

TABLE I

pH	Buffer	Free Ag		Relative areas ^a Free Ab		α -complex ^b		AgAb ^c	$K \times 10^{-3}$	log K
		App.	Cor.	App.	Cor.	App.	Corr.			
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 pH 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.

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STERLING CHEMISTRY LABORATORY,
CONTRIBUTION No. 1229
YALE UNIVERSITY
NEW HAVEN, CONNECTICUT

S. J. SINGER

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

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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 pH 9.0 and extracting countercurrently with methyl isobutyl ketone. The solvent phase is extracted with water at pH 2.0. The acid solution is adjusted to pH 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 C₁₄H₁₉NO₄, m.p. 140–141°, [α]_D²³ –30° (c, 1, methanol). (*Anal.* Calcd. for C₁₄H₁₉NO₄: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.51; H, 7.21; N, 5.22). Ultraviolet light absorption in ethanol: λ_{\max}^m , 224, ϵ 10,800; λ_{\max}^m , 277,

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

ϵ 1800; λ_{\max}^m , 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 pH 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.

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BIOCHEMICAL RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
BROOKLYN 6, N. Y.

BEN A. SOBIN
FRED W. TANNER, JR.

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, (HClO₄) = 1.0 (ClO₄⁻ is the only anion present)

(Cr ⁺⁺) × 10 ⁴	(CrCl ⁺⁺) × 10 ³	k (l. mole ⁻¹ min. ⁻¹) × 10 ⁻³
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

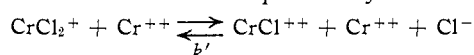
Av. $5 \pm 1 \times 10^3$

The specific rate k (total rate of electron transfer = $k(\text{Cr}^{++})(\text{CrCl}^{++})$) was calculated from the initial rate of exchange as measured by the growth of the specific activity of CrCl⁺⁺ (due to Cr⁶¹). 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

exchange technique was used to separate 60–80% of the CrCl^{++} from all of the Cr^{+++} .

The observed exchange must occur by a bridge activated complex¹ of the configuration $[\text{Cr} \cdots \text{Cl} \cdots \text{Cr}]^{+4}$ which permits transfer of chlorine. Electron transfer via other possible paths, whether by a single H_2O acting as a bridge or over a distance of several water molecules, or by a hydrogen atom transfer leads to net dissociation of CrCl^{++} . The electron transfer is thus *very* much more efficient by the chloride bridged activated complex than by other available paths. Direct measurements show the rate of dissociation of CrCl^{++} catalyzed by Cr^{++} to be lower by a factor of at least 10^4 than the measured exchange. At 0° in 1 *M* HClO_4 , with $(\text{Cr}^{++}) = 6 \times 10^{-3} M$, (CrCl^{++}) decreases by less than 1% in 2 hours. It is also of interest to note the specific rate of exchange in the system $\text{Cr}^{++}-\text{Cr}^{+++}$, $k \cong 0.025 \text{ l. mole}^{-1} \text{ min.}^{-1}$ at 27° .³ A water molecule is probably the bridging group in the activated complex for this electron exchange.

It has been observed that Cr^{++} catalyzes the exchange of Cl^- and CrCl^{++} . This observation is remarkable in view of negligible catalysis of the dissociation of CrCl^{++} by Cr^{++} and the immeasurably low rate of exchange of CrCl^{++} and Cl^- . In *two* experiments at 0° in 1 *M* HClO_4 , with $(\text{Cr}^{++}) = 5.3 \times 10^{-3} M$, $(\text{CrCl}^{++}) = 0.048 M$, $(\text{Cl}^-) = 0.095$ and $0.024 M$, first order dependence upon Cl^- is observed. The observed half times (31 min. and 48 min.) lead to a specific rate in a rate term $k' (\text{Cr}^{++}) (\text{Cl}^-) (\text{CrCl}^{++})$ of $31 \pm 1 \text{ l.}^2 \text{ mole}^{-2} \text{ min.}^{-1}$. The path is almost certainly the reverse of the reaction which has been studied previously.¹ Combina-



tion of the specific rates of the forward and reverse reaction leads to an estimate of 10^2 to 10^3 for $(\text{CrCl}^{++}) (\text{Cl}^-) / (\text{CrCl}_2^+)$, which seems reasonable. Arguments which arise from (1) the necessity of preserving CrCl^{++} in the forward reaction, and (2) making the activated complex readily accessible from Cr^{++} , Cl^- and CrCl^{++} suggest the geometry $[\text{Cr} \cdots \text{Cl} \cdots \text{Cr} \cdots \text{Cl}]^{+3}$ for the activated complex. If this formulation is correct, the observations demonstrate that a negative ion can participate in an electron transfer process other than as a bridging group and that an unsymmetrical activated complex can provide a path for electron exchange.

GEORGE HERBERT JONES LABORATORIES
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

HENRY TAUBE⁴

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

EDWARD L. KING⁵

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- (1) H. Taube and H. Myers, *THIS JOURNAL*, **76**, 2103 (1954).
- (2) The equilibrium quotient $(\text{CrCl}^{++}) / (\text{Cr}^{++}) (\text{Cl}^-)$ has the value 0.77 ± 0.06 at ionic strength 4.9 at 74° (H. S. Gates and E. L. King, unpublished work). Thus, the equilibrium dissociation of CrCl^{++} under these experimental conditions is essentially complete.
- (3) R. A. Plane and H. Taube, *J. Phys. Chem.*, **56**, 33 (1952).
- (4) Acknowledging financial support by the Office of Naval Research.
- (5) Acknowledging financial support by the U. S. Atomic Energy Commission.

RATE OF THE FERROCYANIDE-FERRICYANIDE EXCHANGE REACTION¹

Sir:

The isotopic exchange reaction between the hexacyanoferrate(II) and hexacyanoferrate(III) ions has been the subject of numerous investigations.^{2,3,4,5,6,7} In all except the first,² which is believed³ to be in error because of a faulty separation procedure, complete exchange was observed in the time required to mix and separate the reactants. It was not known whether a very rapid exchange occurred prior to the separation or whether the exchange occurred during the separation. In our studies we have found both that the exchange is very rapid and that the separation-induced exchange is large, often complete.

We have investigated in greatest detail the separation method of Eimer and Dodson⁷ in which about nine-tenths of the hexacyanoferrate(III) ion is extracted into a chloroform solution of tetraphenylarsonium chloride. We found the separation was effective at reactant concentrations as low as 10^{-6} to $10^{-7} M$, but even at these low concentrations complete exchange occurred at 0° during the several seconds required to mix and separate the reactants. That complete exchange could occur during the separation process was shown by heterogeneous exchange experiments ($\text{Fe}(\text{CN})_6^{-4}$ in the water phase, $\text{Fe}(\text{CN})_6^{-3}$ in the chloroform phase) which were complete during shaking times of two seconds and less. This heterogeneous exchange can be slowed to measurable rates (half-times 10 seconds and longer) by addition of hexacyanocobaltate(III) ion in moderate concentrations.

It was found when the reactants were mixed in a 3-way stopcock⁸ and the mixture injected directly into a chloroform solution 0.06 *f* in $(\text{C}_6\text{H}_5)_4\text{AsCl}$ and 0.002 *f* in $\text{Co}(\text{CN})_6^{-3}$, that the exchange was incomplete. At 4° and reactant concentrations of 0.002 *M* the zero-time exchange was 75%. As the reaction time was lengthened by insertion of longer pieces of tubing between the stopcock and the chloroform, the per cent. exchange increased with a half-time of ~ 0.3 sec., which corresponds to a specific rate of $\sim 10^3$ moles/l./sec., if a second-order rate law is assumed.

Working at reactant concentrations in the range $(0.5-3) \times 10^{-4} M$ and starting and stopping the exchange by rapidly injecting the reactant and chloroform solutions into a tube containing a mechanical stirrer, we observed essentially complete

(1) This research was started under the auspices of the U. S. Atomic Energy Commission by A.C.W. while a summer visitor at the Brookhaven National Laboratory during 1952. It has been continued at Washington University with the support of the National Science Foundation.

(2) S. Ruben, G. T. Seaborg and J. W. Kennedy, *J. Applied Phys.*, **12**, 308 (1941); reported by G. T. Seaborg, *Chem. Rev.*, **27**, 199 (1940).

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(4) W. B. Lewis, Tech. Report No. 19, O.N.R. Task Contract N5-ori-07806, "Isotopic Exchange by Electron Transfer Between Complex Ions," Mass. Institute of Tech., Jan., 1949.

(5) C. Haenny and E. Wikler, *Helv. Chim. Acta*, **32**, 2444 (1949).

(6) J. W. Cobble and A. W. Adamson, *THIS JOURNAL*, **72**, 2276 (1950).

(7) L. Eimer and R. W. Dodson, Brookhaven National Laboratory Quarterly Progress Report, (Oct. 1–Dec. 31, 1950), BNL 93 (S-8), p. 69.

(8) F. J. W. Roughton and G. A. Millikan, *Proc. Roy. Soc. (London)*, **A155**, 258 (1936).