Our calculations are preliminary and correspond to the same rough approximation of de Voe and Tinoco (*14*) of similar interactions in the nucleic acids. The numerical values depend upon the distance between the stacked components. Moreover, in the association considered, the distance is small with respect to the molecular dimensions of the components, so that the absolute values of the interactions may be quite different from those obtained from dipoles, if polarizabilities are isotropic and the inverse sixth-power law is applied.

Orientation factors and the influence of the medium have not been taken into account. The role of these possible refinements is not yet known. Although these unknowns may influence appreciably the numerical values of the results, it seems rather improbable that they would introduce an important change in the relative order of the sum of the forces, in particular as this order is largely determined by the values of the polarizabilities of the bases. We may therefore state, with a large degree of confidence, that the combination of the polarization and dispersion forces must influence the physical interactions between purines and the polybenzenoid aromatic hydrocarbons. The relative value of this component with respect to the charge transfer component remains to be determined.

We continue to believe (*7, 8*) that the role of this type of interaction between the aromatic hydrocarbons and the purine and pyrimidine bases for the process of chemical carcinogenesis (*7, 8*) can only be small, owing to the complete nonspecificity of this type of interaction (and of the similar physical interactions of the hydrocarbons with the nucleic acids) with respect to the carcinogenic activity of the hydrocarbons. Thus, it can be easily predicted that such interactions should not distinguish between carcinogenic and noncarcinogenic molecules. That such is actually the case can be seen, in the narrow limits of the available data, by the fact that noncarcinogenic hydrocarbons, like pyrene, seem to be as able to form complexes with purines as the related carcinogens 3,4-benzpyrene or 1,2,5,6-dibenzanthracene (*4*).

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References and Notes

1. H. Weil-Malherbe, *Biochem. J.* **40**, 351 (1946).
2. E. Boyland and B. Green, *Brit. J. Cancer* **16**, 347 (1962).
3. P. O. P. Ts'o, I. S. Melvin, A. C. Olson, *J. Am. Chem. Soc.* **85**, 1289 (1963); E. O. Akinrimisi and P. O. P. Ts'o, *Biochemistry* **3**, 619 (1964); P. O. P. Ts'o, in *Nucleohistones*, J. Bonner and P. O. P. Ts'o, Eds. (Holden-Day, San Francisco, 1964), p. 149.
4. E. Boyland and B. Green, *Biochem. J.* **84**, 54P (1962); E. Boyland and B. Green, *Brit. J. Cancer* **16**, 507 (1962); A.-M. Liquori, B. De Lerma, F. Ascoli, C. Botré, M. Frasciatti, *J. Mol. Biol.* **5**, 527 (1962); P. O. P. Ts'o and P. Lu, *Proc. Natl. Acad. Sci. U.S.* **51**, 17 (1964).
5. E. Boyland and B. Green, *J. Mol. Biol.* **9**, 589 (1964); ———, *Biochim. Biophys. Acta* **87**, 653 (1964).
6. B. C. Giovanella, L. E. McKinney, C. Heldelberger, *J. Mol. Biol.* **8**, 20 (1964).
7. B. Pullman and A. Pullman, *Proc. Natl. Acad. Sci. U.S.* **44**, 1197 (1958); B. Pullman, *Biopolymers Symp.* **1**, 141 (1964); ———, *J. Cellular Comp. Physiol.* **64**, Supp. 1, 91 (1964).
8. B. Pullman and A. Pullman, *Quantum Biochemistry* (Wiley, New York, 1964).
9. See B. Pullman and A. Pullman, *Les Théories Electroniques de la Chimie Organique* (Masson, Paris, 1952).
10. K. Denbigh, *Trans. Farady Soc.* **36**, 9136 (1940).
11. H. Berthod and A. Pullman, *Compt. Rend.* **257**, 2738 (1963); *ibid.* **259**, 2711 (1964); *Biopolymers* **2**, 483 (1964).
12. A. Pullman and M. Rossi, *Biochim. Biophys. Acta* **88**, 211 (1964); B. Pullman, *Compt. Rend.* **259**, 3101 (1964).
13. J. B. Birks and M. A. Slifkin, *Nature* **191**, 761 (1961).
14. H. De Voe and I. Tinoco, Jr., *J. Mol. Biol.* **4**, 500 (1962).

16 December 1964

Bursa of Fabricius in Chickens: Possible Humoral Factor

Abstract: *Chicks bursectomized by testosterone injection on the 5th day of incubation showed a marked inability to produce antibodies to* Salmonella typhimurium. *When portions of the bursa of Fabricius were enclosed in cell-impermeable Millipore diffusion chambers and implanted subcutaneously or intraperitoneally, the antibody-producing capacity of these animals was restored. Evidence strongly suggests that the bursa of Fabricius elaborates a noncellular agent capable of restoring immunologic reactivity in bursectomized chicks.*

The bursa of Fabricius, a lymphoepithelial organ peculiar to birds, arises as a dorsal diverticulum of the cloaca. In the chicken this structure plays a significant role in antibody production. Experimental bursectomy by either surgical (*1*) or hormonal (*2*) means results in marked reduction in antibody response. Isakovic *et al.* (*3*) reconstituted immunological reactivity by intraperitoneal implantation of bursas in bursectomized chicks. It is possible, however, that this reconstituted antibody production was mediated through either cellular (lymphocyte) migration from the bursa or by a "humoral" substance produced by this organ. Evidence suggesting a noncellular agent for inducing antibody formation was reported by Glick (*4*) who injected saline extract of acetone-dried bursas into bursectomized chicks. We have investigated the capacity of the bursectomized chick to form antibodies after implantation of the bursa of Fabricius enclosed within a cell-impermeable, Millipore diffusion chamber, following the technique used in recent studies on the thymus (*5*).

Two groups of fertile eggs from the regional random-bred White Leghorn population were used. The eggs in group A (experimental) were injected on the 5th day of incubation with 0.1 ml (2.5 mg) of testosterone propionate in sesame oil (Schering). This hormonal method of bursectomy was chosen over the surgical procedure done after hatching because the morphological development of the bursa at the time of hatching shows marked lymphocytic proliferation (*6*). Therefore, lymphocyte dissemination or "humoral" release (or both) was considered a distinct possibility if bursa development were allowed to proceed normally in the embryo. By administration of testosterone (or its derivatives) during early embryogenesis—that is, on the 5th day of incubation (*2*), total elimination of the bursa of Fabricius may be accomplished before any lymphocytes or stromal cells are formed. The eggs in group B were not treated with testosterone; they served both as a source of normal bursas used for subsequent implantation and as a source of control animals having normal immunological reactivity.

Bursas were removed surgically from 15 control chicks on the 8th day after hatching. The bursas were cut into four equal pieces and placed in balanced salt solution. Pieces of bursa were placed between two 25-mm plastic cellulose filters and the filters were sealed (*7*). Diffusion chambers were constructed of 0.45-μ porosity filters, known to prevent cellular migration (*8*). Diffusion chambers were constructed also of smaller (0.1-μ) porosity filters and used in comparative studies.

Chicks were anesthetized with ether, the ventral down was removed, and a 1.5-cm mid-ventral incision was made caudal to the keel after the area was

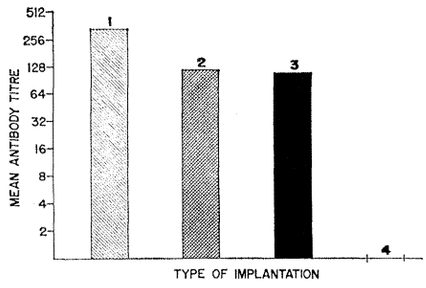

Fig. 1. Mean titers of antibody production in (1) nonbursectomized control chicks; (2) hormonally bursectomized chicks containing bursa; (3) hormonally bursectomized chicks containing bursa-filled diffusion chambers; and (4) hormonally bursectomized chicks containing empty diffusion chambers.

cleansed with alcohol. In each hormonally bursectomized chick either a bursa-filled diffusion chamber or a piece of bursa alone was implanted; for controls, empty diffusion chambers were placed in hormonally bursectomized chicks. A parallel experiment was done in which subcutaneous implants were employed.

On the 9th day after hatching—that is, 1 day after the surgical procedure, all chicks were injected intramuscularly with 1.0 ml of *Salmonella typhimurium* (standardized at 3×10^9 cells per milliliter). Four weeks after the first injection, a second intramuscular injection of 1.0 ml of *S. typhimurium* was given. Two weeks after this second injection blood for bacterial agglutination and serum electrophoretic studies was obtained by cardiac puncture.

Agglutination tests were done by adding 0.25 ml of the standard antigen to 0.25-ml samples of serum in serial dilution from 1 : 2 to 1 : 1025. Tubes were incubated at 45°C for 2 hours and then refrigerated for 24 hours. Agglutination above a titer of 1 : 4 was considered positive—that is, indicating the presence of antibodies to *S. typhimurium*.

A high titer of antibody to *S. typhimurium* (range 1 : 256 to 1 : 512, mean 1 : 330) was obtained from 22 of 30 of the nonbursectomized control chicks (Fig. 1). By contrast, ten chicks hormonally bursectomized and containing empty diffusion chambers were unable to produce demonstrable antibody, this result being in agreement with previous reports for both surgically and hormonally bursectomized chicks which received no further treatment (*1*, *2*). Reconstitution of antibody production (range 1 : 64 to 1 : 256, mean 1 : 105) occurred in 29 of 41 of the hormonally bursectomized chicks bearing bursa-filled diffusion chambers as well as in 15 to 20 of the animals bearing donor bursas not enclosed in diffusion chambers (range 1 : 64 to 1 : 128, mean 1 : 115). No differences were noted in experiments in which the diffusion chambers constructed of 0.45- and 0.1-μ porosity filters were used or in which we used chicks with bursas implanted intraperitoneally or subcutaneously.

Serum electrophoresis added confirmation to our observations in that hormonally bursectomized chicks containing empty diffusion chambers were found to have markedly decreased amounts of γ-globulin, in agreement with reports for bursectomized chicks receiving no further treatment (*9*). In bursectomized chicks containing bursa-filled diffusion chambers and bursas not enclosed in diffusion chambers, γ-globulin concentrations were greater than in the bursectomized chicks, although the concentrations were less than normal.

Our data indicate strongly that in the bursa of Fabricius a noncellular (hormone-like) substance is elaborated which enhances the establishment of immunologic reactivity in bursectomized chicks.

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References and Notes

1. T. S. Chang, B. Glick, A. R. Winter, *Poultry Sci.* **34**, 1187 (1953); B. Glick, T. S. Chang, R. G. Jaap, *ibid.* **35**, 224 (1956); T. S. Chang, M. S. Rheins, A. R. Winter, *ibid.* **36**, 735 (1957); **38**, 174 (1959).
2. A. P. Mueller, H. R. Wolfe, R. K. Meyer, *J. Immunol.* **85**, 172 (1960); M. L. Warner and F. M. Burnet, *Australian J. Biol. Sci.* **14**, 580 (1961); B. Papermaster and R. A. Good, *Nature* **196**, 838 (1962).
3. K. Isakovic, B. Jankovic, L. Papeskovic, D. Milosevic, *Nature* **200**, 273 (1963).
4. B. Glick, *Poultry Sci.* **39**, 1097 (1960).
5. D. Osoba and J. F. A. P. Miller, *J. Exptl. Med.* **119**, 177 (1964); L. W. Law, N. Trainin, R. H. Levey, W. F. Barth, *Science* **143**, 1049 (1964).
6. G. A. Ackerman and R. A. Knouff, *Am. J. Anat.* **104**, 163 (1959).
7. The filters were obtained from Millipore Filter Corp., and were sealed with Millipore Filter Cement Formulation No. 2.
8. E. Shelton and M. E. Rice, *J. Natl. Cancer Inst.* **21**, 137 (1958).
9. P. Long and A. Pierce, *Nature* **200**, 426 (1963); J. Carey and N. Warner, *ibid.* **203**, 198 (1964).
10. This work was supported by PHS fellowship 1-FL-GM-22, 521-01 and research grant HE-04061-07 (HEM) from NIH. One of us (R.L.St.P.) is a predoctoral fellow, National Institute of General Medical Sciences. We thank Dr. Paul H. Aldenderfer and Dr. R. G. Jaap for their invaluable cooperation.

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Sarcoplasmic Reticulum: Ultrastructure of the Triadic Junction

Abstract. *The appositional region between the intermediate element and adjacent cisternae in the triads of the sarcoplasmic reticulum of striated muscle from humans, copepods, ostracods, and barnacles shows a five-layered construction similar to that of a tight junction. Known functions of tight junctions and cisternal elements suggest that a membrane depolarization, conducted by the intermediate element, is transmitted to the cisternae by way of the triadic junction to cause a release of calcium ions from the cisternae.*

The triad, a constant component of the sarcoplasmic reticulum of vertebrate striated muscle, consists of two cisternae of the sarcoplasmic reticulum flanking a transverse tubule called the intermediate element or T-element. The unit is located either at the level of the Z-band or, as in human muscle, near the edge of the A-band, where it encircles each myofibril. The coapted membranes of the triad, that is, the lateral surfaces of the T-element and the mesial membranes of the cisternae, appear modified by virtue of increased electron opacity in addition to their maintaining a rather uniformly tight spacing. Several authors (*1–3*) have commented recently on peculiarities of this junctional region, pointing out both the existence of electron-opaque material in the space between the T-element and cisterna and the scalloping of the cisternal membrane (*4*); one author has suggested that the differentiated region of the dyad—the morphological equivalent of the triad in some invertebrates—may constitute a tight junction (*5*).

In the present study of human rectus abdominis muscle, further details of the junctional region were revealed by means of double-staining with potassium permanganate and lead citrate (*6*). The apposed membranes of the T-element and the cisterna have an average thickness of 45 to 60 Å and are separated by a space of about 110 Å. This gap, in turn, is bisected by a third layer, averaging 40 Å in thickness (Fig. 1). In a tangential section of the triad, this intermediate membrane follows the gap for as long a distance as the triad stays in the plane of section; in transverse section the intermediate membrane occupies the full width of the gap between the flattened, apposed