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.

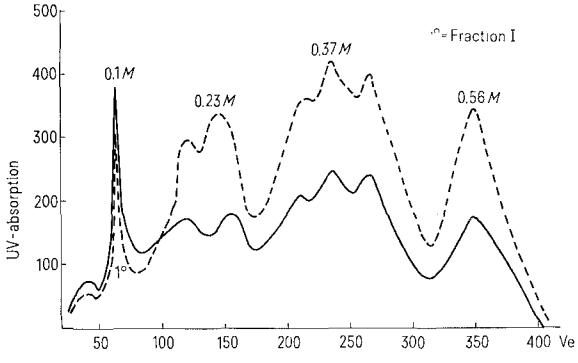

Fig. 4. Elution of filtrate D_3 on Sephadex DEAE – A 50. Eluant: 0.033 *M* phosphate buffer pH 6.3 in continuous gradient salt to 1.0 *M* NaCl. Temp. 4 °C. Operating pressure 10 cm H_2O. ——, 280 nm; – – –, 260 nm.

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).

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them being normal controls. The area gigantopyramidalis as defined by KRIEG[5] (area 4), in the middle third of their cortical layer, was selected in our study. Semi-thin sections (3 μm thick) of Spurr embedded material were made of Cajal's cortical zone V. 3 tissue blocks from each animal were sectioned and stained with toluidine blue. In each block, 3 different microscopical fields (100,068 μm^2) considered as unit surface areas were examined: the contours of the capillary lumina were drawn with a camera lucida and the total capillaries were counted in 2 different ways; Figure 1 gives an example for the use of the two procedures: I, the total number of individual capillary vessels seen in field of view. II, to this were added all lumen profile (particularly the 'en lunette' formations) which clearly belonged to a capillary (which had already been counted under I). The 'convolution factor' was defined as the quotient of the second count (II) to the first one (I). Two-way anovas were used to assess the significance of differences between measurements[6,7].

Results. Figures 1a and 1b show capillaries of the cerebral cortex from control and lead-treated infant rats. An increase of capillary density in lead-treated brain compared to control one, can often be discerned by sight and this is borne out by quantitative analysis (Table Ia, Figure 2). The significance of these observations can be recognized from a two way anova of the measurements showing that the number of capillaries per unit surface area of fronto-parietal cortex (zone V) is significantly great in lead-treated rats ($p < 0.001$) and that this effect augments with age (Table Ib). Thus, after 3 month administration of lead, capillary density is 25% greater

Table Ia) Quantitative analysis of the capillary density in the cerebral cortex of control (Tm) and lead-treated (Pb) rats.

Age 1 month				
Weight (g)	Counting procedures I	II	CF	Thk
Tm. I.1	31.3	32.3	1.03	6.3
44.3	33	35.3	1.07	6.2
	29.6	31	1.05	6.2
Tm. I.2	32	33.6	1.05	5.8
47.2	33.3	34	1.02	6
	29	30	1.03	6
Tm. I.3	33	33.6	1.02	6
42.2	26.3	28	1.06	5.5
	35.6	36	1.01	5.7
Means ± SD				
44.58 ± 2.51	31.4 ± 2.77	32.64 ± 2.58	1.037 ± 0.02	5.96 ± 0.26
Pb. I.1	35	36.6	1.05	6.5
37	38	40.6	1.07	5.8
	33.6	35	1.04	6.3
Pb. I.2	36.1	39.6	1.09	6
47	36.6	37.6	1.03	6.1
	36.3	38	1.05	5.3
Pb. I.3	37.3	41.3	1.1	6.5
42.5	39	41.6	1.07	5.7
	34.3	38	1.1	6.4
Means ± SD				
42.16 ± 5	36.26 ± 1.74	38.7 ± 2.23	1.066 ± 0.03	6.06 ± 0.41
Age 2 months				
Tm. II.1	33.6	34.3	1.02	5.5
130	34	35	1.03	6.8
	38.6	40	1.04	6
Tm. II.2	34.6	36	1.04	–
127	35	35	1	5.3
	28.3	29.3	1.04	4.5
Tm. II.3	36	37	1.03	5.5
124	41.6	43.3	1.04	5.8
	36	38.8	1.08	6
Means ± SD				
127 ± 3	35.3 ± 3.63	36.52 ± 3.96	1.035 ± 0.021	5.67 ± 0.662
Pb. II.1	49.6	50.3	1.01	4.7
35	39.3	41	1.04	4.1
	49.6	51	1.03	5.1
Pb. II.2	37.6	40	1.06	3.8
34	37.3	39.3	1.05	5.1
	41.6	43	1.03	5.1
Pb. II.3	37.6	39	1.04	4.5
35	37	39.6	1.07	5.6
	47.6	50.3	1.06	5.8
Means ± SD				
34.66 ± 0.57	41.91 ± 5.48	43.72 ± 5.24	1.043 ± 0.018	4.86 ± 0.657
Age 3 months				
Weight (g)	Counting procedures I	II	CF	Thk
Tm. III.1	30.8	32	1.04	5.8
178	32.2	34.4	1.07	4.6
	28.2	29	1.03	6.3
Tm. III.2	30.3	32	1.06	5.8
171	35	36.3	1.03	5.3
	34	35	1.03	4.8
Tm. III.3	31.3	31.6	1.01	5.7
176	30.6	33.3	1.09	6.5
	31	32	1.03	5.7
Means ± SD				
175 ± 3.6	31.48 ± 2.17	32.84 ± 2.17	1.043 ± 0.025	5.61 ± 0.62
Pb. III.1	43	45.8	1.07	4
58	42.4	45.8	1.08	–
	40.6	43.2	1.06	3.7
Pb. III.2	39.3	41.3	1.05	5.3
51	32	34.3	1.07	4
	42.3	44	1.04	5.2
Pb. III.3	41	44.3	1.08	4.5
37	44.6	47.6	1.07	4.2
	42	44.3	1.05	4.2
Means ± SD				
48.66 ± 10.7	40.76 ± 3.68	43.4 ± 3.84	1.063 ± 0.014	4.38 ± 0.58

Abbreviations: I and II: the 9 data in each of column I and II represent the mean numbers of capillaries counted from camera lucida drawings of 3 different microscopical fields of the same tissue block. 3 tissue blocks, prelevated in Cajal's cortical zone V, were examined for each animal. The difference between type I and II measurements is explained in Material and Methods and in Figure 1c, d. CF, 'convolution factor' (= II/I); Thk, cortex thickness (one measurement per tissue block) expressed in arbitrary units (1 arbitrary unit = 357 μm). The measurement is made along the neuronal axis, from the pial surface to the beginning of white matter.

[5] W. J. S. KRIEG, J. comp. Neurol. *84*, 221 (1946).

[6] R. R. SOKAL and F. J. ROHLF, *The Principles and Practice of Statistics in Biological Research* (W. H. Freeman, San Francisco 1969).

[7] P. DAGNELIE. *Théorie et Méthodes Statistiques* (J. Duculot S.A., Gembloux 1970), vol. 2.

Ib) Two-way anova study: Effect of treatment and age on capillary density in the cerebral cortex (Type I measurements)

Source of variation	*df*	*ss*	*ms*	*F*	*P*
Groups (9 data)	5	890.909	178.18	15.04	<0.001
Age	2	202.719	101.35	8.55	<0.001
Treatment	1	642.729	642.73	54.28	<0.001
Interaction age-tmt	2	45.46	22.73	1.91	ns
Error	48	568.361	11.84		
Total	58	1459.27			

Ic) Two-way anova study: Effect of treatment and age on the 'con volution factor'

Source of variation	*df*	*ss*	*ms*	*F*	*P*
Groups (9 data)	5	0.007972	0.001594	3.66	
Age	2	0.002144	0.001072	2.46	ns
Treatment	1	0.004816	0.004816	11.07	<0.005
Interaction age-tmt	2	0.001011	0.000505	1.16	ns
Error	48	0.020877	0.000434		
Total	58	0.036820			

Id) Two-way anova study: Effect of treatment and age on cerebral cortex thickness

Source of variation	*df*	*ss*	*ms*	*F*	*P*
Groups (8 data)	5	18.508	3.7		
Treatment	1	5.948	5.948	19.67	<0.001
Age	2	9.198	4.599	15	<0.001
Interaction age-tmt	2	3.362	1.68	5.55	<0.01
Error	42	12.7	0.302		
Total	52	49.716			

Ie) One-way anova study: Effect of age on cerebral cortex thickness in controls

Source of variation	*df*	*ss*	*ms*	*F*	*P*
Age	2	0.723	0.361	1.14	ns
Error	21	6.64	0.316		
Total	23	7.37			

Abbreviations: *df*, degrees of freedom; *ss*, sum of squares; *ms*, mean squares; *P*, significance level.

[8] A. HIRANO and J. A. KOCHEN, Lab. Invest. *29*, 659 (1973).
[9] S. ROY, A. HIRANO, J. A. KOCHEN and H. M. ZIMMERMANN, Acta neuropath. *30*, 287 (1974).
[10] A. J. STARR, R. A. CLASEN, S. PANDOLFI, I. LAING and G. M. HASS. Am. J. Path. *59*, 8a (1970).

than in controls. Moreover, the weights of 1-, 2- and 3-month old rats (Table Ia) indicate that growth is considerably retarded by lead treatment (in agreement with the observations of PENTSCHEW[3]).

In view of the severe growth retardation on the one hand and the increased capillary density of the CNS on the other, one may presume that vascular growth continues (almost normally) whereas volume growth of the brain might be impaired. Indeed, measurements of the thickness of the cerebral cortex (Table Ia, Thk) demonstrate that the grey matter is not only retarded in growth but even undergoes a real involution during the time of lead-treatment (Table Id). The 'convolution factor', defined earlier, is also significantly higher ($p < 0{,}005$) in lead-treated animals, although this effect appears not to depend on age or on capillary density.

Discussion. There is a considerable body of information on the effect of lead on brain capillaries. HIRANO et al.[8]. predicted serious vascular lesions in the encephalon of the chick embryo treated with lead, and indeed such lesions were described afterwards[9]. In the rat, hemorrhage, serous transudations and tissue necrosis were demonstrated[3]. Nevertheless, many capillaries appeared normal and some even proliferated ('activation'). STARR et al.[10] and CLASEN et al.[11] reported vascular changes in young rats treated with lead, particularly the presence of abundant vascular strands deprived of lumina which they interpreted as collapsed capillaries whose development was arrested.

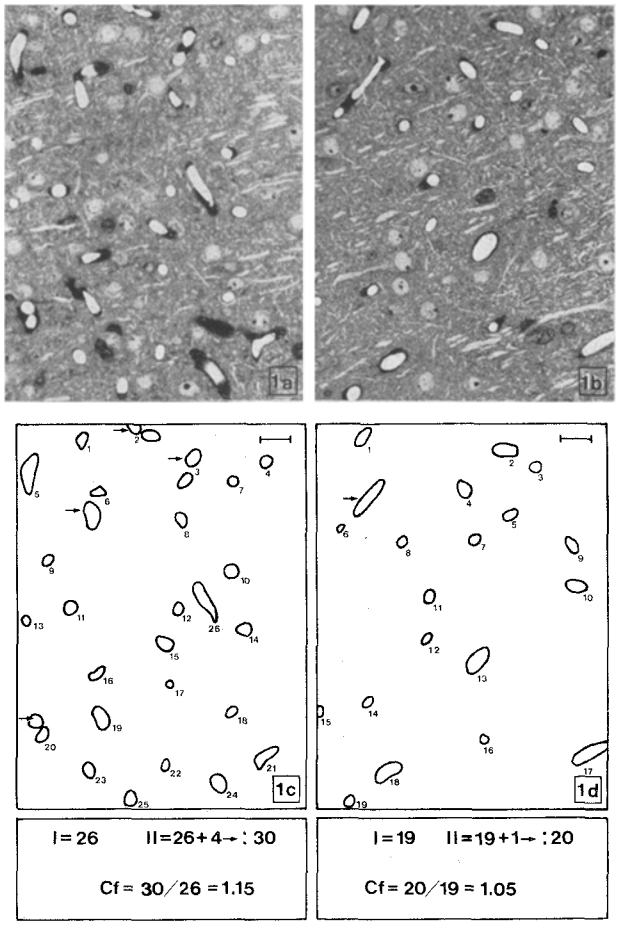

Fig. 1. Photographs and camera lucida drawings of the same microscope fields, from lead treated (1a, 1c) and control (1b, 1d) 3-month-old rats. The difference between type I and II measurements is emphasized. Cf: 'convolution factor' = II/I. Scale = 20 μm.

Table II.

Age (months)	D_c	Thk_c (from Table Ia)	Thk_{Pb}	D_{Pb} theo-retical	D_{Pb} ob-served	Δ obs.–th.
1	31.4	5.96	6.06	30.37	36.26	+ 5.88
2	35.3	5.67	4.86	48.04	41.91	− 6.13
3	31.48	5.61	4.38	51.64	40.76	−10.88

Abbreviations: D_c and D_{Pb}: mean capillary densities in control and leadtreated animals. Thk_c and Thk_{Pb}: mean cortex thickness. Δ: difference between observed and predicted capillary density values after lead treatment.

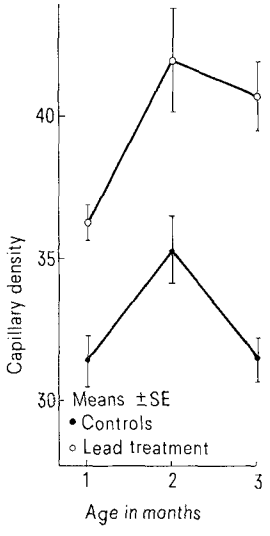

Fig. 2. Influence of age on the capillary density. The capillary density values correspond to type I measurements of Table Ia.

The cerebral cortex of the lead-treated animals in this light microscopic study does not display evident qualitative lesions. Vascular strands are, however, more frequent than in control animals, although the lead dose was lower than that used by other investigators[3,10,11]. Contrary to CLASEN[11], we consider these vascular strands as tangential sections of capillaries: indeed, their number can be considered as proportional to the characteristic high convolution factor seen in lead-treated animals (Table Ic). Moreover in the majority of cases, the use of 3 µm thick sections makes it possible to discern the presence of vascular lumina, which evidently occur more rarely in ultra-thin sections destined to electron microscopy.

In addition, the capillaries in lead-treated brain are not only more convoluted but also more numerous than in the controls. A possible reason for the enhancement of small vessels density and convolution is the significant reduction in thickness of the cortex in lead-treated rats (Table Id). Nevertheless, other factors may play a role also for the following reasons: 1. the involution of the brain is not yet noticeable in the 1-month-old animals (at the end of the suckling period) but still, their capillary density is already significantly increased. 2. Although the capillary density is much enhanced, it is lower than to be expected from the reduction in cortical size. Such a prediction was made on the assumption that as the thickness of the cortex decreases, the density of the vascular supply would increase according to a simple equilateral hyperbolic function: $D_{Pb} = \frac{Thk_c^2 \times D_c}{Thk_{Pb}^2}$ where D_{Pb} is the evaluated capillary density for the lead-treated animals; Thk_c and Thk_{Pb} the thickness of the cerebral cortex in both kinds of animals; D_c the observed density of capillaries in controls. However, the capillary density observed in lead-treated rats was consistantly lower than the predicted one (D_{Pb}) and this difference (Δ in Table II) increased with time of lead treatment. The difference may indicate that the disturbed relationships between nervous and vascular components in the cerebral cortex begin to readjust slowly according with the time elapsed. The present data would thus suggest that lead acts primarily on the grey matter and that the quantitative and conformational changes in the vascular supply represent mainly a sequel of this effect.

Whatever the case, the vulnerability of brain blood vessels to lead still deserves further investigation, principally at low dose levels and electron microscopy studies, using the same material, have now been brought to bear on this particular problem of the equilibrium relations between vascular, glial and nervous elements in the lead-treated cerebral cortex of the infant rat.

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Adenosine Promoted Accumulation of Adenosine 3′,5′-Monophosphate in Rabbit Vagus Nerve

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Summary. Desheathed rabbit vagus nerve has been found to form cyclic AMP when incubated with adenosine. This accumulation of cyclic AMP is inhibited by theophylline but not by antiadrenergic agents, anticholinergic agents or local anaesthetics. Depolarizing media are not able to promote cyclic AMP accumulation in this preparation.

Nervous tissues synthesize adenosine 3′,5′-monophosphate (cyclic AMP) under various conditions[2–5]. In peripheral nervous tissue, attention has been focused on sympathetic ganglia, especially the superior cervical ganglion, where cyclic AMP accumulation has been related to the formation of the slow inhibitory postsynaptic potential[6]. Tests on peripheral nerve have shown only an absence of cyclic AMP accumulation in response to electrical stimulation[7]. Such a negative result, however, is not sufficient to conclude that peripheral axons do not accumulate cyclic AMP. It is of particular importance to re-investigate this possibility, because of