was observed in four cats, the dose range being 30–40 U/kg. Using SP 10,000 U/mg, the same phenomenon was seen in six cats, the dose range being from 20–70 U/kg. Where possible, the duration of these actions of SP was followed and found to be in excess of 30 min. No attempt was made to determine the minimal effective dose.

Discussion. These experiments were undertaken for the express purpose of determining if highly 'purified' SP has neurotropic activity, and with the ultimate goal of determining whether or not this activity is lost during purification of the leiotropic fractions, thereby explaining the discrepancy between STERN and HUKOVIĆ[7] and HAEFELI and HÜRLIMANN[8]. However, the data presented here show that SP, of relatively high potency, still contains neurotropic activity. Unfortunately, the nature of the test preparation used precludes quantitation of the information obtained, and it is not possible to suggest whether the leiotropic and neurotropic actions are due to the same component.

These data are in support of the conclusions of STERN and HUKOVIĆ[7] that purified SP has neurotropic activity[11].

Zusammenfassung. Gereinigte Präparate von Substanz P mit Aktivitäten von 10, 1000 und 10000 E/mg verstärken das vierte Potential der dorsalen Nervenwurzel im Rückenmark der Katze (Verabreichung nach LSD). Unsere Befunde bestätigen frühere Beobachtungen und legen die Vermutung nahe, dass bei fortschreitender Reinigung ein Teil der neurotropen Aktivität zusammen mit der leiotropen angereichert wird.

W. KRIVOY and D. KROEGER

Baylor University College of Medicine, Houston (Texas), University of Texas Dental Branch, Houston (Texas, U.S.A.), April 18, 1963.

[11] *Acknowledgments.* This research was supported by funds from research grants MY 3477 and AM 04138 of the United States Public Health Service.

PRO EXPERIMENTIS

Microdetermination of Magnesium and Calcium in Animal Tissue

In connection with studying the metabolism of kidney cortex slices, the necessity arose to determine microgram quantities of Mg and Ca in this material. None of the known methods can satisfactorily remove all the difficulties associated with such determination, which necessitates complete separation of Mg and Ca from each other, and of the two from phosphate and from interfering heavy metals, in particular Fe.

The method presented here is based on the separation of metals on ion exchanger[1]. The technique of purifying the resin and the effect of cross-linkage on the strength of the bond of the two metals to the resin was assessed.

Experimental. Reagents: The water used was redistilled from a quartz apparatus. The solutions were kept in polyethylene bottles.

$5 \times 10^{-3} M$ EDTA.

Buffer, pH 10.5, $1M$ NH_4Cl–NH_4OH.

Buffer, pH 5.6 (20 ml $1N$ CH_3COOH + 180 ml $1N$ CH_3COONa).

0.06% methanolic solution of Eriochrome Black T or Calcon.

0.1% ethanolic solution of 8-hydroxyquinoline.

0.018% aqueous solution of sodium naphthalohydroxamate[2].

Dowex 50-X8 (200–400 mesh).

All chemicals were of analytical purity.

Method. The method was tested on standard solutions and applied to the determination of Mg and Ca in the ash of rabbit and rat kidney cortex.

Dry Dowex 50 was left to swell for 24 h and then washed several times by decantation with water. The resin was transferred into 5×90 mm columns. Each column was purified by successive washing with: 80 ml $6N$ HCl, 15 ml H_2O, 80 ml $6N$ HCl, 15 ml H_2O, 40 ml $6N$ HCl and water to a neutral reaction of the eluate, the rate of flow due to gravity being 5 ml/h. This procedure took several days. The resin was then in the H^+ cycle and ready for use.

The test solution contained 7.3 μg Mg, 2.4 μg Ca, and further 5 μg Fe, 2 μg Zn, 0.5 μg Cu, 0.1 μg Mn and 0.523 mg KH_2PO_4 in 1 ml.

Elution of Mg and Ca. Three types of Dowex 50 with different cross-linkage were investigated for the separation of Mg and Ca. With increasing cross-linkage the strength of bond of Ca rose more rapidly than that of Mg so that, whereas both metals were eluted with $2N$ HCl in close sequence from Dowex 50-X4, Dowex 50-X8 permitted elution of Mg selectively with 5 ml $2N$ HCl, no Ca being present in the subsequent 4 ml of the eluate and appearing only after elution with 5 ml $3N$ HCl. On the other hand, the elution zones of Mg and Ca on Dowex 50-X12 were wide and diffuse.

Determination of Mg and Ca. After adding an aliquote of the test solution, the column of Dowex 50-X8 was washed with 5 ml $1N$ HCl; Mg was eluted with 5 ml $2N$ HCl and Ca with 5 ml $3N$ HCl. The eluates were collected in quartz tubes and evaporated in an electric oven at 100°C. The residues were dissolved in water.

Since the interfering metals are eluted together with Mg, they were removed by adding 0.1% 8-hydroxyquinoline (pH 5.6, acetate buffer) and extracting the chelates formed with chloroform[3]. Mg was then determined in the aqueous phase by titration with EDTA, using an Agla microsyringe, to Eriochrome Black T (pH 10.5, ammonium buffer)[4]. The equivalence point was determined graphically from curves of extinction changes followed on a Hilger Spekker photocolorimeter at 650 mμ.

Calcium was determined photometrically with sodium naphthalohydroxamate at 390 mμ (CF 4 Spectrophotometer) by the indirect method[2] or by complexometric titration to Calcon (pH 12.5, diethylamine)[5] similarly as

[1] R. L. GRISWOLD and N. PACE, Anal. Chem. *28*, 1035 (1956).
[2] D. K. BANERJEE, C. C. BUDKE, and F. D. MILLER, Anal. Chem. *33*, 418 (1961); *34*, 440 (1962).
[3] J. BITTEL, Ann. Inst. Nat. Rech. Agronom. Ser. 2, 144 (1951).
[4] G. SCHWARZENBACH, *Die komplexometrische Titration* (Stuttgart 1955).
[5] R. BELCHER, R. A. CLOSE, and T. S. WEST, Talanta *1*, 238 (1958).

Table II. Determination of Mg and Ca in kidney and rat cortex slices

	Weight of fresh tissue (mg)	Mg found µg	Mg found µg/100 mg of fresh tissue	Arithmetic mean ± S.E.	Ca found µg	Ca found µg/100 mg of fresh tissue	Arithmetic mean ± S.E.
Rabbit	122.8	26.7	22.0	22.3 ± 0.30	10.75	8.75	8.73 ± 0.20
	119.0	27.2	22.9		10.35	8.70	
	116.0	25.8	22.2		10.95	9.45	
	128.2	28.3	22.1		10.25	8.00	
	156.8	36.8	23.4		14.12	9.07	
	101.0	21.5	21.3		8.48	8.40	
Rat	92.0	19.4	21.1	21.7 ± 0.26	6.65	7.30	7.10 ± 0.32
	157.0	34.2	21.8		9.96	6.35	
	159.0	33.9	21.3		12.55	7.90	
	118.5	26.8	22.6		9.00	7.60	
	162.0	34.8	21.5		10.20	6.30	

Mg. Results of Ca and Mg determinations in the test solution are shown in Table I.

Determination of Mg and Ca in the ash of kidney cortex. Accurately weighed tissue (about 150 mg fresh weight) was ashed for 9 h in platinum crucibles in an electric oven at 600–650°C. After cooling, the white melt was dissolved in 0.1 ml 1*N* HCl and transferred to a Dowex 50-X8 column. The further procedure was the same as described above. The results of Mg and Ca determination in rabbit and rat kidney cortex are shown in Table II. The results agree with values previously reported[6].

The method is applicable without special modifications to other animal tissues[7].

Table I. Determination of Mg and Ca in the test solution

Mg used	Ca (µg)	Mg found µg	Mg found Δµg	Mg found Δ%	Ca found µg	Ca found Δµg	Ca found Δ%
14.6	4.8	14.4	−0.2	−1.3	4.9	+0.1	+2.0
29.2	9.6	29.5	+0.3	+1.1	9.6	0.0	0.0
36.5	12.0	36.45	−0.05	−0.1	11.8	−0.2	−0.7
Standard deviation of each determination			< 4%			< 5%	

The values shown are averages from 3 determinations.

Zusammenfassung. Eine Methode zur Bestimmung von 10–40 µg Mg und 5–15 µg Ca in etwa 150 mg Tiergeweben wird beschrieben. Nach Trennung auf Dowex 50-X8 wird Mg mit Komplexon III und Ca mit Na-naphthalohydroxamat bestimmt.

M. HÖFER

Laboratory for Cell Metabolism, Institute of Microbiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia), January 10, 1963.

[6] *Handbook of Biological Data* (Ed. by W. S. SPECTOR, Philadelphia and London 1956).

[7] *Acknowledgment.* My thanks are due to Dr. A. KLEINZELLER for an unflagging interest shown in the work described.

STUDIORUM PROGRESSUS

Preliminary Notes on the Ostracoda of the Gulf of Naples[1]

MÜLLER[2] published his classic monograph on the Ostracoda of the Gulf of Naples. It is also one of the basic works on the ecology of Ostracoda and has been extensively used by later workers. During the spring and summer months of 1961 and 1962, the author occupied MÜLLER's stations. The Zoological Station, Naples, obtained on loan MÜLLER's syntype material deposited in the Zoological Museum of Greifswald, East Germany, for examination in Naples. Some syntype material of MÜLLER is deposited in Humboldt University, Berlin[3], and this material will also be utilized in preparation of a revised monograph on the sediments, ecology, and microfauna of the Bay of Naples.

In cooperation with the Zoological Station, Naples, 400 cores were collected on 1-mile grids in the Gulf of Naples by a deep-sea coring device. Physical (temperature, transparency, depth, bottom topography, and currents), chemical (salinity, hydrogen ion concentration [pH], dissolved oxygen [O_2], NO_2–N, and PO_4–P), and biological data (food supply and gross relationship with other marine life) were obtained during the time of collection. The ostracodes are being studied by the author and J. MALLOY. The section of Foraminifera will be prepared by Dr. MONCHARMONT-ZEI, and the sediments will be studied by Professor A. LAZZARI, both of the University of Naples. It is hoped that this monograph will include not only a revision of MÜLLER's ostracodes but also a study

[1] Supported by NSF Grant No. G-14562.

[2] G. W. MÜLLER, *Fauna und Flora des Golfes von Neapel*, Naples Sta. Zool., 21 Monographien (1894), p. 1.

[3] K. DIEBEL, Geol. Jahrgang II, 2, 238 (1962).