it is to be expected that quantitative comparative studies of protein structure should aid in providing this measure of genetic relationship (*4*).

Proteins appear to evolve over time, as do the organisms of which they are a part. Thus, we may speak of the common ancestor of, for example, the human and chimpanzee serum albumin molecules, this ancestral molecule being present in the common ancestor of man and the chimpanzee. From the time that the human and chimpanzee lineages separated, their albumins have had the opportunity of evolving independently until today they are recognizably different, but homologously related, molecules. Such homologies may be studied by immunological techniques, the magnitude of the immunological cross-reaction serving as a measure of the degree of structural similarity between the two kinds of albumin (*5*).

The immunological methods used in this investigation were similar to those described earlier (*5*, *6*). Serum samples were obtained from all the living genera of apes and from six representative genera of Old World monkeys and stored at −10°C (*7*). Albumin was purified from individual chimpanzee, gibbon, and human serums by the method of Hoch and Chanutin (*8*). Groups of three or four rabbits were immunized by three courses of injections with each of the purified albumins. The antiserums were tested for purity by immunodiffusion, immunoelectrophoresis, and microcomplement fixation (MC'F) with whole serum and purified albumin. Antibodies to components of serum other than albumin were always detectable with the first two methods, but they were too low in concentration to interfere with the MC'F analysis of the cross-reactions discussed below (*9*). Pooled antiserums were made by mixing the individual antiserums in reciprocal proportion to their MC'F titers (*10*). The degrees of cross-reaction were expressed quantitatively as the index of dissimilarity or immunological distance (ID) that is, the relative concentration of antiserum required to produce a complement fixation curve whose peak was as high as that given by the homologous albumin.

These antiserums were used to obtain the data summarized in Table 1. Some of these results have already been published (*5*, *6*). With the antiserum pool prepared against human serum albumin, the albumins of the African apes (gorilla and chimpanzee) reacted more strongly than those of the Asiatic apes (orang, siamang, and gibbon). The antiserum pool directed against chimpanzee (*Pan troglodytes*)