is thought to bridge the H- and L-chains of the immunoglobulins (*7*).

The NH_2-terminal peptide of this type I Bence Jones protein is the peptide previously identified by us as B_3 (*5*). In accord with the composition reported earlier, its probable sequence is: Asp-Ileu-GluNH_2-Met-Thr-GluNH_2-Pro-Ser-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp-Arg. There is some uncertainty still about the Pro-Ser and Gly-Asp positions, but the remainder has been verified by comparison of overlaps in five peptic peptides derived from B_3. The NH_2-terminal position of this peptide is in accord with the presence of aspartic acid as NH_2-terminal in Bence Jones protein Ag, with the proximity of methionine to the amino end as cleavage by cyanogen bromide indicates, and with the presence of DNP-aspartic acid, isoleucine, and glutamic acid in the DNP-peptide obtained by pronase digestion of the DNP-protein. The significant features of the composition of the NH_2-terminal peptide are the absence of aromatic amino acids and cysteine, the presence of the single methionine, and the concentration of one-fifth of the serine residues of the whole protein within one heptapeptide sequence.

Although B_3 has been identified in the tryptic peptide maps of a number of type I Bence Jones proteins, the myeloma globulin from the same patient (Ag), and in normal 7*S* γ-globulin (*3, 4, 5*), it is absent in several type I Bence Jones proteins including specimen Lo, which has NH_2-terminal glutamic acid and lacks methionine (*4*). This indicates that the NH_2-terminal portion of type I L-chains is one locus subject to individual variation. Other peptides present in Ag but absent in some type I proteins are B_{16} which tentatively has been assigned the sequence Leu-Glu-Ileu-Lys and B_8 which has the sequence Thr-Phe-Gly-GluNH_2-Gly-Thr-Lys.

Variation in the NH_2-terminal portion but constancy in the COOH-terminal portion may have general structural significance for both type I and type II Bence Jones proteins. In the type II proteins the NH_2-terminal amino acid is either undetectable or varies considerably with the individual specimen (*9*). On the other hand, the acidic peptide A_1 is present in all 11 type II Bence Jones proteins that we have examined (*4*). Apparently A_1 is COOH-terminal since it lacks lysine and arginine; its composition is: Thr_2,Ser,Glu,Pro,Ala,-Val,CyS. Milstein (*7*) has suggested that the COOH-terminal cysteine in type I L-chains provides the disulfide bridge to the H-chain in type I globulins. Whether the cysteine in the COOH-terminal peptide of type II proteins has a function similar to that of the terminal cysteine in type I proteins remains to be established.

The heterogeneity of normal γ-globulin (*1, 2*) and the nonidentity of Bence Jones proteins (*9*) have hitherto diminished the incentive for sequence analysis of these related proteins. However, the successful elucidation of the sequence of the NH_2- and COOH-terminal peptides of a type I Bence Jones protein and the identification of these and other peptides in normal γ-globulin verifies the value of this approach to structural study of the immunoglobulins. For this purpose complete amino acid sequence analysis of several Bence Jones proteins of each antigenic type will be essential.

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Intermolecular Forces in Association of Purines with Polybenzenoid Hydrocarbons

Abstract. *The interactions in solution between purine or pyrimidine bases and polybenzenoid aromatic hydrocarbons probably consist in a vertical, stacking-type physical association. By molecular orbital calculations the role of the Van der Waals-London intermolecular forces in these interactions is determined. The electrostatic dipole-dipole forces are negligible, the polarization (or induction) dipole–induced dipole forces are contributory, but most important are the dispersion (or fluctuation) forces. This loose, physical type of interaction should not show any specificity with respect to the carcinogenic activity of the hydrocarbons.*

The molecular associations between purine and pyrimidine bases or their nucleosides and polycyclic aromatic hydrocarbons correspond, to a large extent, to the long-known phenomenon in which aromatic molecules are made soluble by purines, first studied by Weil-Malherbe in 1946 (*1*) and investigated by Boyland and Green (*2*). From studies, particularly those of Ts'o and co-workers (*3*), on the interaction and association of purine bases with themselves or the aromatic amino acids of proteins or hydrocarbons, it appears that these interactions consist in a vertical, stacking-type association of the parallel-oriented partners, with an intermolecular separation of 3 to 4 Å. A similar type of interaction seems to exist also between the aromatic hydrocarbons and the nucleic acids themselves (*4*). Some authors (*5*) but not others (*6*) consider that it corresponds to the intercalation of the hydrocarbon into the nucleic acid between successive base-pairs.

With regard to the nature of these physical interactions between the purines and the hydrocarbons a correlation has been shown to exist (*7, 8*) between the solubilizing power of the purines and their electron donor properties. This correlation indicates that charge transfer forces represent a significant component in the overall binding forces. However, as no charge transfer band is observed in these interactions, and as the compounds taking part are not particularly outstanding electron donors or acceptors, one could

expect that the more classical Van der Waals-London intermolecular forces also play a role in these associations.

Our report is an evaluation of this last type of forces, which are built up of three main components: the electrostatic (dipole-dipole) interactions, the polarization or induction (dipole–induced dipole) interactions, and the dispersion or fluctuation interactions.

In relation to these three divisions, the interactions between the hydrocarbons and the purines have, from the theoretical viewpoint, some characteristics which make their study advantageous and relatively simple. Unsubstituted aromatic polybenzenoid hydrocarbons are with very few exceptions devoid of dipole moments (*9*). This means that the purely electrostatic dipole-dipole interaction (E) represented by

$$E_{\mu\mu} = \frac{1}{r^3}\left[\mu_1 \mu_2 - \frac{3}{r^2}(\mu_1 \bar{r})(\mu_2 \bar{r})\right]$$

(where μ_1 and μ_2 are dipole moments and $\bar{r}$ is the distance between their sites of localization, these sites being the midpoints of the segments joining the centers of gravity of the net positive charges with the net negative ones) cannot play any essential role in the association of hydrocarbon and purine. For the same reason the dipole–induced dipole interaction forces will be reduced to only one of their components, the one involving the dipole moment of the base and the polarizability of the hydrocarbon and which if the polarizabilities are isotropic, will be represented by:

$$E_{\mu\alpha} = -\frac{1}{2}\frac{\mu_1^2 \alpha_2}{r^6}\left[3\cos^2(\mu_1 \bar{r}) + 1\right]$$

where μ_1 is the dipole moment of the base and α_2 the polarizability of the hydrocarbon. This factor, whatever its numerical contribution, cannot by itself account for the observed order of the solubilizing power of the different purines studied. Thus available results indicate the relative solubilizing power of a series of purines toward a given hydrocarbon, in particular 3,4-benzpyrene. Under these conditions, the polarizability being that of the hydrocarbon, remains constant, and if the induction forces played a decisive role in ensuring the solubilization, the degree of interaction would be related to the dipole moments of the bases. This is not the case.

For example, the solubilizing power of the purines is generally much greater than that of the pyrimidines, and in the purines themselves increases with methyl substitution. Also there is no general relation between this behavior and the values of the corresponding dipole moments (Table 1). The purines and pyrimidines are listed in order of decreasing solubilizing power. The polarizability of the hydrocarbon, evaluated with the aid of the usual additivity rules (*10*), is taken as equal to 35.7 Å^3. The dipole moments of the bases are evaluated by calculations especially calibrated for this purpose, and the results agree very well with the known dipole moments of certain derivatives of some of the bases (*11*).

Table 1. Intermolecular forces in the interactions between the bases and 3,4-benzpyrene (intermolecular distance 4 Å).

Base	Dipole moment base (D)	$E_{\mu\alpha}$ (kcal/mole)	Ionization potential (ev)	Polarizability (Å^3)	E_L (kcal/mole)	$E_{\mu\alpha} + E_L$
Tetramethyluric acid	3.3	−0.7	5.8	21.8	−21.1	−21.8
Caffeine	3.4	−0.7	6.8	19.3	−20.3	−21.0
6-Dimethylaminopurine	3.3	−0.7	7.2	17.6	−19.1	−19.8
Guanine	6.8	−2.9	7.2	14.4	−15.7	−18.6
Adenine	3.2	−0.6	7.8	13.9	−15.6	−16.2
Hypoxanthine*	5.2	−1.7	7.5	13	−14.4	−16
Thymine	3.6	−0.8	7.8	12	−12.8	−13.5
Cytosine	7.2	−3.2	8.1	11	−12.7	−15.9
Uracil	3.9	−0.9	8.1	10.2	−11.7	−12.6

* According to Weil-Malherbe (*1*), this compound has a smaller solubilizing power than adenine; according to Boyland and Green (*2*) it has a greater solubilizing power than adenine.

There is no correlation between the values of these polarization interactions and the solubilizing power of the purines (Table 1). Nevertheless, this component is significant for the total result.

We may now consider the dispersion (or London) forces, E_L, represented with the assumption of isotropic polarizabilities, by

$$E_L = -\frac{3}{2}\frac{1}{r^6}\frac{I_1 I_2}{I_1 + I_2}\alpha_1\alpha_2$$

where I_1 and I_2 are the ionization potentials and α_1 and α_2 the polarizabilities of the partners.

An a priori evaluation of the significance of these forces is difficult because the forces depend on a somewhat complex interplay of the ionization potentials and the polarizabilities of the partners. This situation remains true even if the problem is somewhat simplified by considering again the solubilization of a given hydrocarbon, say again 3,4-benzpyrene, by a series of different bases. Thus, the ionization potentials are, as a mean, greater for the pyrimidines than for the purines but the polarizabilities of the purines are greater than those of the pyrimidines. Only numerical calculations can therefore give an answer to this problem (Table 1). The ionization potentials of the bases are deduced from the coefficient of their highest filled molecular orbital, and a reference curve established for a series of standard molecules (*8*). This procedure gives results in substantial agreement with those obtained by an appropriate self-consistent field calculation (*12*). The ionization potential of benzpyrene, obtained in a similar way is 7.2 ev, in excellent agreement with a semi-empirical value deduced from charge-transfer spectroscopy (*13*).

The results indicate parallelism between the solubilizing power of the bases toward 3,4-benzpyrene and the dispersion forces taking part in the intermolecular interactions between those entities. It is the value of the polarizabilities which plays the essential role in determining the relative values of these forces corresponding to different bases, and the order of the polarizabilities themselves corresponds to the order of the solubilizing power of the bases.

If the energies of the interactions for each item in the table are summed, one obtains the values of the total energies of interaction between the bases and 3,4-benzpyrene. The correlation observed then becomes somewhat refined. The combination of the polarization and dispersion interactions increases the advantage of guanine over adenine, which is in agreement with the greater solubilizing power of guanine. Similarly, it brings closer together the values for adenine and hypoxanthine, whose solubilizing powers are relatively indistinguishable.

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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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