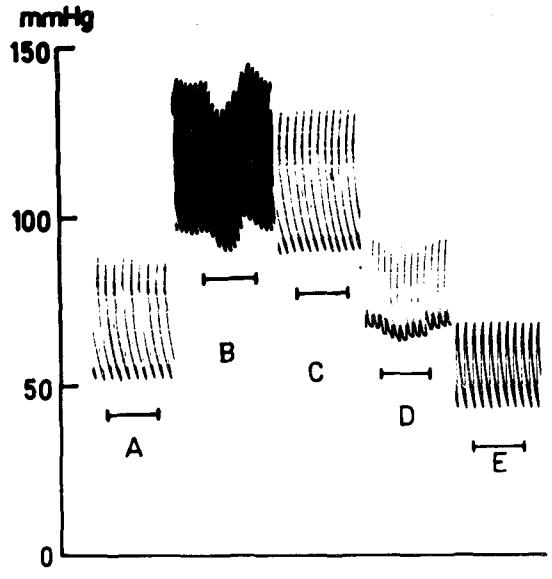
rats; d) atropine-treated rats; e) phentolamine-treated rats. Except the control animals, all the other groups had the carotid and aortic nerves excised. The drugs were given through the jugular vein in the following doses: 1.5 mg/kg atropine and 5 mg/kg phentolamine, 15 min prior to aortic occlusion. Results are stated as mean ± SE and compared with those obtained in control animals.



A) Control animal. B) Animal without aortic and carotid nerves. C) Vagotomized animal. D) Atropine treated animal. E) Phentolamine-treated animal. Segment: period of aortic occlusion.

Results. The results obtained are to be found in the Table and one experiment of each group is shown in the Figure. The cardiac distention in 11 tests carried out on 6 control animals does not show modifications in the perfusion pressure of the aorta. In 14 experiments on 7 animals without carotid and aortic pressoreceptor nerves, the occlusion of the ascending aorta produced a significant decrease in the perfusion pressure.

Similar results were obtained in 10 experiments carried out in 6 atropine-treated rats. Incision of the vagus nerve inhibited the vasodilatation obtained in 9 tests done in 6 rats and the same inhibition was reached in 14 experiments made in 6 animals treated with phentolamine.

Discussion. The decrease in the perfussion pressure obtained through aortic occlusion indicates that there are receptors in the heart, probably in the left ventricle[1,2], that respond to the distention with vasodilatation. The persistence of the reflex in atropine-treated animals, and the inhibition obtained by means of the vagotomy, would indicate that the afferent tract is to be found in the vagus nerve. The suppression of the vasodilatation obtained with phentolamine makes it possible to think that the efferent tract is the sympathetic nervous system.

The absence of this reflex in the presence of the aortic and/or carotid pressoreceptor nerves would indicate that its function, under normal conditions, is less important than the carotid or aortic reflexes. Similar conclusions were reached by OBERG and THORÉN in the cat[3], although it is possible that this reflex has a more important function in the regulation of blood pressure in the hypertensive state[4].

[2] D. M. AVIADO JR. and C. F. SCHMIDT, Physiol. Rev. *35*, 247 (1955).
[3] B. OBERG and P. THORÉN, Acta physiol. scand. *85*, 145 (1972).
[4] A. MARTINEZ SEEBER, M. I. SALLEMI, M. PEREZ, E. KELLY and A. C. TAQUINI, JR., Pharmacology *2*, 1114 (1974).

Ox-Spleen Erythropoietic Factor: Chromatographic Investigations and Dosages

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Summary. The constant proteic fraction, obtained from ox-spleen homogenates by chromatographic elutions, is the main seat of erythropoietic activity in our assay with polycythemic mice.

In a preceding work[1], we reported a preliminary purification method for the erythropoietic factor from ox-spleen, while in this study we report the results of further investigations of the incoagulable proteins present in the filtrate 'D', obtained with our purification method[1], and their probable correlations with the erythropoietic effect elicited after injection in polycythemic mice.

Materials and methods. The preliminary purification method reported[1] was used for spleens removed from 3 calves (about 12 months old) immediately after death of the animal. The obtained filtrates D_1, D_2, D_3 were tested after dialysis against tap-water at 4 °C and lyophilization for their UV-absorption (proteic concentration 100 µg/ml in 0.033 *M* phosphate buffer pH 6.3) and then analyzed by elution on Sephadex DEAE-A 50 using 0.033 *M* phosphate buffer pH 6.3 in continuous gradient salt to 1.0 *M* NaCl. The swollen-equilibrated Sephadex was packed in a column of 1.5 × 50 cm at 4 °C under an operating pressure of about 1.0 cm H_2O/cm height of bed. The materials, before and after chromatographic elution, were assayed for their erythropoietic activity employing CF/1 strain female mice, 8–10 weeks of age, made polycythemic by discontinuous hypoxia according to FISHER's method[2]. The s.c. injections of the substances were made on the 4th and 5th day following the hypobaric chamber treatment and the erythropoietic increase was determined, whether using ^{59}Fe or by reticulocyte calculation. In the first case, 0.5 µCi of ^{59}Fe was injected i.v. 24 h after the material administration; 48 h later each mouse was bled via cardiac puncture, microhematocrits and haemoglobin percentage were determined (microhematocrits = 74 ± 1.38; haemoglobin = 19%) and 1.0 ml of blood counted for calculation of percent RBC ^{59}Fe incorporation in red cells. We treated 8 groups of polycythemic mice (10 mice

[1] P. DE FRANCISCIS, A. M. GRECO, S. BERTUGLIA and A. NASTASI, Experientia *31*, 1221 (1975).
[2] J. W. FISHER, J. J. L. LERTORA, J. ESPADA, P. TAYLOR and B. L. ROH, in *Regulation of Erythropoiesis* (The Publishing House 'Il Ponte', Milano, Italy 1972), p. 121.

Table I. Effect of filtrates D_1, D_2 and corresponding proteic fractions I on % RBC ^{59}Fe incorporation in polycythemic mouse

Group No.	Treatment	Injected proteins % RBC ^{59}Fe incorporated	
		mg	Mean ± SEM
1[a]	0.25 ml × 2 saline		2.30±0.42
2	0.25 ml × 2 filtrate D_1	1.0	7.40±0.98[b]
3	0.25 ml × 2 filtrate D_2	1.0	10.60±1.21[b]
4	0.25 ml × 2 frac. I from D_1	0.70	9.41±1.60[b]
5	0.25 ml × 2 frac. I from D_2	0.70	9.40±1.38[b]
6	0.05 IU erythropoietin		4.27±0.98[b]
7	0.10 IU erythropoietin		8.03±1.08[b]
8	0.25 IU erythropoietin		21.50±1.09[b]

[a]Control mice. [b]$p<0.01$.

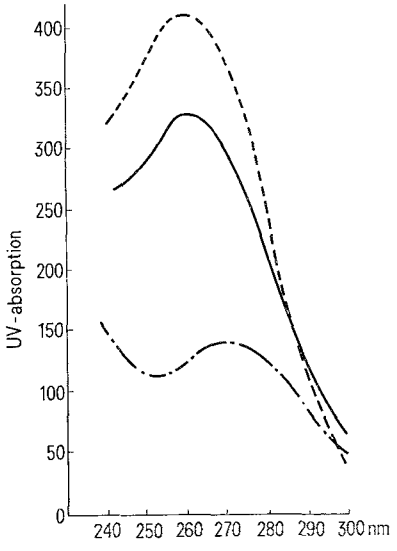

Fig. 1. UV-absorptions of filtrates D_1, D_2 and D_3 in 0.033 *M* phosphate buffer pH 6.3. Proteic concentration 100 µg/ml. —·..·—·—, D_1; ——, D_2; – – –, D_3.

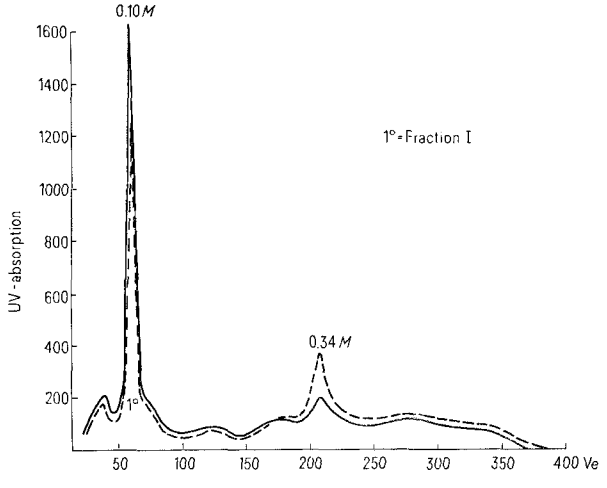

Fig. 2. Elution of filtrate D_1 on Sephadex DEAE – A 50. Eluant: 0.033 *M* phosphate buffer pH 6.3 in continuous gradient salt to 1.0 *M* NaCl. Temp. 4 °C. Operating pressure 10 cm H_2O. ——, 280 nm; – – –, 260 nm.

each): the first was the control group; 4 groups were treated with the substances to assay; the other 3 groups were treated with 0.05 – 0.1 – 0.25 IU of erythropoietin (step I, Arnold R. Horwell, England) respectively.

For reticulocyte determination the accounts were made for the following 7 days, beginning 24 h after the injections of the substances when microhematocrits and haemoglobin percentage, determined on blood samples withdrawn from the animal's tails, were 67 ± 1.38 and 17.31% respectively. We treated 3 groups of polycythemic mice (10 mice each): the first was the control group; the other 2 groups were treated with the substances to be tested.

Results. The UV-absorptions of filtrates D_1, D_2 and D_3 are reported in Figure 1. These graphs show some differences i.e.: the ratio $\frac{OD\ 260}{OD\ 280}$ for D_1, D_2 and D_3 is 1.04, 1.46 and 1.60 respectively; also maximum absorption for D_2 and D_3 is at 260 nm, while for D_1 at 270 nm. Figure 2 reports the chromatographic resolution for D_1 obtained by recording at 280 and 260 nm; remarkable is the fraction I eluted by molarity ranging between 0.09 and 0.13. This fraction shows maximum absorption at 280 nm and appears to contain 40% of total proteins applied to the column.

The chromatographic elution for D_2 is shown in Figure 3. In this case the resolution provides many peaks with maximum absorption at 260 nm, except the fraction I with a greater absorption at 280 nm and eluted between 0.097 and 0.13 values of molarity. Protein dosages indicate that 37% of total protein applied to the column is present

Table II. Effect of filtrates D_3 and corresponding proteic fraction I on % reticulocytosis increase in polycythemic mouse

Group No.	Treatment	Injected proteins ‰ reticulocytes	
		mg	Mean ± SEM
1[a]	0.25 ml × 2 saline		17±3.34
2	0.25 ml × 2 filtrate D_3	1.0	30.50±1.94[b]
3	0.25 ml × 2 frac. I from D_3	0.60	40.33±4.14[b]

[a]Control mice. [b]$p<0.01$.

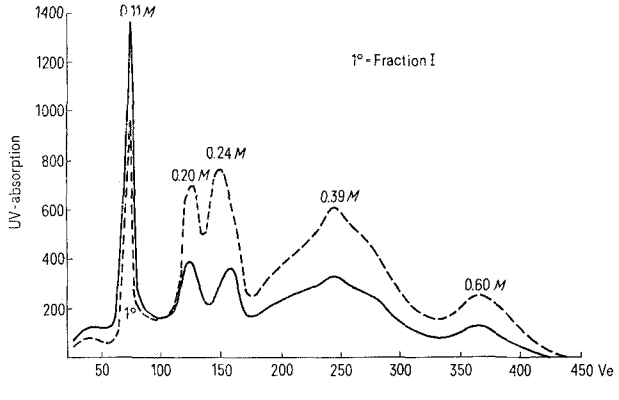

Fig. 3. Elution of filtrate D_2 on Sephadex DEAE – A 50. Eluant: 0.033 *M* phosphate buffer pH 6.3 in continuous gradient salt to 1.0 *M* NaCl. Temp. 4 °C. Operating pressure 10 cm H_2O. ——, 280 nm; – – – –, 260 nm.

in fraction I. The graph obtained for D_3 (Figure 4) is like that for D_2 but with an enhanced absorption at 260 nm; moreover the fraction I, eluted for 0.1 value of molarity, includes 20% of the total proteins put on the column.

These reports suggest that the proteic fraction I is a common element of different ox-spleens; electrophoresis on cellogel by veronal-buffer pH 8.6 of fraction I revealed the presence of 2 components at least. The erythropoietic activity of filtrates D_1, D_2 and the corresponding proteic fractions I in comparison with 0.05, 0.1 and 0.25 IU of erythropoietin are grouped in Table I. The values reported show an expressive increase of % RBC ^{59}Fe incorporation when polycythemic mice were given the substances for examination. We valued these erythropoietic effects at 0.086 (D_1), 0.11 (fraction I from D_1), 0.12 (D_2) and 0.11 (fraction I from D_2) IU of erythropoietin respectively.

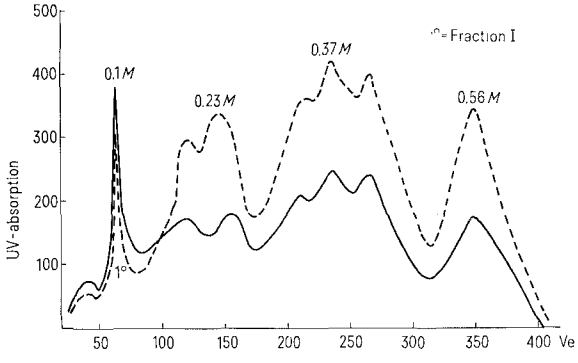

Fig. 4. Elution of filtrate D_3 on Sephadex DEAE – A 50. Eluant: 0.033 *M* phosphate buffer pH 6.3 in continuous gradient salt to 1.0 *M* NaCl. Temp. 4 °C. Operating pressure 10 cm H_2O. ——, 280 nm; – – –, 260 nm.

The reticulocyte increases of polycythemic mice after injection of filtrate D_3 and its fraction I are grouped in Table II. The values reported show that 600 μg of proteic fraction I elicite a greater erythropoietin activity than 1000 μg of filtrate D_3.

Discussion. Considering the chromatographic elution of D_1 (Figure 2), where the fraction I appears to be the most important component, and comparing the erythropoietic effect elicited in mice by injection of filtrate D_1 (1000 μg) and its fraction I (600 μg), the erythropoietic activity of ox-spleen could be correlated entirely to this proteic fraction. Otherwise the comparison of erythropoietic activity for filtrate D_2 (1000 μg) and its proteic fraction I (600 μg) and examination of chromatographic elution (Figure 3), suggest that other substances take part perhaps in erythropoietic action. Nevertheless it appears interesting that both fraction I elicite the same stimulating effect on the rate of erythropoiesis since they appear to be the seat of a steady activity. Our assumptions are confirmed by examination of Table II because, in this case, the proteic fraction I appears responsible for erythropoietic activity measured in D_3, inspite of many fractions obtained at greater values of molarity (Figure 4). Hence we think erythropoietic factor of spleen is correlated with one or many substances which constitute the proteic fraction I.

Hypervascularization of the Cerebral Cortex in Lead-Induced Encephalopathy[1]

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Summary. Pregnant rats were fed a diet containing 1.8% lead acetate for 8 days before delivery until the young were 3 month old. The density of the cerebral cortex capillaries of the infant rats and their convolution rate were studied morphometrically and noted to increase significantly according to the duration of lead treatment, as demonstrated by two-way analysis of variance. On the other hand, the thickness of the cortex reduced progressively. The increase of both capillary density and convolution rate is supposed to be related with this involution of cortex. This provides a quantitative insight of the previously described 'capillary activation' phenomenon, caused by lead encephalopathy and reveals it as a significant sequel of saturnine action.

Lead is known to affect particularly the nervous system of the developing organism. PENTSCHEW et al.[3] demonstrated, in 1966, that lead encephalopathy can be produced readily in young rats when the heavy-metal is added to the maternal diet during the period of suckling, and this procedure has been used in many investigations on lead toxicology.

Several mechanisms have been discussed as potentially responsible for the great sensitivity of the developing central nervous system (CNS) towards lead; blood vessels have been often considered as a most likely candidate for the target of lead action. In this paper, the density of capillaries in the cerebral cortex of the young rat after lead poisoning has been studied by a quantitative method and its relation to saturnine damage to the cortex is evaluated.

Materials and methods. Lead encephalopathy was induced in suckling R rats by the method of PENTSCHEW et al.[3]. A smaller dose of lead was, however, used (1.8% of lead acetate, in food) in order to approach more closely natural environmental conditions. Mothers and their litter were supplied continuously with food containing lead from 1 week before delivery until 3 months thereafter. The brains of 1-, 2- and 3-month-old rats were fixed by retrograde perfusion through the descending aorta and processed using the method of PALAY et al.[4]. Six female animals were used in each fixation, three of

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[3] A. PENTSCHEW and F. GARRO, Acta neuropath. *6*, 266 (1966).

[4] S. L. PALAY and V. CHAN-PALAY, *Cerebellar cortex. Cytology and Organization* (Springer-Verlag, Berlin 1974).