HERWEGH, VAN ROY and DE GROOTE[8] added sodium bilirubinate to rat liver homogenate at 4 °C and found that most of the bilirubin was present in the microsomes and cell sap fraction. If human serum or albumin was used as the suspending vehicle for bilirubin then most of the bilirubin was found in the cell sap fraction, the distribution being similar to that observed here in the GUNN rat. The distribution of bilirubin in liver in vitro depended on the suspending medium in which bilirubin is added. HERWEGH et al.[8] interpreted their results as showing that albumin and serum competed for bilirubin with the subcellular fractions and so prevented the uptake of bilirubin by the fractions. In the in vivo experiments bilirubin is presented to the liver attached to rat serum proteins, and we would expect that the bilirubin could be found mainly in the cell sap because of this binding to serum proteins.

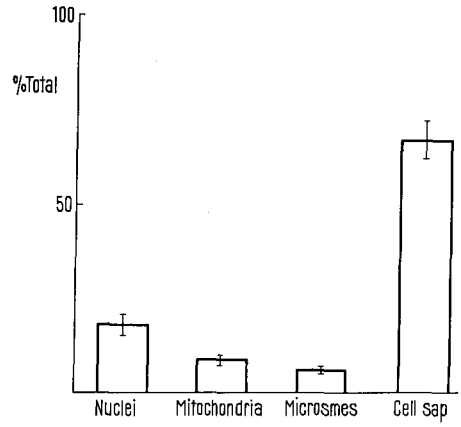

Fig. 1. Bilirubin content of the liver of jaundiced male and female GUNN rats. Results are means of 14 animals ± I.S.D.

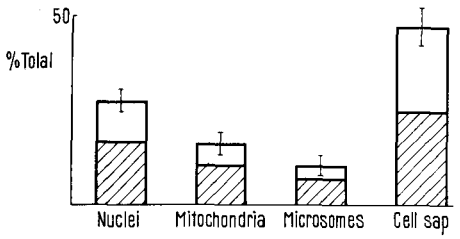

Fig. 2. Bilirubin content of fractions from rat liver after bilirubin infusion. Results are means of 5 experiments ± I.S.D. $\overline{/7/}$, Direct reacting bilirubin.

Zusammenfassung. Die Bilirubinmengen in den Geweben ikterischer GUNN-Ratten werden zusammen mit den in den subzellulären Anterilen vorliegenden Bilirubinmengen ikterischer GUNN-Rattenlebern bestimmt. Die Bilirubinbestimmung erfolgte nach i.v. Infusion von Bilirubin in die Rattenleber: es findet sich vorwiegend im Leberzellensaft der ikterischen GUNN-Ratte, verteilt sich jedoch nach Bilirubininfusion gleichmässig über alle Leberanteile. Die teils bekannten, teils bestätigten Unterschiede können durch Plasma-Eiweiss-Bindung des Bilirubins erklärt werden.

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January 31, 1966.

[8] K. HERWEGH, F. VAN ROY, and J. DE GROOTE, Tijdschr. Gastroent. *7b*, 184 (1964).

[9] We would like to thank Professor N. H. MARTIN for his advice and encouragement and the British Empire Cancer Campaign for financial assistance.

Amperometric Study on Lanthanum Polytungstates

The formation and composition of lanthanum polytungstates obtained by the interaction of lanthanum nitrate and different alkali tungstates have been investigated by means of amperometric titrations between the reactants at different pH values in aqueous-ethanolic media. The sharp breaks obtained provide cogent evidence for the formation of $La_2O_3 \cdot 3WO_3$ and $La_2O_3 \cdot 7WO_3$ in the pH ranges 5.5–6.5 and 4.2–5.5 respectively. Amperometric titrations provide a simple and rapid method of determination of La^{3+} ions.

An outstanding characteristic of tungstates is their strong tendency to form condensed complex ions in solution[1], and from these polytungstates crystallize depending upon the pH, which plays an important role in the aggregation process. The formation of polytungstates is usually attributed to combination with corresponding acids, as if they were separate chemical individuals. This led the earlier workers to believe that similar salts of heavy metals may be precipitated as a result of metathesis[2]. BRITTON and GERMAN[3], however, contended that heavy metal tungstates are not precipitated by interaction of metal salts and alkali tungstates.

In the literature, there are few references to the study of La^{3+}-tungstates and most workers had based their conclusions on analytical results. HITCHCOCK[4] obtained La^{3+}-tungstate by adding $LaCl_3$ to Na_2WO_4. ROGERS and SMITH[5] prepared ammonium La-tungstate having the composition $2(NH_4)_2O \cdot La_2O_3 \cdot 16WO_3 \cdot 16H_2O$. HOGBOM[6] reported the formation of $Na_8La_2(WO_4)_7$ by dissolving

[1] G. JANDER and H. JAHR, Z. anorg. allg. Chem. *194*, 383 (1930).
[2] F. ABEGG, *Handbuch der Anorganischen Chemie* (Leipzig 1921), p. 577, 636.
[3] H. T. S. BRITTON and W. L. GERMAN, J. chem. Soc. Lond. *1429* (1931).
[4] F. R. M. HITCHCOCK, J. Am. chem. Soc. *17*, 483 (1898).
[5] A. ROGERS and E. F. SMITH, J. Am. chem. Soc. *26*, 1774 (1904).
[6] A. J. HOGBOM, Bull. Soc. chim., Paris *42*, 2 (1884).

La_2O_3 and WO_3 in a fused mixture of NaCl and an excess of Na_2WO_4. As the nature of the WO_4^{2-} ion is a function of $[H^+]$, precipitation of tungstates is mainly governed by the pH, and hence simple gravimetric methods fail to indicate the correct composition of the precipitate. Formation of La^{3+}-polytungstates has therefore been studied at specific pH levels by applying electrometric techniques, which have provided more conclusive results on the compositions of metallic tungstates[7].

Experimental. The solutions of lanthanum nitrate, sodium tungstate and gelatin (AnalaR[BDH] quality) were prepared in air-free conductivity water. HCl was of the reagent grade. Solutions of the various alkali tungstates (*di-*, *para-*, *tri-* and *meta-*) were prepared by the action of requisite quantities of HCl on normal sodium tungstate at 100 °C.

A manual polarograph with a Scalamp galvanometer was used for amperometric titrations. A dropping mercury electrode having the characteristics m = 2.416 mg/sec, t = 3.58 sec, and $m^{2/3}t^{1/6}$ = 2.226 $mg^{2/3}$ $sec^{1/2}$– at $E_{d.e.} = -1.0$ V (vs. SCE) was used in conjunction with saturated calomel electrodes connected to the cell by a low resistance salt bridge. All titrations were performed at 30 ± 0.05 °C in the presence of 25% alcoholic medium, using 0.01% gelatine as the maximum suppressor at an applied potential of −1.5 V (vs. SCE) at which the limiting current is produced. The titre solution (20.0 ml) was taken in the cell each time and it was swept and stirred by bubbling hydrogen. After each addition of titrant, the observed current was corrected for dilution effect and plotted against the volume of titrant. The end-points were located graphically. The results of amperometric titrations have been summarized in the Table, and only one Figure has been given for the sake of brevity.

Discussion. The authors[8] have shown that the formation of polytungstates corresponds to various stages in the anomalous neutralization of sodium tungstate by different acids, as indicated by the changes in H^+ ion concentration. It is considered, therefore, that any interaction between alkali tungstate and metal salt in solution would be largely governed by the $[H^+]$ that would be set up by the reactants and these in turn would have pronounced influence on the compositions of precipitates. A series of reactions between La-salt and each of the alkali tungstates has, therefore, been studied amperometrically.

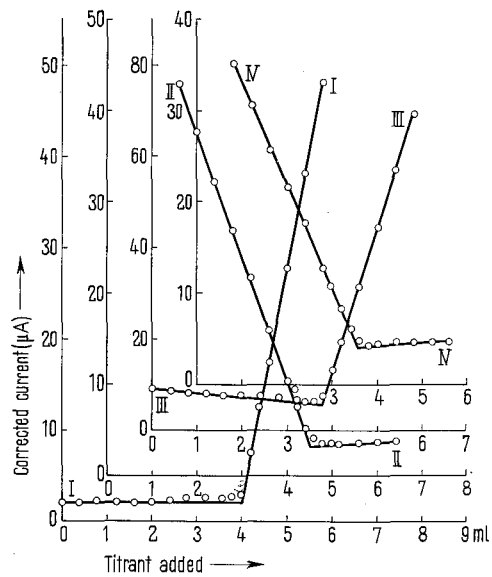
Normal tungstate titrations. The pH of $La(NO_3)_3$ and Na_2WO_4 was measured and found to be 5.20 and 7.80 respectively. In direct titrations, when lanthanum salt was added to sodium tungstate taken in the cell (Figure, curve I) the diffusion current almost remained constant until the stoichiometric end-point was reached, beyond which it increased with the increase in $[La^{3+}]$. In the case where sodium tungstate was used as the titrant, the reverse phenomenon was observed (Figure, curve II). The equivalence point obtained from titration curves occurred at a stage where the molecular ratio of the reacting species La_2O_3:WO_3 was 1:3, corresponding to the formation of $La_2O_3 \cdot 3WO_3$ (normal) in the pH range 5.5–6.5 according to the following equation:

$$2La(NO_2)_3 + 3Na_2WO_4 = La_2O_3 \cdot 3WO_3 + 6NaNO_3.$$

Di- and para-tungstate titrations. Amperometric titrations of sodium *di*-tungstate (prepared as described earlier) showed an interesting phenomenon: instead of the well-defined breaks occurring for the precipitation of lanthanum *di*-tungstate found to coincide with stoichiometric end-point corresponding to the formation of lanthanum *para*-tungstate $La_2O_3 \cdot 7WO_3$. Its formation has been further confirmed by performing a series of ampero-

Results of amperometric titrations

Molarity of solutions		Equivalence points (ml)	
$La(NO_3)_3$	Na_2WO_4	Calculated	Observed see Figure 1
Normal tungstate		Direct titrations, curve I	
M/30	*M*/100	4.00	4.00
M/50	*M*/150	4.44	4.40
M/80	*M*/300	3.55	3.60
		Reverse titrations, curve II	
M/200	*M*/30	4.50	4.50
M/400	*M*/40	3.00	3.00
M/700	*M*/80	3.43	3.40
para-tungstate			
$La(NO_3)_3$	$3Na_2O \cdot 7WO_3$	Direct titrations, curve III	
M/20	*M*/70	3.81	3.80
M/50	*M*/200	3.33	3.35
M/75	*M*/350	2.85	2.80
		Reverse titrations, curve IV	
M/300	*M*/35	3.50	3.50
M/500	*M*/56	3.36	3.40
M/750	*M*/70	3.00	3.05



Direct (curves I and III) and reverse (curves II and IV) amperometric titrations between lanthanum nitrate and alkali tungstates. I, ml of *M*/30 $La(NO_3)_3$ added to 20.0 ml of *M*/100 Na_2WO_4; II, ml of *M*/30 Na_2WO_4 added to 20.0 ml of *M*/200 $La(NO_3)_3$; III, ml of *M*/20 $La(NO_3)_3$ added to 20.0 ml of *M*/70 $Na_2O \cdot 2 \cdot 33WO_3$; IV, ml of *M*/35 $3Na_2O \cdot 7WO_3$ added to 20.0 ml of *M*/300 $La(NO_3)_3$.

[7] R. S. SAXENA and O. P. SHARMA, J. Indian chem. Soc. *42*, 449 (1965). – R. S. SAXENA and C. M. GUPTA, J. Indian chem. Soc. *36*, 345 (1959), and *35*, 850 (1958); Z. phys. Chem. *19*, 94 (1959); Curr. Sci. *27*, 487 (1958); J. scient. ind. Res. *17B*, 505 (1958).

[8] R. S. SAXENA and O. P. SHARMA, Z. anorg. allg. Chem. *333*, 154 (1964).

metric titrations between $La(NO_3)_3$ and $3Na_2O \cdot 7WO_3$ solutions (Figure, curves III and IV). This reaction taking place at pH range 4.2–5.5 can be represented as:

$$7(Na_2O \cdot WO_3) + 8HCl = 3Na_2O \cdot 7WO_3 + 8NaCl + 4H_2O$$

$$3Na_2O \cdot 7WO_3 + 2La(NO_3)_3 = La_2O_3 \cdot 7WO_3 + 6NaNO_3.$$

Similar titrations performed between lanthanum salt and other alkali polytungstates failed to provide any dependable results.

Each titration takes a little time for the diffusion current values to become steady. Thorough stirring in the vicinity of the end-point has a favourable effect. The presence of 25% ethanol has been found to improve the end-point as it decreases the dielectric constant of the medium, with the consequent decrease in the solubilities of the precipitates.

The individuality of either the *di-*, *tri-* or *meta*-compounds of lanthanum was not established, but the electrometric investigations confirm the formation of normal and *para*-tungstates of lanthanum. The accuracy and reproducibility of these titrations have been found to be excellent over the range of concentrations studied.

Zusammenfassung. Es wurde die Zusammensetzung von Lanthanwolframat-Niederschlägen untersucht, die sich aus Lanthannitrat- und Natriumwolframat-Lösungen verschiedener Polymerisationsgrade bilden. Die Reaktionen wurden bei verschiedenem pH, in rein wässrigem oder wässrig-alkoholischem Milieu durch amperometrische Titration verfolgt. Im pH-Bereich von 5,5–6,5 bildet sich $La_2O_3 \cdot 3WO_3$, bei einem pH von 4,2–5,5 $La_2O_3 \cdot 7WO_3$.

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Department of Chemistry, Malaviya Regional Engineering College, Jaipur (India), November 1, 1965.

[9] Acknowledgment: O. P. Sharma is grateful to the Council of Scientific and Industrial Research, New Delhi, for financial assistance and to Principal V. G. Garde for providing research facilities.

Bestimmung des α-2-Makroglobulinumsatzes beim Menschen[1]

In einer vorhergehenden Arbeit wurde am Beispiel des Ratten-Serumalbumins eine spezielle Methode zur Bestimmung des Umsatzes von Plasmaproteinen durch quantitative Immunpräzipitation beschrieben[2]. Mit Hilfe dieser Versuchungsanordnung kann man die Dynamik von Serumeiweissen auch dann erfassen, wenn man nicht über das radioaktiv markierte Reinprotein verfügt. Die Umsatzbestimmung erfolgt durch quantitative Immunpräzipitation des speziellen Eiweisses aus einem Gemisch radioaktiv markierter Eiweisskörper. Diese Methode ist deswegen von besonderem Interesse, da bekanntlich durch Anwendung von Absorptionsverfahren die Zahl hochspezifischer Immunseren die der Reinantigene übersteigt. Nach dieser Methode wurde nun mit Hilfe eines hochspezifischen Antihuman-α_2-Makroglobulinserums von Kaninchen (Behringwerde, Marburg) erstmalig der Umsatz des α_2-Makroglobulins beim Menschen bestimmt. Dieser ist im Hinblick auf die Aufklärung des Pathomechanismus spezieller Eiweißspektren (Nephrose, Rheumatismus u.a.) wie auch der Bedeutung als Transportprotein von besonderem Interesse.

Methodik. α_2-Makroglobulin wurde aus Blutspendeserum (200 mg%) mit Hilfe einer Gelfiltration über Sephadex G-200 auf etwa das 5fache der Ausgangskonzentration angereichert. Die quantitative Bestimmung des α_2-Makroglobulins erfolgte nach Heidelberger[3,4].

[1] Mit Unterstützung durch die Deutsche Forschungsgemeinschaft.

[2] R. Kluthe, B. Riebow, F. Richter und N. Kleine, Nuklearmedizin *5*, 107 (1965).

[3] M. Heidelberger und F. E. Kendall, J. exp. Med. *61*, 563 (1935).

[4] E. A. Kabat und Ch. C. Meyer, *Experimental Immunochemistry* (Ch. C. Thomas, Springfield 1961).

Übersicht über wesentliche Messgrössen und Berechnung des Umsatzes von α_2-Makroglobulin

Name	Alter Geschlecht	Körpergrösse cm	Körpergewicht kg	α_2-M (Serum) mg%	Plasmavolumen *l*	α_2-M intravasculär g	α_2-M *t*/2 Tage	Katabolische Rate in %/Tag	Katabolische Rate absolut (in % des intravasculären α_2-M)	Katabolische Rate in mg/kg pro Tag	α_2-M Urinausscheidung mg/Tag
D. Veih.	30 J. ♂	174	71	220	3,39	8,58	9	7,5	654	9,10	–
E. Vo.	25 J. ♂	179	63	281	2,34	6,56	8	8,6	567	8,95	–
P. Schn.	24 J. ♂	191	90	325	3,38	11,0	11	6,4	703	7,80	–
A. Wo.	24 J. ♂	178	65	325	2,75	8,95	10	7,1	640	9,80	–
U. Ha.	27 J. ♂	186	83	275	3,16	8,7	9	7,2	627	7,55	–
G. Pe.	27 J. ♂	192	81	225	3,10	7,0	11	6,2	437	5,40	–