whether the base behaves as a tetradentate or hexadentate ligand. When (IV) behaves as a tetradentate ligand (as in the copper and palladium compounds obtained by the first two methods of preparation), the metal complexes have an asymmetric center in the metal ion, due to the two aromatic rings lying in different planes, irrespective of whether the arrangement be planar or tetrahedral. There is of course a possible meso-form.

Some of the compounds prepared are tabulated:

Compound	Color and crystal form	Solubility Water	Solubility EtOH	Magnetic moment	Melting point, °C.
$[CO^{II}TS_2]\cdot 0.5H_2O$	Brownish yellow powder	insol.	sol.	4.37	dec. 226
$[Co^{III}TS_2]Cl\cdot 2.5H_2O$	Dark black-brown hexagonal plates	sol.	sol.	0	240
$[Fe^{III}TS_2]I\cdot 1.5H_2O$	Dark purple rectangular prisms	sol.	sol.	1.83	117–118
$[Cu^{II}TS_2]$	Bluish green prismatic needles, green when anhydrous	sol.	sol.	2.01	78
$[Al^{III}TS_2]I\cdot 0.5H_2O$	Colorless, hexagonal	sol.	sol.	...	<285

The cobalt, iron and aluminum complexes have been resolved through their *d*-antimonyl tartrates and *d*-bromocamphor sulfonates (in aqueous solution for the first and methanol–water solutions for the others), $[\alpha]^{30}D$ being 300, 357 and 68°, respectively.

The Co(III) compound is quite stable even in aqueous solution, while the other two racemize in solution—more quickly in water than in methanol. The half-life for the aluminum compound is 1.5 hours in 75% ethanol and 2.5 hours in 95% methanol.

The cupric compound, when prepared by the action of (II), (III) and alkali in methanol on an aqueous solution of the *d*-tartrate complex of copper, comes out as an active compound, with $[\alpha]_D$ −65°. (Tartaric acid, tartrates and Cu *d*-tartrates are all dextrorotatory.)

The details of the work together with that on other metal compounds will be shortly communicated.

UNIVERSITY OF ILLINOIS BASUDEB DAS SARMA
URBANA, ILLINOIS JOHN C. BAILAR, JR.

RECEIVED JUNE 30, 1954

THE INFLUENCE OF PH ON ANTIGEN-ANTIBODY EQUILIBRIA

Sir:

Continuing our studies of soluble complexes of antigen (Ag) and antibody (Ab),[1,2] we have investigated the effect of *p*H on solutions containing crystalline bovine serum albumin (BSA) as antigen and rabbit antibodies to BSA. A solution of complexes was preapred[1] containing 21 mg. of protein/ml., consisting of 63.0% total Ag and 37.0% total Ab by weight.[3] Aliquots of this solution were dialyzed against buffers of different *p*H, all at 0.1 ionic strength, and were then ultracentrifuged at about 25°. Between *p*H 7.5 and 4.6, the ultracentrifuge diagrams were essentially unchanged, closely resembling those of Fig. 2c of reference (1). From *p*H 4.6 to 3.1 progressively larger amounts of a component with sedimentation rate corresponding to *free* antibody γ-globulin appeared in the diagrams, while the peaks due to complexes diminished in area. At *p*H 2.4, the diagram was that of a corresponding mixture of BSA and normal γ-globulin. A solution at *p*H 3.1 dialyzed back to *p*H 7.5 exhibited an ultracentrifuge pattern indistinguishable from that of a solution kept at *p*H 7.5, indicating that the acid dissociation of the complexes was completely reversible under these conditions.

The apparent and corrected[4] relative areas of free Ag, free Ab, and of the slowest-sedimenting complex peak (*a*-complex) only, are given in Table I. At *p*H 7.5, the *a*-complex peak was shown[1] to be due largely to the $(Ag)_2Ab$ complex. At acid *p*H values, however, where considerable amounts of free Ab appear in the ultracentrifuge diagrams, we expect appreciable amounts of the AgAb complex to be present as well. We infer that the sedimentation rates of $(Ag)_2Ab$ and AgAb are similar enough so that the two complexes are not resolved in these experiments, and that both together constitute the *a*-complex area at acid *p*H values. That *fraction* of the *a*-complex area attributable to AgAb may be calculated, as a good first approximation as follows. If all Ag reactive sites have equal affinity for Ab sites, and *vice versa*, regardless of the size or shape of the complex in which these sites are bound, it must follow[5,6,2] that $c_{(Ag)_2Ab} = c^2_{AgAb}/4c_{Ab}$, where *c* represents molar concentration. This permits evaluation of the quasi-experimental relative areas of AgAb which are given in column 9 of Table I.

With these data we may evaluate apparent equilibrium constants, *K*, which are almost entirely experimental, for the reaction Ag × Ab ⇄ AgAb, as a function of *p*H. *K* and log *K* are listed in the last two columns of Table I. In view of the approximations made, the *K* values may be uncertain to ±25%, but this introduces an uncertainty of only ±0.1 unit in log *K*. We conclude therefore that in this *p*H range log *K* is a linear function of *p*H

(1) S. J. Singer and D. H. Campbell, THIS JOURNAL, **74**, 1794 (1952).

(2) S. J. Singer, and D. H. Campbell, *ibid.*, **75**, 5577 (1953).

(3) Analyses for total Ag and total Ab were performed by electrophoresis in glycine–HCl buffer, *p*H 2.4, ionic strength 0.1, using known mixtures of BSA and normal γ-globin for calibration. This method will be described in detail elsewhere.

(4) The empirical corrections are made to take account of the anomaly described by J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946).

(5) L. Pauling, D. Pressman, D. H. Campbell and C. Ikeda, THIS JOURNAL, **64**, 3003 (1942).

(6) R. J. Goldberg, THIS JOURNAL, **74**, 5715 (1952).

TABLE I

		Relative areas[a]								
		Free Ag		Free Ab		a-complex[b]			K	
pH	Buffer	App.	Cor.	App.	Cor.	App.	Corr.	AgAb[c]	$\times 10^{-3}$	log K
4.22	Lactate	50	42	5.3	5.3	26	30	17	18	4.25
3.90	Lactate	57	49	9	10	31	35	23	11	4.04
3.88	Acetate	57	49	9	10	25	30	20	9.4	3.97
3.60	Lactate	66	57	13	16	17	22	18	4.6	3.66
3.42	Lactate	68	59	19	22	12	17	15	2.8	3.45
3.31	Lactate	71	62	23	27	10	14	13	1.9	3.28
3.12	Glycine-HCl	68	59	29	34	5	10	10	1.2	3.08
2.40	Glycine-HCl	73	63[d]	27	37[d]	..	..	..	...	...

[a] Given as per cent of total area expected for total protein content of solution, 21 mg./ml. [b] Taken as constituted of AgAb and $(Ag)_2Ab$. [c] Calculated as described in text. [d] As determined by electrophoresis.[3]

with slope unity. This unit slope, together with the fact that in solutions more alkaline than *p*H 4.6 no further extensive changes occur in the sedimentation diagrams, are the two principal results of this study. Although other possibilities are not as yet eliminated, an explanation which is consistent with these results is that there is *one carboxyl* group either in each of the specific reaction sites of the antibody or the BSA molecule which must be ionized in order for the antigen-antibody bond to form in this particular system.

A more detailed description of these and other results will be presented in the near future.

The authors gratefully acknowledged the support of grants from the Rockefeller Foundation and from the United States Public Health Service.

STERLING CHEMISTRY LABORATORY,
CONTRIBUTION NO. 1229
YALE UNIVERSITY S. J. SINGER
NEW HAVEN, CONNECTICUT

GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CONTRIBUTION NO. 1918
CALIFORNIA INSTITUTE OF TECHNOLOGY DAN H. CAMPBELL
PASADENA, CALIFORNIA

RECEIVED MAY 7, 1954

ANISOMYCIN,[1] A NEW ANTI-PROTOZOAN ANTIBIOTIC

Sir:

A new monobasic antibiotic has been isolated from two different species of *Streptomyces* and found to exhibit a high degree of activity *in vitro* against *Trichomonas vaginalis* and *Endamoeba histolytica*. The identity of the two preparations was established by a comparison of the infrared and ultraviolet spectra, elementary analyses, and mixed melting point.

Anisomycin may be recovered by adjusting the filtered culture broth to *p*H 9.0 and extracting countercurrently with methyl isobutyl ketone. The solvent phase is extracted with water at *p*H 2.0. The acid solution is adjusted to *p*H 9.0, and extracted countercurrently with chloroform. The antibiotic crystallizes on concentration of the chloroform extract. On recrystallization from hot ethyl acetate or water, Anisomycin is obtained as long white needles.

Titration data and analyses are in agreement with the formula $C_{14}H_{19}NO_4$, m.p. 140–141°, $[\alpha]^{23}D$ −30° (*c*, 1, methanol). (*Anal.* Calcd. for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.51; H, 7.21; N, 5.22). Ultraviolet light absorption in ethanol: $\lambda_{max.}^{m\mu}$ 224, ϵ 10,800; $\lambda_{max.}^{m\mu}$ 277, ϵ 1800; $\lambda_{max.}^{m\mu}$ 283, ϵ 1600. The infrared spectrum in chloroform shows a series of maxima at 3545, 3450, 3320, 2890, 2800, 1725, 1610, 1582, 1515, 1470, 1447, 1380, 1320, 1302, 1242, 1178, 1036, and 962 $cm.^{-1}$.

Crystalline anisomycin can be stored for long periods of time with no loss of potency. Aqueous solutions are quite stable over a wide *p*H range at room temperature. The compound can be distilled *in vacuo* a few degrees above its melting point.

At the present time studies are under way investigating the efficacy of anisomycin in systemic *Trichomonas* infections in mice and *Endamoeba histolytica* infestation in guinea pigs.

Acknowledgement.—The authors wish to acknowledge the invaluable aid of Dr. A. R. English, Dr. J. E. Lynch, Dr. T. M. Lees, and Dr. J. B. Routien.

BIOCHEMICAL RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC. BEN A. SOBIN
BROOKLYN 6, N. Y. FRED W. TANNER, JR.

(1) The trade name of Chas. Pfizer & Co. for anisomycin is flagecidin.

THE BRIDGED ACTIVATED COMPLEX FOR THE ELECTRON EXCHANGE OF CHROMIUM(II) AND MONOCHLOROCHROMIUM(III) ION

Sir:

We have found the rate of electron exchange in the system Cr^{++}–$CrCl^{++}$ to be rapid but measurable; our results are summarized in Table I.

TABLE I

EXCHANGE OF CHROMIUM IN THE SYSTEM Cr^{++}–$CrCl^{++}$ AT *ca.* 0°

Ionic Strength = 1.0, $(HClO_4)$ = 1.0 (ClO_4^- is the only anion present)

$(Cr^{++}) \times 10^4$	$(CrCl^{++}) \times 10^3$	k(l. $mole^{-1}$ $min.^{-1}$) $\times 10^{-2}$
7.5	2.15	4.3
3.6	1.08	5.6
1.8	1.06	5.6
0.19	0.55	4.2
0.17	0.28	5.0
		Av. $5 \pm 1 \times 10^2$

The specific rate k (total rate of electron transfer = $k(Cr^{++})$ $(CrCl^{++})$) was calculated from the initial rate of exchange as measured by the growth of the specific activity of $CrCl^{++}$ (due to Cr^{51}). Initial rates were used since the data indicated that Cr^{++} was being consumed during the exchange reaction. Aliquots of reaction mixture were quenched with Fe^{+++} which converts Cr^{++} to Cr^{+++} without inducing any exchange. An ion