diate reaction and yielded an amorphous, gray-blue solid which was not obtained sufficiently pure for analysis.

The mild conditions found to be necessary and the short reaction times observed in these experiments indicate azulene to be markedly more reactive with respect to electrophilic substitution than the isomeric naphthalene. The rapid reaction of mercuric chloride with azulene is noteworthy as the chloromercuration of thiophene requires several days.[4]

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(4) F. C. Whitmore, "Organic Compounds of Mercury," Am. Chem. Soc. Monograph No. 3, Chemical Catalog Co., New York, N. Y., 1921.

(5) Shell Oil Fellow, 1949-1950.

## BACTERIAL DESOXYPENTOSE NUCLEIC ACIDS OF UNUSUAL COMPOSITION

*Sirs:*

Previous work in this laboratory[1] has shown that the desoxypentose nucleic acids (DNA) of different species differ in composition. All specimens examined so far, however, were, with one exception, considerably richer in adenine and thymine than in guanine and cytosine. Only in the DNA of avian tubercle bacilli[2] were the molar ratios of adenine to guanine or to cytosine and of thymine to cytosine lower than 1.

Two other instances of microbial DNA of unusual composition have now been found, namely in preparations from *Serratia marcescens* and from a facultatively autotrophic hydrogen organism, *Bacillus Schatz*, kindly given us by Drs. van Niel and Schatz.

Because of the strong DNA depolymerase activity of *Serratia marcescens* cultures a modification of a previously described procedure[3] was used. Washed cells from a 48-hr. culture on nutrient agar at room temperature were ground in 3.5 *M* aqueous NaCl with pyrex powder. The supernatants (20,000 x g, 0.5 hr.) from repeated extracts of the ground material (0°, 3.5 *M* NaCl) were injected into cold alcohol, the precipitated fibers deproteinized and processed as described before[3]; yield 0.08% of wet cells, 92–97% DNA, 1.1–2.6% RNA.

Similar procedures led to the isolation of DNA from the hydrogen organism, *Bacillus Schatz*. The fibrous preparations were freed of tenaciously held RNA (9%) by dialysis of their solution against dilute NaOH of *p*H 13.5 for 18 hr. at 30°; yield, 0.1%, 100–108% DNA, 0.5–3% RNA.

Three independently prepared samples from each of the organisms were analyzed for purines and pyrimidines by a modification[4] of previously published procedures.[5] The molar ratios reproduced in Table I represent averages for these preparations.

TABLE I

MOLAR RATIOS IN TWO BACTERIAL DNA

| | *Serratia marcescens* | Hydrogen organism, *Bacillus Schatz* |
|---|---|---|
| Adenine/guanine | 0.76 | 0.68 |
| Thymine/cytosine | .63 | .59 |
| Adenine/cytosine | .64 | .63 |
| Purines/pyrimidines | .92 | .98 |

Several generalizations can be made. DNA, apart from smaller differences in composition characteristic of the species, appears to occur in two main groups: the "*AT type*" (DNA from animal tissues, yeast, etc.) in which adenine and thymine preponderate, and the "*GC type*" (DNA of tubercle bacilli and compounds discussed here) in which guanine and cytosine are the major constituents. Adenine and thymine occur mostly in almost equimolar quantities, as do guanine and cytosine and also total purines and pyrimidines.

This work was supported by a research grant from the U. S. Public Health Service.

(1) Chargaff, *Experientia*, **6**, 201 (1950).

(2) Vischer, Zamenhof and Chargaff, *J. Biol. Chem.*, **177**, 429 (1949).

(3) Chargaff and Zamenhof, *J. Biol. Chem.*, **173**, 327 (1948).

(4) Chargaff, Green and Lipshitz, unpublished experiments; Zamenhof and Chargaff, *J. Biol. Chem.*, in press.

(5) Vischer and Chargaff, *J. Biol. Chem.*, **176**, 703, 715 (1948).

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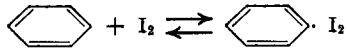
## THE HEAT OF FORMATION OF THE BENZENE–IODINE COMPLEX

*Sir:*

Benesi and Hildebrand[1] have recently reported the existence of an intense ultraviolet absorption peak for solutions of iodine in benzene with a maximum at $\lambda = 297$ m$\mu$. This characteristic absorption serves as part of the evidence for the formation of a 1:1 complex:



The absorption by this complex for various concentrations of iodine and benzene in a non-complexing solvent, such as carbon tetrachloride or *n*-heptane, has been used by Benesi and Hildebrand for determining equilibrium constants for the above reaction. They assumed that the equilibrium constant is the same for all concentrations of the benzene, and evaluated $K$ and the extinction coefficient of the complex $\epsilon_c$ by a linear extrapolation. This led to radically different values of $\epsilon_c$ in carbon tetrachloride and *n*-heptane and involved extrapolation of two curves through a single point (pure benzene, $x_a = 1$).

(1) H. A. Benesi and J. H. Hildebrand, THIS JOURNAL, **71**, 2703 (1949).

We have made what seems to us a more reasonable approximation: that $\epsilon_c$ is the same in all solvents. By calculating the activity coefficients of the various species,[2] we are able to account for the Benesi–Hildebrand measurements with $\epsilon_c$ = 14,000. With this extinction coefficient we calculate from their data concentration equilibrium constants $K'$ (at about 20°) of 2.3 in pure benzene, 1.9 at infinite dilution in carbon tetrachloride, and 1.4 at infinite dilution in *n*-heptane.

We have measured the 297 mμ absorption of the complex in carbon tetrachloride solution at various temperatures ranging from 0° to 44.5°, and for various concentrations of benzene; and using $\epsilon_c$ = 14,000, have calculated concentration equilibrium constants. By plotting log $K'$ against $1/T$, the heats of formation were obtained from the slope and are given in Table I.

TABLE I

| $x_a$ mole fraction benzene | $[I_2]$ mole/liter at 25.0° | $\Delta H$, calories | $K'$ at 25.0° |
|---|---|---|---|
| 1.00 | $17.35 \times 10^{-5}$ | $-1452 \pm 80$ | 2.17 |
| 0.620 | $5.04 \times 10^{-5}$ | $-1416 \pm 14$ | 1.87 |
| 0.0434 | $141 \times 10^{-5}$ | $-1349 \pm 34$ | 1.84 |
| 0.0217 | $264 \times 10^{-5}$ | $-1317 \pm 50$ | 1.91 |

The standard deviations for each concentration (using at least four temperatures) are given in Table I. We estimate the probable error, allowing for uncertainties in the measurement of temperature and optical density, to be about 50 calories. The regular-solution calculation referred to above predicts a difference of approximately 90 calories between the heat of formation in benzene and in carbon tetrachloride, which is in the right direction and in reasonable agreement with the measured values. It should be pointed out that with increasing dilution of the benzene, the calculation of $\Delta H$ becomes less sensitive to the choice of $\epsilon_c$, and below $x_a = 0.05$ is essentially independent of it.

All the solutions of benzene and iodine in carbon tetrachloride were prepared and thermostatted at 25.0°. By using such a procedure it was easy to make the appropriate corrections for the variation of the volume concentration with temperature. Spectrophotometer measurements were made in a Beckman Quartz Spectrophotometer, equipped with the attachment for thermostatting the solutions described by McCullough and Barsh.[3]

We have investigated the shape of the absorption curve at 1.0 and 23.8° and found no measurable temperature broadening.

We are continuing to study these interactions between iodine and aromatic hydrocarbons and will discuss these and further experiments and calculations in a more detailed report at a later date.

(2) J. H. Hildebrand and R. L. Scott, "Solubility of Non-electrolytes," third edition, 1950.

(3) J. D. McCullough and M. K. Barsh, THIS JOURNAL, 71, 3029 (1949).

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## THE NUCLEIC ACID OF AVIDIN

*Sir:*

Avidin, the biotin-binding protein of egg white, has been characterized[1,2,3] as a water-insoluble protein, isoelectric at approximately *p*H 10, containing 10–13% N, and also phosphorus, carbohydrate and ash. A contaminant, iso-electric near *p*H 4.3, was regularly detected in the Tiselius apparatus and could be separated successfully only by this technique.[2]

By an entirely different method of isolation (bentonite adsorption, elution with *M* $K_2HPO_4$, dialysis and refractionation of the water-insoluble protein with ammonium sulfate) we have regularly obtained water-insoluble avidin (avidin NA) of an activity similar to the highest previously reported.[1,2,3] The electrophoretic behavior of such preparations corresponded to that of Woolley and Longsworth's preparations.

The ultraviolet absorption spectrum of avidin NA showed a steep maximum near 260 mμ. The presence of desoxypentose nucleic acid was thereupon demonstrated by a variety of methods. Most of the 1% of phosphorus occurring in avidin NA could be split off by hot, but not cold, trichloroacetic[4] or perchloric acid,[5] or precipitated with acid after treatment with alkali.[6] The Dische reaction[7] accounted for over 50% of the phosphorus in the various nucleic acid extracts. Paper chromatography combined with U.V. spectral analysis clearly indicated the presence of thymine, adenine, cytosine and probably guanine, as well as a small amount of uracil, and pentose.

Electrophoretic fractionations of avidin NA (free, or as the biotin complex), performed in 0.2 *M* $K_2HPO_4$, has yielded the main protein moiety showing the typical ultraviolet spectrum of a protein containing tryptophane and tyrosine, with a maximum at 281 mμ. In contrast, a material containing almost all the phosphorus, and absorbing much more strongly in the ultraviolet, with a maximum only at 258 mμ was found to make up the minor peak of the "contaminant"[2] (Table I). Both components were non-dialyzable and, in contrast to the unfractionated material, largely water-soluble in both experiments.

The biotin-binding activity of avidin is inherent in the protein and independent of the nucleic acid

(1) Eakin, Snell and Williams, *J. Biol. Chem.*, **140**, 535 (1941).
(2) Woolley and Longsworth, *ibid.*, **142**, 285 (1942).
(3) Pennington, Snell and Eakin, THIS JOURNAL, **64**, 469 (1942).
(4) Schneider, *J. Biol. Chem.*, **161**, 293 (1945); **164**, 747 (1946).
(5) Ogur and Rosen, *Arch. Biochem.*, **25**, 262 (1949).
(6) Schmidt and Tannhaeuser, *J. Biol. Chem.*, **161**, 83 (1945).
(7) Hoagland, *J. Exp. Med.*, **72**, 139 (1940).