

ing, there develops a peculiar vicious circle: on the one hand, the tissues accumulate copper which is not involved in metabolism due to ceruloplasmin deficiency; on the other, the excess of copper in liver cells further inhibits ceruloplasmin biosynthesis, impairing the state of the patient's metabolic system. A reasonable way to treat this disease appears to consist in overcoming the vicious circle.

Zusammenfassung. Aus Affenserum (*Macacus rhesus*) ist das Zeruloplasmin in Form einer homogenen Eiweiss-substanz isoliert und in ihren physikalisch-chemischen Konstanten näher bestimmt worden. Der Vergleich der Eigenschaften des Zeruloplasmins des Menschen und des Affen ergibt eine Ähnlichkeit dieser beiden Substanzen. Bei der Untersuchung mittels spezifischer Präzipitation im Agar haben sich beim Antiserum des

Kaninchens Beweisgründe für eine antigene Identität beider Zeruloplasmine ergeben. An Gewebeschnitten der Affenleber inkubiert bei aktiv oxydativer Phosphorylierung ergab sich die Bindung des Zeruloplasmins *in vitro*. Resynthetisierte Eiweissubstanz bildet einen spezifischen Niederschlag mit dem Antiserum des Kaninchens, welches gegen das Zeruloplasmin des Menschen immunisiert ist. Mit Hilfe lumineszierender Antikörper ist die spezifische Lumineszenz des Zeruloplasmins nur in parenchymatösen Leberzellen gefunden worden. Die Biosynthese des Zeruloplasmins ist in der Leber lokalisiert. Aus *In-vitro*-Versuchen an Gewebeschnitten der Leber beim Affen und entsprechenden *In-vivo*-Versuchen ergibt sich, dass Kupfersalze in geringen Konzentrationen die Biosynthese verstärken, in grossen Konzentrationen hingegen die Biosynthese des Zeruloplasmins hemmen.

SPECIALIA

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Kinetics of Discharge of Cadmium (II) at Dropping Mercury Electrode in KNO_3 -Thioglycolic Acid System

The reduction of Cadmium (II) at dropping mercury electrode (D.M.E.), studied in KNO_3 -TGA mixtures of constant ionic strength (0.5μ), has been found to be irreversible. Kinetics of the electrode reaction has been investigated, adopting the treatment followed by KORYTA, which gave the values of transfer coefficient (α) and rate constant (K°) as 0.506 and $2.544 \times 10^{-3} \text{ cm/sec}$ (22°C) respectively.

Polarographic behaviour of Cd^{++} at D.M.E. in presence of complexing and non-complexing electrolytes had been of considerable interest¹, but the nature of its reduction in medium comprised of thioglycolic acid (TGA), which figures prominently in the discussion of sulphur containing ligands and provides 2 possible co-ordination sites, viz. $-\text{COOH}$ and $-\text{SH}$ groups, has not yet been explored. The present investigation has, therefore, been initiated.

Experimental. All inorganic chemicals and thioglycolic acid were either reagent grade or Merck's guaranteed extra-pure, which were used without further purification. Gelatin was used as maximum suppressor, and purified and equilibrated nitrogen was used to remove oxygen from the solutions.

A Cambridge (G.P.) polarograph was used for recording $c-v$ curves in conjunction with a thermostated H-cell ($22 \pm 0.05^\circ\text{C}$) containing a saturated calomel reference electrode. All the polarograms were run with the damping control in the off position and keeping current sensitivity constant. The capillary used has a constant of $m^{2/3} t^{1/6} =$

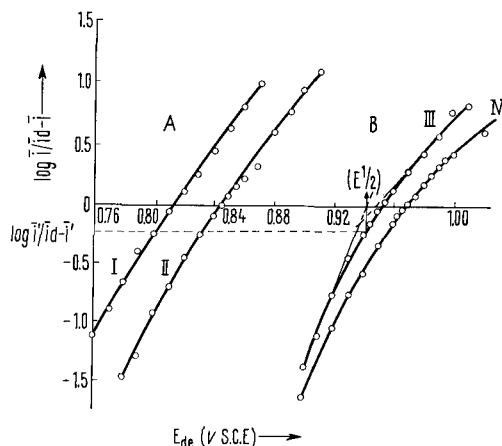
$2.035 \text{ mg}^{2/3} \text{ sec}^{-1/2}$ in $0.5 \text{ M } \text{KNO}_3$ and 0.01% gelatin at 1.0 V (Vs. S.C.E.).

Various solutions containing 0.5 mM cadmium sulphate, different amounts of sodium thioglycolate (0.05 M) and 0.01% gelatin were prepared. Potassium nitrate was added to maintain the ionic strength at 0.5 M. The thoroughly mixed solutions were transferred to the cell and deoxygenated for 15 min with nitrogen. The mercury head was adjusted to 29.5 cm and polarograms were run. Necessary corrections for IR drop and residual current were made in determining half wave potentials and diffusion current data respectively.

Results and discussion. A series of $c-v$ curves, obtained as a result of the discharge of Cd^{++} at D.M.E. in TGA media of constant ionic strength, were analysed for determining the number of electrons taking part at the electrode and for ascertaining the reversibility or irreversibility of the electrode reaction. In all cases, except one in which the concentration of TGA was zero, the plots of $\log \bar{i}/(\bar{i}_d - \bar{i})$ v. $E_{d,e}$ yielded parabolic curves and indicated considerable shift in $E_{1/2}$ values with the increase in TGA concentration, revealing the irreversible reduction of Cd (II) in presence of TGA and complexation between them; in absence of TGA however, cadmium (II) reduces

¹ L. MEITES, in *Polarographic Techniques* (Interscience Publication, New York 1965), p. 623.

reversibly with the transfer of 2 electrons ($n = 2$). In view of the irreversible discharge of Cd (II) it was considered worthwhile to investigate the kinetics of electrode reaction.



Plots of $E_{d.e.} v. \log \frac{i}{i_d - i}$. Cd^{2+} in (I) 0.05 M TGA, (II) 0.06 M TGA, (III) 0.4 M TGA and (IV) 0.5 M TGA.

S. No.	TGA Concentration (M/l)	α	K° cm/sec (22°C)
1	0.05	0.540	2.770×10^{-3}
2	0.06	0.50	2.288×10^{-3}
3	0.40	0.505	2.343×10^{-3}
4	0.50	0.484	2.774×10^{-3}

α , mean = 0.506. K° , mean = 2.544×10^{-3} .

Determination of K° and α . From the logarithmic analysis of the $c-v$ curves of Cd^{++} in different concentrations of thioglycolate the kinetic parameters K° and α are calculated according to the relations proposed by KORYTA².

The Figure represents the plots of $E_{d.e.} v. \log \frac{i}{i_d - i}$. From the slope of the asymptote drawn at the negative part (marked A) of the log plots the value of n was evaluated, which in each case was found to be 2, and also the values of reversible half wave potential ($E_{1/2}$) given by the point of intersection of asymptote at voltage axis. The values of transfer coefficient (α) were calculated from the slope of the asymptote at the positive portion (marked B) of the log plots. After knowing the values of α and i' (obtained from ($E_{1/2}$) as shown in the Figure) (curve II) the values of K° were determined. The Table illustrates the concentrations of TGA employed and the values of α and K° obtained.

It is apparent from the Table that the values of α and K° are almost constant. The rate of discharge of Cd (II) at D.M.E. is independent of thioglycolate concentration.

Zusammenfassung. Das polarographische Verhalten von Cd^{2+} wird beschrieben.

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² J. KORYTA, *Electrochim. Acta* 6, 67 (1964).

³ Acknowledgment. I wish to express my gratitude to my teacher, Prof. R. S. SAXENA, who interested me in polarography, put the best working facilities at my disposal and stimulated this work by many valuable discussions.

Isolation of Canine Gastrin

The hormone gastrin has now been isolated from the gastric antral mucosa of hog, man, sheep and cow¹; the present account describes its isolation from antral mucosa of the dog. The chief difficulty, as anticipated, was the collection of sufficient antral mucosa to provide the minimal amounts of the purified peptides required for a definitive study of their constitution. On the basis of previous experience it was expected that about 10 μg gastrin would be obtained from one antrum, so that at least 100 antrums would be required for a single preparation; 2 such batches were processed. As opportunity offered, the antral mucosa was dissected from the stomachs of freshly killed animals and the strips boiled in a small volume of water for 5 min to inactivate enzymes; the mixture was then stored in the deep-freeze. When 100 such preparations had been obtained, the isolation was carried out following the general procedure already described for hog and human antral mucosa^{2,3}. As in the latter case, the boiled antral strips were homogenised and re-extracted with water to obtain the maximal yield. The final stage of isolation, as before, was by gradient elution chromatography on a small column (1 \times 5 cm) of amino-

ethylcellulose, using a concentration gradient of 0.04 to 0.4 M ammonium bicarbonate buffer. The Figure shows the result. The fractions corresponding to the peaks DI and DII were pooled and dried in vacuo until free from ammonium bicarbonate; they were then rerun in similar conditions to those described above to remove traces of contaminants.

Throughout the isolation procedure, the behaviour of the activity was followed by testing small aliquots of appropriate fractions for their power to stimulate gastric acid secretion in conscious dogs provided with denervated pouches of the gastric fundus. The final products showed the expected high potency in this respect; but owing to the very small amount of material available, no studies were made of the actions on other gastro-intestinal struc-

¹ R. A. GREGORY, *Proc. R. Soc. B* 170, 81 (1968).

² R. A. GREGORY and H. J. TRACY, *Gut* 5, 103 (1964).

³ R. A. GREGORY, HILDA J. TRACY and M. I. GROSSMAN, *Nature* 209, 583 (1966).