youngest part of the sequence. A comparison of the entire sequence with sections of borings from the neighboring continent suggests a stratigraphic range from the Jurassic to the Lower Cretaceous. These results, which indicate that the eastern Canaries are underlain by continental crust, are supported by geophysical evidence (*8*).

Rifting parallel to the present continental margin is suggested by tremendous basaltic dikes that cut the Mesozoic sedimentary rocks of Fuerteventura in a north-northeastern direction. The relation between the sedimentary rocks and the dikes indicates that rifting could have started in the Upper Cretaceous. It cannot be stated with absolute certainty that rifting was the mechanism of the separation of the eastern Canaries from the continent. The flightless ratites could have gained access to the Lanzarote area across volcanic land bridges, which would have been destroyed later. As the remains of different kinds of ratites were found on Lanzarote, one can envision successive waves of immigration, most likely with a considerable time gap in between. Thus, the possibility of the birds' access across volcanic land bridges is only slight.

Dietz and Sproll (*9*) add further support to the idea of a continental origin of the eastern Canaries. They suggest that this part of the Canary Islands formed a microcontinent or sialic continental fragment that became detached from the African margin of what is named the "Ifni Gap" in the Africa–North America drift fit. If the opening of this gap happened 200×10^6 years ago, the eastern Canaries block must not have become completely separated from Africa until millions of years later. It could have occurred at a time when the struthious and aepyornithoid birds had established themselves in the Lanzarote region of West Africa.

Dietz and Sproll (*9*) mention a mid-Triassic detachment of the eastern Canaries, but they do not force the issue. They rather suggest that this event might have taken place in the early Cenozoic, associated with the orogeny of the Alps and the creation of the Atlas foldbelt.

Biological and geological evidence brings new validity to the controversial concept of bird distribution and continental drift. It makes it plausible that the isolation of the struthious and aepyornithoid ratites of Lanzarote was the direct result not of the original rifting process between the North American and African continents but of the eventual detachment of the eastern Canaries from the African mainland.

Whether the distribution patterns of birds can be attributed to continental drift has long been a subject of discussion, with seemingly unsatisfactory results. The new and substantial evidence projects the separation of the eastern Canaries from Africa into a period of abundant bird life. One might think that Wolfson's (*10*) ideas on bird migration and its correlation with continental drift can not be rejected on the grounds of a temporal discrepancy between drift and the origin or modification of migration.

The question arises whether the Malagasy elephant birds (Aepyornithiformes) might have experienced an evolutionary development comparable to the one that separated the Lanzarote ratites from their ancestral stock on the African mainland. It is very likely that the aepyornithoids from Lanzarote will eventually become identified as ancestral to the Malagasy ratites, and that both bear a direct relationship to the Asiatic species of aepyornithoid character whose eggshell remains have been found in Punjab and Inner Mongolia (*2*).

E. G. FRANZ SAUER
Zoologisches Forschungsinstitut, and
Museum Alexander Koenig, and
Universität Bonn, 53 Bonn 1, Germany
PETER ROTHE
Laboratorium für Sedimentforschung,
Universität Heidelberg,
69 Heidelberg 1, Germany

References

1. P. Rothe, *Natur. Mus.* **94**, 175 (1964).
2. E. G. F. Sauer, in preparation; *Bonn. Zool. Beitr.* **23**, 1 (1972).
3. ———, *Cimbebasia* **A1**, 27 (1968).
4. A. Abdel-Monem, N. D. Watkins, P. W. Gast, in *Abstr. Int. Symp. Volcanol.*, Canary Islands (1968), p. 1.
5. P. Rothe, *Commentat. Phys.-Math.* **31** (No. 13) (1966).
6. ———, *Neues Jahrb. Mineral. Monatsh.* **1967**, 71 (1967).
7. ———, *Geol. Rundsch.* **58**, 314 (1968).
8. B. P. Dash and E. Bosshard, *Proc. Int. Geol. Congr., 23rd*, Prague (1968), sect. 1, p. 249.
9. R. S. Dietz and W. P. Sproll, *Nature* **226**, 1043 (1970).
10. A. Wolfson, *Science* **108**, 23 (1948).

26 October 1971; revised 15 December 1971 ■

Production of Specific Antibody by Lymphocytes of the Bursa of Fabricius

Abstract. *Lymphocytes in the bursa of chickens have been found to produce hemolytic antibodies to sheep erythrocytes that are introduced into the cloaca. These lymphocytes also react with* Escherichia coli *and form bacterial adherent colonies, but not with gamma streptococci to which they have not been previously exposed. Thymic and splenic lymphocytes do not bind either organism.*

Removal of the bursa of Fabricius from chickens, at or before the time of hatching, interferes with subsequent development of immunological competence, as is shown by decreases in concentration of several classes of serum immunoglobulins and of specific antibody (*1*). The major function ascribed to the bursa, therefore, is that of seeding immunocompetent cells to peripheral sites where they become antibody-producing cells (*2*).

The presence of immunoglobulins G and M has been demonstrated, by immunofluorescent methods, in bursal follicles of mature chickens (*3*); μ and light chains have been found in bursae from 14-day embryos (*4*). In culture in vitro, bursal lymphocytes from 18-day-old embryos synthesize small amounts of IgM (*5*). There is, at best, inconclusive evidence of specific antibody production in or by bursal cells following systemic antigenic challenge (*6*). Neither rosette nor hemolytic plaque formation by bursal cells has been obtained in vitro after parenteral injection of sheep red blood cells (sheep RBC) (*7*). It was also not possible to detect specific antibodies to bovine serum albumin in the bursa after immunization (*8*).

It is enigmatic that lymphoid cells in the bursa actively produce immunoglobulins but not specific antibody, at least not against antigens introduced by the usual methods of immunization. We present evidence here that the bursa produces specific antibodies to sheep RBC's introduced into the cloaca and bursal duct. The bursa is also shown to be a site for antibody production to bacterial antigens that reside in the cloaca.

Table 1. Hemolysin response by bursal and splenic lymphocytes of 4- to 6-week-old chickens after injection of 10^9 sheep red blood cells. The numbers in parentheses indicate the number of organs with plaque-forming cells over the number tested; the number of plaque-forming cells are shown as means ± standard error of the mean per 10^6 leukocytes.

Injection	Time after injection (days)	Plaque counts per 10^6 leukocytes			
		Bursa		Spleen	
Uninjected	—	15	(1/7)	150.7 ± 60	(5/7)
Intramuscular	4	<15	(1/6)	1175.5 ± 158.3	(6/6)
Bursal lumen	4	158.7 ± 26	(8/10)	< 150	(3/10)
Bursal lumen	5	426.3 ± 119.9	(11/12)	167.2 ± 23.0	(3/12)
Bursal follicles	4	<15	(3/16)	< 150	(10/15)

To determine whether bursal lymphocytes could be made to produce antibody to a specific antigen, 4- to 6-week-old chickens were immunized with 10^9 washed sheep RBC's, either by injection into the cloaca and bursal duct, or by direct injection into the bursa. The number of cells producing hemolyzing antibody was determined at days 2, 3, 4, and 5 by use of a monolayer of cells in the absence of agar (*9*). Bursal cells (0.5 ml), containing between 2.5×10^6 and 1×10^7 lymphoid cells, were mixed with 0.5 ml of a 15 percent suspension of sheep RBC's and 0.03 ml of chicken serum. This mixture was pipetted into a hemocytometer and was incubated in a moist chamber at 37°C for 90 minutes; the number of clear areas surrounding a lymphocyte was then determined. In all cases, serum from a single chicken was used as a source of complement and the sheep RBC's were aged in Alsever solution for at least 1 week.

To determine whether bursal cells were reacting with bacteria resident in the cloaca, we used the method described by Diener (*10*). Single-cell suspensions from the bursa, spleen, and thymus were prepared by gentle disruption and sedimentation, were washed three times in medium 199, and were incubated at a concentration of 5×10^6 cells in 1 ml, at 4°C for 30 minutes, in medium containing 85×10^5 *E. coli* or γ streptococci per milliliter. (The *E. coli* were obtained from the cloaca of experimental chickens, and were used as pure cultures. The γ streptococci had been isolated from a hospital patient and were maintained for teaching purposes.) After incubation, the lymphocytes were plated on nutrient agar (0.5 percent), and were incubated at 37°C for 3 hours; colonies derived from the coated lymphoid cells were then counted after flooding the plates with 0.5 percent methylene blue and washing off the excess stain with 70 percent alcohol. In all experiments, one petri dish of nutrient agar containing 85×10^5 bacteria only was included as a growth control.

In contrast to previous studies, the results presented in Table 1 show that a substantial number of lymphocytes of the bursa are capable of secreting specific hemolytic antibodies to sheep RBC's on days 4 and 5 after erythrocytes were introduced into the cloaca of 4- to 6-week-old chickens. In contrast, if the sheep RBC's were injected directly into the bursal tissue, then the numbers of plaque-forming cells (PFC) did not exceed that in uninjected chickens. Further, in Table 1 it can be seen that when sheep RBC's were injected into the bursa by either method, the number of splenic lymphocytes synthesizing hemolyzins did not increase. When the antigen was injected intramuscularly, bursal lymphocytes failed to produce antibody although splenic lymphocytes did. These experiments demonstrate that lymphocytes of the bursa, but not of the spleen, are capable of producing specific antibody to sheep RBC's when this antigen is introduced into the cloaca.

Table 2 is a summary of results of experiments that indicate that bursal lymphocytes of chickens from 4 days through 6 months of age are capable of producing bacterial adherent colonies (BAC) with *E. coli* isolated from the chicken cloaca. In 18-day embryos and newly hatched chicks, BAC were not produced by bursal lymphocytes. At these ages the cloaca was sterile, and it was not until 2 days after hatching that *E. coli* from chicks could be cultured. When the chickens were 8 to 9 days and 5 weeks of age, most of their bursae tested were positive for BAC, but the splenic lymphocytes of these chickens were not. In other experiments, it was not possible to demonstrate BAC with thymic lymphocytes in five 4- to 6-week-old chickens, although their bursal lymphocytes were positive for BAC. No colonies were observed if lymphocytes were not first incubated with bacteria.

To assess the specificity of the BAC reaction, bursal and splenic lymphocytes were incubated with either γ streptococci that were not isolated from the chicken or with *E. coli* that were isolated from the cloaca. Bursal lymphocytes never produced BAC after incubation with the γ streptococci, but they consistently produced BAC with *E. coli* (Table 2). In only one of the 6- to 7-week-old chickens did the spleen contain lymphocytes which reacted with either *E. coli* or γ streptococci. These results confirm the belief that the BAC formed by bursal lymphoid cells with *E. coli* are specific for this organism.

These experiments provide evidence that after the chickens hatch, their bursal lymphocytes have the ability to initiate an immunological reaction. When sheep RBC's were introduced into the cloaca and bursal duct, a significant number of bursae produced PFC, but no such increase in PFC formation occurred after injection of antigen intramuscularly or into the bursal follicle. Bursal cells also reacted specifically by forming BAC to microorganisms

Table 2. Numbers of chicken lymphocytes in the bursa and spleen that bind *E. coli* and γ streptococci as measured by bacterial adherence colonies (BAC). Numbers in parentheses indicate the number of organs reacting over the number tested; NT, not tested; the number of BAC is expressed as the mean ± standard error of mean per 10^6 leukocytes.

Age	Number of BAC per 10^6 leukocytes			
	E. coli		γ Streptococci	
	Bursa	Spleen	Bursa	Spleen
18-day embryo	0 (0/4)	NT	NT	NT
Newly hatched	0 (0/4)	NT	NT	NT
4 to 5 days	936.5 ± 153.6 (6/6)	NT	NT	NT
8 to 9 days	1059.0 ± 57.1 (8/8)	(0/4)	(0/8)	(0/4)
5 weeks	671.3 ± 97.5 (7/9)	(0/9)	NT	NT
6 to 7 weeks	1609.7 ± 452.1 (6/7)	263* (1/2)	(0/6)	571* (1/7)
4 to 6 months	614.5 ± 372.6 (2/3)	351.5 ± 248.2 (2/3)	NT	NT

* Mean not calculated; data of one reactive organ.

that reside in the gut but not to γ streptococcus that does not reside there. Diener (*10*) has interpreted the specific binding of microorganisms on the surface of lymphoid cells as an indication of production of specific antibody by these cells. Friedman *et al.* (*11*), with *E. coli*, substantitated the fact that such surface binding of bacteria was due to production of specific antibody. We conclude that soon after hatching, bursal lymphocytes are involved in recognition of antigen present in the gut and they carry out active antibody synthesis in response.

We have shown previously that immunoglobulin synthesis takes place primarily in the medulla of bursal follicles after the lymphoid cells have undergone a proliferative cycle in the follicular cortex (*3*). Other studies failed to demonstrate specific antibody formation by the bursa (*7, 8*). These investigators considered the bursa to be exclusively a site where cells can differentiate before emigrating to peripheral sites for active antibody synthesis. This view is too restrictive and must be modified to include our new data. The bursa, in addition to being a site for differentiation of antibody forming cells at a critical period in ontogenesis, begins functioning actively in the first week after hatching in the local defense system of the gut; it produces antibodies against the environmental antigens encountered by this well-developed lymphoid organ. That this is a local defense system was clearly shown by the marked increase in the production of PFC by bursae, and not by spleen, when the sheep RBC's were introduced into the bursal lumen. Further, although bursal cells reacted with *E. coli* and produced BAC, both splenic and thymic lymphocytes failed to react with this organism. We postulate that antigens reach the follicular cells by direct passage from the gut by way of the epithelium overlying the lymphoid follicles. Only when the sheep RBC's were introduced into the lumen did we detect antibody-producing cells. The epithelium associated with the follicle has the capability of pinocytosis and the necessary fine structure for this function (*12*). Therefore, only when antigens gain entrance to the follicle by way of the lumen are they capable of setting in motion a chain of events that culminates in production of specific antibodies. Thus, although unrecognized up to now, one of the major functions of the bursa, shortly after hatching of the chicken and until resorption of the organ, is related to local immunological defense against bacterial organisms present in the gut.

PIERSON J. VAN ALTEN
Department of Anatomy, University of Illinois at The Medical Center, Chicago 60680

HILAIRE J. MEUWISSEN
Kidney Disease and *Birth Defects Institutes, New York State Department of Health, Albany, 12208*

References and Notes

1. T. S. Chang, B. Glick, A. R. Winter, *Poultry Sci.* **34**, 1186 (1955); B. Glick, *ibid.* **37**, 240 (1958).
2. P. J. Van Alten, W. A. Cain, R. A. Good, M. D. Cooper, *Nature* **217**, 358 (1968); M. D. Cooper, W. A. Cain, P. J. Van Alten, R. A. Good, *Int. Arch. Allergy* **35**, 242 (1969); W. A. Cain, M. D. Cooper, P. J. Van Alten, R. A. Good, *J. Immunol.* **102**, 671 (1969); E. J. Motika and P. J. Van Alten, *ibid.* **107**, 512 (1971).
3. H. J. Meuwissen, K. Gajl-Peczalska, G. T. Kaplan, D. Frommel, T. J. Linna, R. A. Good, in preparation.
4. P. W. Kincade and M. D. Cooper, *J. Immunol.* **106**, 371 (1971).
5. G. M. Thorbecke, N. L. Warner, G. H. Hochwald, S. H. Ohanian, *Immunology* **15**, 123 (1968).
6. T. H. Kerstetter, I. O. Buss, H. A. Went, *J. Exp. Zool.* **149**, 233 (1962).
7. P. B. Dent and R. A. Good, *Nature* **207**, 491 (1965).
8. B. Glick and S. Whatley, *Poultry Sci.* **46**, 1587 (1967).
9. A. J. Cunningham and A. Szenberg, *Immunology* **14**, 599 (1968).
10. E. Diener, *J. Immunol.* **100**, 1062 (1968).
11. H. Friedman, J. Allen, M. Landy, *ibid.* **103**, 204 (1969).
12. D. E. Bockman and M. D. Cooper, *Fed. Proc.* **30**, 511 (1971).
13. This work was supported by grant AI-08289 from the National Institutes of Health. We thank Mrs. Zanet F. Gill for technical assistance, Mr. James Robinson for providing the γ streptococci, and Dr. R. A. Good for helpful discussions.

29 July 1971; revised 27 September 1971

Epoxides of Carcinogenic Polycyclic Hydrocarbons Are Frameshift Mutagens

Abstract. *K-region epoxides of the carcinogens benz*[a]*anthracene, dibenz*[a,h]*anthracene, and 7-methylbenz*[a]*anthracene are mutagenic in strains of* Salmonella typhimurium *designed to detect frameshift mutagens. Parent hydrocarbons, K-region diols and phenols and some other epoxides are inactive as mutagens in these tests. Polycyclic hydrocarbon epoxides, and other presumed proximal carcinogens, are discussed as examples of intercalating agents with reactive side chains. It has been shown previously that intercalating agents with reactive side chains are potent frameshift mutagens.*

Polycyclic planar aromatic compounds, such as the acridines, which intercalate in the DNA base pair stack (*1*) are mutagens that cause additions and deletions of bases in a gene (*2–4*). The mechanism is thought to be that the intercalation stabilizes a mispairing in the DNA during replication or repair synthesis, or during recombination, which causes the errors of addition or deletion (*3, 5*). These mutagens are called frameshift mutagens because the reading frame of the messenger RNA (mRNA) is shifted by the addition or deletion of a base, and this effect distinguishes them from the usual mutagens that cause base pair substitutions (*3*). When an intercalating agent also has a side chain attached to it that can react with DNA, it is a more potent frameshaft mutagen by one or two orders of magnitude (*4*). Polycyclic hydrocarbons, which are known to intercalate into DNA (*6*), are converted into epoxides by microsomal enzyme systems (*7*). These epoxide intermediates are chemically and biologically active compounds (*8*) that may well be involved in hydrocarbon carcinogenesis (*9*). We report here that polycyclic hydrocarbon K-region epoxides cause frameshift mutations in bacteria but that the hydrocarbons themselves and the hydroxylated derivatives are inactive. K-region epoxides are also mutagenic to T2 bacteriophage and to mammalian cells in culture, but the types of mutation produced have not been characterized (*10*).

We have used a set of tester strains (*11*) that have been developed in *Salmonella typhimurium* for detecting and classifying mutagens. We score, by a simple back mutation test, the reversion from histidine requirement to growth on minimal medium. Three of the strains (TA1531, TA1532, and TA1534) are designed for detecting frameshift mutagens of varying specificity. Each strain has a frameshift mutation in one of the genes of the histidine operon thus causing the strain to have a histidine requirement. Another strain (TA1530), used to detect mutagens that cause base pair substitutions, has a base pair change in the histidine *G* gene. All four