Die Zellen der Kragendrüse überlappen sich und bilden schlanke Fortsätze aus. Diese stellen typische Pedicellen dar, wie sie sonst nur von vielen Nierenorganen bekannt sind. Benachbarte Pedicellen werden wie dort durch Diaphragmata verbunden (Figuren 2 und 3, Pfeile). In allen Zellen sind cytotische Vesikulationen (V) häufig. In ihnen ist die fädige Glykokalyx deutlich. Die Nuclei liegen im zentralen Zellbereich und sind überwiegend kugelig. Die stets nur cristären Mitochondrien kommen in grosser Zahl vor. Charakteristisch sind viele Lysosomen (Figuren 3 und 4, Ly) verschiedener Grösse und Dichte sowie schlauchförmige und oft gewundene Konfigurationen (W), die im Cytoplasma verstreut liegen. Auch bezüglich dieser Strukturen wird man sehr an das Bild von Sacculuszellen des Maxillarnephridiums von *Polyxenus lagurus* erinnert[5].

Setzt man experimentell eine Häutung in Gang[6], dann werden die Kragendrüsenzellen deutlich aktiviert. Die vorher weiten Interzellularspalten (Figuren 1, 2 und 3) verschwinden wegen einer starken Volumenzunahme der Zellen fast vollständig (Figur 4). Im Cytoplasma treten häufiger freie Ribosomen auf. Lysosomen und Mitochondrien sind deutlich vermehrt. Die Zisternen des ursprünglich schwach entwickelten granulären ER sind jetzt stellenweise blasig aufgetrieben und mit flockigem Inhalt gefüllt. Besonders bemerkenswert ist, dass nun auch agranuläres ER in der Nähe von Golgifeldern sichtbar wird. Die Zahl der Golgikomplexe pro Zelle scheint vermehrt; an den freien Enden ihrer Zisternen werden Prosekretgrana abgeschnürt (Figur 4). Ausführlichere Untersuchungen werden demnächst an anderer Stelle veröffentlicht.

Summary. A hitherto unidentified endocrine 'glandula perioesophagealis' in *Polyxenus lagurus* is described. Its cells are typical podocytes. Between the pedicells there are many intercellulary spaces. In animals close to molting, the gland cells are activated.

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[5] E. EL-HIFNAWI und G. SEIFERT, Z. Zellforsch. *113*, 518 (1971).
[6] G. SEIFERT, Zool. Anz. *177*, 258 (1966).

Lead Binding to Human Haemoglobin

Lead is known to combine with erythrocytes[1]. Previously it was thought to combine with the erythrocyte cell membrane[2] but recent work has suggested that it reacts with the cell contents, notably with haemoglobin[3]. The nature of the reaction is still in doubt and this report describes an attempt to determine the association constant for the lead haemoglobin complex and the number of binding sites available for lead on the haemoglobin molecule.

Stroma free haemolysates were obtained from adult and fetal blood red cells, previously washed 3 times with normal saline, by ultracentrifugation[3]. Each haemolysate was diluted with twice its volume of tris-maleic acid buffer, pH 7.0 and samples obtained for haemoglobin[4] and protein[5] determinations. Varying concentrations of lead in the range 50 to 500 ppm were prepared in the buffer and labelled with carrier free Pb-203 to give solutions of known specific activity. Volumes of the lead solutions were mixed with samples of the haemolysate in bags of Visking dialysis tubing which had previously been prepared by boiling in several changes of distilled water. The sealed bags were centrifuged for 2 h at 2500 *g* in a temperature controlled centrifuge at 25°C using a technique previously described[3]. Aliquots of the ultrafiltrate and residual haemolysate were obtained and the lead content of each derived by measurement of the radioactivity with a Hewlett-Packard Auto-γ-Spectrometer. The lead bound to haemoglobin was determined by the difference between the free and total lead activities.

Analysis of the results by the method of SCATCHARD[6] gave a linear relationship between 'v' (moles of bound lead per total moles of protein) and 'v/M' (where M is the molar concentration of free metal; Figure 1). This supports the view that the sites available for lead binding are equivalent and that there is no interaction between them. The intercept on the X-axis indicates that the adult haemoglobin molecule has 8 sites available for lead binding although the standard error of the slopes of both regression lines would allow values between 7 and 10 (Figure 2). The symmetry of the haemoglobin molecule ($\alpha_2\beta_2$) would suggest that the number of lead binding sites would be given by an even whole number.

The association constant (K) for the lead haemoglobin complex was calculated from the value given by the intercept of the line on the Y-axis and was such that log $K = 4.08$. This value has been related to other known values for lead association constants (Table I). The disparity between the Pb-EDTA and the Pb-Hb association constants was such that exposure of the Pb-Hb to free EDTA would result in the transfer of lead and the formation of a Pb-EDTA complex. This was verified in lead-containing haemolysates, in which the lead was completely removed by EDTA; by contrast little lead was removed from intact erythrocytes (Table II). These

Table I. Comparison of Log K values for Pb^{++} ions in different systems

Ligand	Log K
EDTA	18.0 (Ref.[14])
Haemoglobin	4.08
Bovine serum albumin	2.3 (Ref.[15])
Gelatin pH 5.5	1.84 (Ref.[16])
Gelatin pH 6.5	2.21 (Ref.[16])

[1] R. A. MORTENSEN and K. E. KELLOG, J. Cell. comp. Physiol. *23*, 11 (1944).
[2] B. LINDEMANN and H. PASSOW, Pflügers Arch. ges. Physiol. *271*, 369 (1960).
[3] D. BARLTROP and A. M. SMITH, Experientia *27*, 92 (1971).
[4] A. J. HAINLINE, in *Statistical Methods in Clinical Chemistry* (Academic Press, New York 1958), vol. 2. p. 49.
[5] W. H. DAUGHADAY, O. H. LOWRY, N. J. ROSEBROUGH and W. S. FIELDS, J. Lab. clin. Med. *39*, 663 (1952).
[6] G. SCATCHARD, Ann. N. Y. Acad. Sci. *51*, 660 (1949).

Table II. The effect of extracellular EDTA on the partition of lead between erythrocytes and saline

Molar content in 0.9% saline		Erythrocyte lead (%)
Lead	EDTA	
10^{-5}	–	95.87
10^{-3}	–	94.20
10^{-5}	10^{-3}	1.02
10^{-3}	10^{-3}	1.16
–	10^{-3}	95.17[a]
–	10^{-2}	92.42[a]

1 ml of washed erythrocytes added to 4 ml of solution, mixed for 10 min. Lead content of erythrocytes determined after washing ×3 with 0.9% saline. The lead solutions were labelled with carrier-free Pb-203. [a] Washed erythrocytes previously equilibrated with $10^{-3}M$ lead.

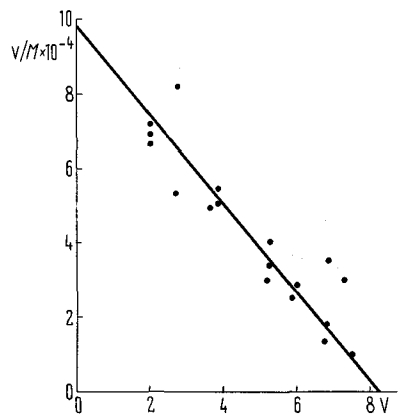

Fig. 1. Plot and regression line for v/M against v, where v denotes moles of bound lead per total moles of haemoglobin and M is the molar concentration of free metal (after SCATCHARD[6]).

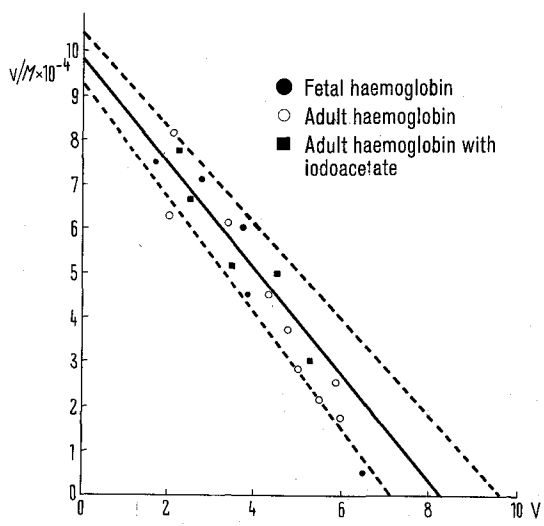

Fig. 2. Graph from Figure 1 superimposed on the results from fetal haemoglobin, normal adult haemoglobin controls and adult haemoglobin containing added iodoacetic acid. The standard errors of the slope of the regression line are included.

findings support the suggestion that EDTA does not cross the erythrocyte membrane and in vivo removes skeletal rather than erythrocyte lead[7]. This was further substantiated by studies with C-14 labelled EDTA, which showed that the EDTA added to whole blood remained extracellular.

The nature of lead binding sites on the haemoglobin molecule is not known. Previous work with heavy metals (mercury, silver) has suggested that binding occurred with up to 6 sulphydryl groups[8–11] although only two of these are normally available for binding[12]. An average of 7.5 sulphydryl groups available for heavy metal binding has been reported for haemoglobin H ($\alpha_2\beta_2$). However, it has been suggested that spurious results might occur with divalent mercury ions due to the formation of Hg-Hg-SH linkages thus giving an incorrect estimate of the number of available groups[13]. Blocking the free -SH groups of haemoglobin by the incorporation of iodoacetic acid in the haemolysates prior to the addition of lead resulted in little difference in the lead binding (Figure 2) suggesting that free sulphydryl groups are not essential in the formation of the lead-haemoglobin complex.

Analysis of the data obtained from experiments using fetal haemoglobin gave similar results showing the sites available for lead binding to be independent of the β-chain component of the haemoglobin[17].

Zusammenfassung. Die Verbindung von Blei mit menschlichem Hämoglobin wurde in vitro mittels Pb-203 untersucht, wobei man die Anzahl der zugänglichen Verbindungsstellen am Molekül des Hämoglobins und der Verbindungskonstante für den Blei-Hämoglobinkomplex bestimmte. Die Verbindung war weder von der Anwesenheit der β-Ketten noch von freien Sulphydrylgruppen abhängig. Mittels EDTA konnte Blei von Hämolysaten, nicht aber aus intektan Erythrozyten entfernt werden.

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[7] P. B. HAMMOND, A. L. ARONSON and W. C. OLSON, J. Pharm. exp. Ther. *157*, 196 (1967).
[8] R. E. BENESCH, H. A. LARDY and R. BENESCH, J. biol. Chem. *216*, 663 (1955).
[9] A. C. ALLISON and R. CECIL, Biochem J. *69*, 27 (1958).
[10] V. A. INGRAM, Biochem. J. *59*, 653 (1955).
[11] G. BRAUNITZER, J. cell. comp. Physiol. *67*, Suppl. 1, 1 (1966).
[12] A. RIGGS, J. biol. Chem. *236*, 1948 (1961).
[13] R. BENESCH, H. M. RANNEY and R. E. BENESCH, Fedn. Proc. *20*, 706 (1961).
[14] G. SCHWARZENBACH, R. GUT and G. ANDEREGG, Helv. chim. Acta *37*, 937 (1954).
[15] C. TANFORD, J. Am. chem. Soc. *74*, 211 (1954).
[16] W. V. MALIK and M. R. JINDAL, Chemy. Ind. *1968*, 1401.
[17] Lead-203 was kindly supplied by the M.R.C. Cyclotron Unit, Hammersmith, London. This work was supported by grants from the Medical Research Council and the Spastics Society.