We have made what seems to us a more reasonable approximation: that ϵ_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 ϵ_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 ϵ_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°	ΔH, calories	K' at 25.0°
1.00	17.35×10^{-5}	-1452 ± 80	2.17
0.620	5.04×10^{-5}	-1416 ± 14	1.87
0.0434	141×10^{-5}	-1349 ± 34	1.84
0.0217	264×10^{-5}	-1317 ± 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 ΔH becomes less sensitive to the choice of ϵ_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).

TABLE I

ELECTROPHORETIC FRACTIONATION OF AVIDIN NA

Component	Mobility[a]	N/P ratio	Ultraviolet absorption E	E_p[b]	E_N[b]	Activity %[c]	Soly. in water
Main	− 2.0	100	Max. 281 Max. 290 Min. 250		130	91	Sol.
Minor	−15.4	2.8[d]	Max. 258 Min. 232	8,100	1360	4	Sol.
Unfractionated material		14	Max. 260 Min. 243	10,000	310	100	Insol.

[a] Mobilities × 10^5 cm.^{2} volt^{-1} at 0° and *p*H 8.9 in 0.2 *M* K_2HPO_4. Similar mobilities were observed for avidin NA and its biotin complex at approximately 0.5 and 1% protein concentration, respectively. The mobilities vary with the solvent; in veronal-chloride buffers at 0.1 ionic strength, mobilities similar to those reported by Woolley and Longsworth[2] have been observed. [b] E_p calculated according to Vischer and Chargaff (*J. Biol. Chem.*, 176, 703, 715 (1949)), E_N correspondingly on the basis of the N content of the preparations. [c] In terms of % of the unfractionated material, 1 mg. of which inactivated 7.4 γ biotin. [d] Upon fractionation of the biotin complex of avidin NA the minor component obtained had N/P ratio of 2.1, which indicates that it is largely nucleic acid.

moiety. This is evident not only from the electrophoretic fractionations, but also from the finding that a readily water-soluble biotin-binding fraction can be isolated from egg white free from nucleic acid, yet almost as active as avidin NA. This albumin (avidin A) is probably identical with the protein component of avidin NA.

The question whether avidin is naturally associated with a nucleic acid, or whether the complex is formed only during the isolation, regardless of the method employed, is under investigation. Nucleoproteins appear not to have been previously recognized and isolated from egg white. Their possible embryological significance, particularly in association with avidin, invites speculation.

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STROPHANTHUS AGLYCONES

Sir:

In a search for sources of sarmentogenin and other 11-oxygenated steroidal aglycones, we have examined a number of different species of *Strophanthus*. Because of the current interest in the *Strophanthus* species, we should like to report our findings at this time. We employed a modification of the procedure of Katz[1] for the isolation of the steroidal aglycones. With seeds of *S. sarmentosus*, *S. hispidus*, *S. kombe* and *S. eminii* our data agree in general with those published previously by other investigators.

(1) A. Katz, *Helv. Chim. Acta*, **31**, 993–1004 (1948).

Sarmentogenin (0.15%; m. p. 270–274°; mixed with authentic sample, no m. p. depression) and a new aglycone, sarverogenin (0.10%), recently described by Reichstein and his associates,[2] were obtained from seeds of *S. courmontii* (sample number 50R344). Strophanthidol (0.15% was isolated from seeds of *S. mortehanii* (50R1241) and from seeds of *S. arnoldianus* (9R7837) in very small yields. No sarmentogenin was isolated from the seeds of either *S. thollonii* (9R9113 and 9R9114) or *S. preussii* (50R581 and 50R582). The samples, however, were small and further work on larger samples is in progress.

Isolation studies with seeds of *S. congoensis* (5OR580) yielded a major Legal-positive steroid (1.02%) and two minor Legal-positive steroids (0.03% and 0.01%). The major steroid has been characterized and it appears to be sarverogenin; m. p. 219–222°C. (micro-block); *Anal.* Found: C, 65.84, 65.91; H, 7.48, 7.27; $[\alpha]^{23}D$ +49.5° (c = 1.0; methanol); $\lambda_{max.}$ 2160 Å. (ethanol), E% 433. The molecular weight by the ebullioscopic method in methanol was 437 ± 14. An infrared spectra comparison of our steroid with sarverogenin showed the two to be almost identical, exhibiting bands at 5.78, 5.88, 6.12 and 2.97 μ as solids in Nujol mull. A mixture of the two compounds gave no melting point depression and each gave the same sequence of color changes when a few crystals were contacted with sulfuric acid.[3] Attempts to prepare the acetyl derivative failed to give a crystalline product. However, a crystalline benzoate was prepared: m. p. 187–191° (micro-block); *Anal.* Found: C, 70.61; H, 6.69; $[\alpha]^{23}D$ +30.7° ± 1° (c = 1.301; acetone). These properties and the sequence of colors produced with this derivative in sulfuric acid are in agreement with sarverogenin dibenzoate.[2]

In addition to seeds, other parts of plants (branchlets with leaves, stem bark, root bark, stem wood, root wood, inner shells of fruits and awns of seeds) were examined for steroid content. From numerous samples of *S. sarmentosus*, *S. barteri*, *S. gratus*, *S. hispidus* and *S. preussii* minute quantities of Legal-positive reacting fractions were obtained in only a few cases. Apparently the glycoside resides almost exclusively in the seeds, at least in the species which were investigated. A detailed report of our work will be presented at a later date.

The samples investigated in this work were collected and identified by B. A. Krukoff. The botanical information and the places of deposit of the herbarium specimens have been reported in

(2) von Euw, Katz, Schmutz and Reichstein, "Festschrift Prof. Paul Casparis" S. 178 (Zürich, 1949); Buzas, von Euw and Reichstein, *Helv. Chim. Acta*, **33**, 465 (1950). Since submission of this manuscript, von Euw and Reichstein reported the isolation of Saveroside, sarmentocymarin and sarmentogenin from *S. courmontii*; *Helv. chim. acta*, **33**, 1006 (1950).

(3) We are indebted to Dr. A. Katz, presently at The Experimental Biology and Medical Institute, National Institute of Health, Bethesda, Maryland, for carrying out these comparisons.