Biochemical Pharmacology, Vol. 37, No. 23, pp. 4539–4547, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

THE EFFECT OF CHRONIC LOW LEVEL LEAD EXPOSURE ON BLOOD–BRAIN BARRIER FUNCTION IN THE DEVELOPING RAT

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(*Received* 30 *March* 1988; *accepted* 1 *July* 1988)

Abstract—Blood–brain barrier (BBB) function was assessed in 19–21-day-old rats exposed to low level lead from birth. Newborn rats received lead via milk from lactating dams given drinking water containing 0.1% lead acetate [$Pb(Ac)_2$]. The treatment regime produced lead levels in the neonates within the range 20–80 $\mu g\ dl^{-1}$ blood, without affecting growth. Cerebrovascular permeability (PS-product) to the diffusion-limited solute mannitol was unchanged in six regions of the cerebral hemisphere, the cerebellum and the brainstem, suggesting that barrier integrity was not affected by the low dose lead treatment. Regional cerebrovascular permeability to nutrient tracers representing seven BBB transport classes was not impaired by lead treatment. However, the PS estimates for the amino acids lysine and histidine and for thiamine were greater than control in some regions of the cerebral hemisphere. These alterations in nutrient supply to the brain may reflect altered substrate utilization associated with repair processes or delayed maturation of the CNS.

It has long been known that the developing central nervous system (CNS) can be irreparably damaged by exposure to high levels of lead [1–3]. In young children, the onset of signs of lead encephalopathy, which can lead to permanent neurological impairment and sometimes death, have been associated with blood lead levels in excess of 80–100 $\mu g\ dl^{-1}$ [4]. In recent years, attention has focused on the possible consequences to CNS development of chronic exposure to the levels of lead encountered in the urban environment. In infant population studies, behavioural and cognitive deficits, and altered reaction time patterns have been associated with blood lead levels as low as 13–35 $\mu g\ dl^{-1}$ [5–8], suggesting a low threshold for neurotoxic effects. However, the methodological problems associated with this type of investigation, e.g. the difficulty in controlling for confounding covariates, has led to disparity in the results between different studies and controversy over the interpretation of findings [9, 10]. Given the limitations of epidemiological surveys, the development and study of an appropriate animal model of low level lead exposure is particularly important. Modifications of the suckling rat model first introduced by Pentschew and Garro [11] have been used in recent years to investigate possible mechanisms underlying lead induced neurotoxicity that are pertinent to human childhood exposure [12, 13].

Both clinical and experimental studies have indicated that the brain microvasculature is a primary target for lead in the CNS. The pathological changes seen in post-mortem brain tissue taken from fatal cases of childhood lead encephalopathy (oedema, vacuolation, haemorrhages, reactive gliosis) appear to be the consequence of selective injury to small blood vessels [12 for review]. A similar pattern of microvascular lesions leading to haemorrhages, oedema and tissue necrosis has been described in animal studies using high dose lead regimes [14 for review]. Other experimental studies have shown that lead accumulates in the endothelial cells of brain capillaries [15–18].

The cerebral capillary endothelium serves as a selective barrier to the free movement into brain of blood-borne substances. Ultrastructural studies have established that the barrier is formed by the tight junctions between endothelial cells [19]. The entry into brain of plasma proteins, ions and many hydrophilic non-electrolytes is severely restricted, whereas the exchange of substrates essential for normal cerebral metabolism is facilitated by specific endothelial membrane transporters [20]. Since transport at the blood–brain barrier (BBB)† is an important determinant of substrate availability in brain [21], selective changes in barrier permeability to essential metabolic substrates could influence substrate-limited pathways of cerebral metabolism. Although there is no evidence of gross structural damage to the cerebral capillary endothelium or change in vascular density with low-level lead exposure [14, 22], it is possible that a more specific functional abnormality, particularly during the critical period of post-natal brain growth [23], might have serious consequences for the structural and functional maturation of the brain. At this time the endothelial transport systems are geared to the increased metabolic demands of the CNS for essential nutrients from the circulation [24–27].

There have been relatively few studies on the

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† Abbreviations used: BBB, blood–brain barrier; PS product, cerebrovascular permeability–surface area product.

functional integrity of the developing BBB at blood lead levels below those associated with gross microvascular lesions and their sequelae. Lorenzo and Gewirtz [28] found that blood-to-brain transfer of tryptophan was reduced in lead-exposed neonatal rabbits. However, Lefauconnier *et al.* [29] were unable to demonstrate a change in BBB transport of several important nutrients in suckling rats, and Michaelson and Bradbury [30] found no effect on choline or tyrosine transport in 8–10-week-old rats exposed to low levels of lead from birth. In these studies BBB function was assessed in a single gross brain sample, and it is possible that alterations localized to particular anatomical and functional regions may not have been detected. In addition, the blood lead levels reported in two of the studies [28, 29], although below the threshold for lead encephalopathy, were within a range where undernutrition is often a complicating factor [31, 32].

In the present study the effect of low-level lead exposure on the functional integrity of the developing BBB has been investigated in rats. Newborn rats received lead throughout the suckling period via maternal milk. The treatment regime produced blood lead concentrations within the range 20–80 $\mu g\ dl^{-1}$, levels reported to be below those associated with frank lead poisoning in children. Cerebrovascular integrity and transport function were assessed by measuring BBB permeability to various radiolabelled solutes.

MATERIALS AND METHODS

Animal maintenance. Pregnant Ola:Sprague–Dawley rats from a specified-pathogen-free colony were obtained from OLAC 1976 (Bicester, Oxfordshire, U.K.) at 16–19 days gestation. They were housed singly in plastic cages with stainless-steel wire tops, and held in a room with an ambient air temperature of 20°, relative humidity in the range 39–83%, and a 12 hr light–dark cycle. The animals were given free access to Rat and Mouse No. 3 breeding diet (Special Diet Services Ltd., Witham, Essex, U.K.; lead content $< 1.6\ mg\ kg^{-1}$) and tap water, and were provided with nesting material.

Lead exposure regime. Within 12 hr of parturition the dams and their litters were randomly assigned to experimental or control groups, and litters were standardized to nine pups. The drinking water of the experimental group was replaced by 0.1% lead acetate [$Pb(Ac)_2$] solution. Control animals received deionized water (containing $< 1\ mg\ Pb\ l^{-1}$). The 547 ppm lead drinking solution was prepared by dissolving 5 g $Pb(Ac)_2$ in 5 l. of deionized water, with the addition of 2.0 ml hydrochloric acid to prevent the precipitation of insoluble lead salts. Glucose (5% w/v) was added to each water bottle to improve palatability. Similar quantities of acid and glucose were added to the drinking water provided for the control animals. Food and fluid intake and body weights of the dams, and total litter weights were recorded daily throughout the three-week suckling period. Individual pup weights were recorded just prior to BBB function studies. At 7 days and 14 days post-partum, blood samples for lead analysis were taken from several lead-treated and control litters under pentobarbitone anaesthesia by cardiac puncture. Blood samples were also taken from pups at 19–21 days just prior to BBB function studies.

Lead analyses. Lead in whole blood or plasma was measured by graphite furnace atomic absorption spectrophotometry (Perkin–Elmer 2380 and HGA 400 with Deuterium Arc background correction) using the method of Fernandez and Hilligoss [33]. Standards and blanks were matrix matched with test samples. The lower limit of sensitivity was 2.5 $\mu g\ Pb\ dl^{-1}$. Lead in drinking water was determined using flame atomic absorption spectrophotometry. Analysis of each batch of lead drinking solution was within 3% of the calculated value. Laboratory analytical quality of the blood lead determinations was monitored by the United Kingdom External Quality Assessment Scheme (UKEQAS, Wolfson Research Laboratories, Birmingham, U.K.).

The integrity of the blood–brain barrier. At 19–21 days post-partum, the suckling rats were anaesthetized ($40\ mg\ kg^{-1}$ i.p. pentobarbitone Na) and cerebrovascular integrity was evaluated from measurements of regional BBB permeability to D-[^{14}C]mannitol using the steady-state programmed infusion technique [34]. Briefly, a steady level of D-[^{14}C] mannitol tracer was maintained in the circulating plasma for an interval (t) of 10 min by programmed i.v. infusion. The arterial tracer concentration, R_p ($dpm\ ml^{-1}$), was monitored, and at the end of the infusion period the blood was rapidly flushed out of the cerebral vessels [35] and the amount of tracer in discrete regions of the CNS, R_t ($dpm\ g^{-1}$), was determined. The apparent permeability – surface area (PS) product was calculated from the tissue and mean plasma radioactivity data:

$$PS = R_t/R_p\ t.$$

This relationship assumes that back diffusion of the tracer from brain to blood is negligible relative to influx during the experimental period. Since the specific gravity of brain tissue is approximately $1.0\ g\ ml^{-1}$, values for PS product ($ml\ sec^{-1}\ g^{-1}$) are presented in units of sec^{-1}.

Blood–brain barrier transport function. Transport function was assessed by measuring regional cerebrovascular permeability to radiotracer substrates representing some of the classes of compounds for which transport systems have been identified and characterized at the BBB. The substrates were D-glucose (hexose system) L-histidine and L-lysine (neutral and basic amino acids, respectively) choline (amine), thiamine (vitamin), adenine (purine base), pyruvate and β-hydroxybutyrate (monocarboxylic acid and ketone body, respectively). The BBB permeability measurements of all substrates except glucose were made using the *in situ* brain perfusion technique essentially as described by Takasato *et al.* [36]. The basic perfusion fluid was bicarbonate-buffered physiological saline (pH 7.4), containing either the [^{14}C]-labelled test solute ($0.3–1.2\ \mu Ci\ ml^{-1}$) and [^{3}H]inulin ($1.0\ \mu Ci\ ml^{-1}$) as an intravascular marker, or [^{14}C]diazepam ($0.5\ \mu Ci\ ml^{-1}$) for measurement of regional cerebral perfusion fluid flow [36]. One second prior to perfusion of the right cerebral hemisphere, the right common carotid artery was ligated.

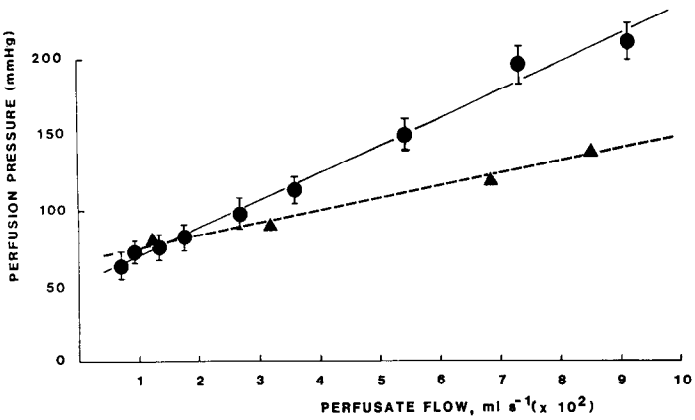

Fig. 1. Pressure-flow calibration for *in situ* brain perfusion. Relation of carotid perfusion pressure to perfusion rate of HCO_3-buffered physiological saline. (——) best fitting regression line ($y = 17.9x + 52.2$, $r^2 = 0.990$). Each point represents a mean (±SE) of six 19 to 21-day-old rats. (- - -) For comparison, data for adult rats taken from Takasato *et al.* [36].

The perfusate was then administered retrogradely into the right external carotid artery at a constant flow rate of 5.42×10^{-2} ml sec^{-1} using a syringe pump (Sage Instruments, Orion Research Inc., MA, U.S., Model 351). This rate was found by pressure-flow calibration of 19–21-day-old rats, using similar methods to Takasato *et al.* [36], to maintain a carotid perfusion pressure between 130–150 mm Hg (Fig. 1), sufficient to minimise collateral arterial supply. Following perfusion the rat was decapitated, the brain quickly excised and sliced into 2-mm coronal sections that were rapidly frozen on the blades using solid CO_2. Discrete regions of the right cerebral hemisphere were sampled, weighed and prepared for scintillation counting together with aliquots of the perfusate. Regional cerebrovascular PS-product was calculated from the equation [36]

$$PS = -F_{pf} \ln (1-(C_{brain} T/F_{pf} T C_{pf}))$$

where C_{brain} is the parenchyma concentration of [^{14}C]-test substrate (dpm g^{-1}) corrected for intravascular tracer content using the [^{3}H]inulin space determined for each sample [36]; C_{pf} is the concentration of [^{14}C]-test solute in perfusate (dpm ml^{-1}); F_{pf} is the regional cerebral perfusion fluid flow (ml sec^{-1} g^{-1}) determined in lead-treated and control pups using [^{14}C]diazepam [36]; T is the net perfusion time (18.5 sec). The perfusion time was corrected for the 1.5 sec period that was required for perfusion fluid to reach the cortical arteries after the syringe drive was started.

Blood–brain glucose transfer. Regional blood-to-brain transfer of glucose was determined at endogenous plasma glucose levels in lead-treated and control rats using methods previously described [37, 38]. Briefly, D-[^{14}C]glucose tracer was maintained constant in the circulating blood plasma for 40 sec by programmed i.v. infusion. Following a controlled washout of cerebral blood, tracer levels were measured in discrete regions of the CNS and in plasma separated from small blood samples taken during the infusion. The plasma glucose concentration was also measured. Blood-to-brain transfer of glucose, J, was calculated from the relationship;

$$J = R_t C_p / R_p t$$

where R_t is the level of radioactivity in cerebral tissue (dpm g^{-1}); R_p is the mean level of radioactivity in plasma (dpm ml^{-1}); C_p is the mean concentration of glucose in plasma (μmol ml^{-1}); t is the duration of infusion.

Counting procedures. Tissue samples (15–20 mg) and aliquots (20–50 μl) of plasma or perfusate were placed in tared counting vials containing 0.5–1.0 ml of solubilizer (Soluene 350, Packard Instrument Ltd., Berkshire, U.K.), weighed, sealed and allowed to dissolve. Glacial acetic acid (0.1 ml) was added, followed by 10 ml of scintillant (5 g PPO and 0.3 g POPOP/litre of toluene). Radioactivity was measured using a Model 2650 automatic Tri-Carb liquid scintillation spectrometer with automatic quench correction.

Chemicals and reagents. Radiochemicals were purchased from Amersham International plc (Aylesbury, Bucks, U.K.). These were [^{3}H]-labelled inulin (sp. act. 1980 mCi mmol^{-1}), [^{14}C]-labelled diazepam (54 mCi mmol^{-1}), D-mannitol (55 mCi mmol^{-1}), D-glucose (250–283 mCi mmol^{-1}), choline (50 mCi mmol^{-1}), L-lysine (348 mCi mmol^{-1}), L-histidine (336 mCi mmol^{-1}) adenine (235 mCi mmol^{-1}) pyruvate (131 mCi mmol^{-1}), D-3-hydroxybutyrate (58 mCi mmol^{-1}), thiamine (25.7 mCi mmol^{-1}). Reagents and chemicals used in the preparation of buffers, drinking solutions and in scintillation counting were of the highest purity available. Glucose assays were performed using a hexokinase kit from Boehringer Mannheim GmbH (F.R.G.).

Statistics. Data were analyzed for statistical significance by ANOVA using the GENSTAT program [39]. In all cases the criterion for statistical significance was $P < 0.05$.

RESULTS

Growth

Fluid and food intake were depressed in some

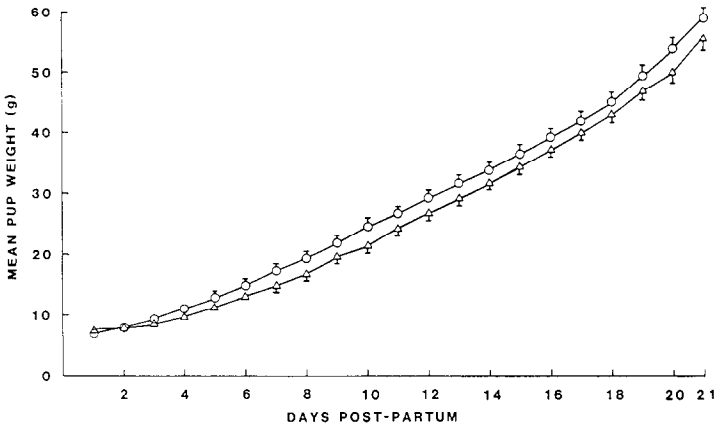

Fig. 2. Weight gain of neonates suckling dams receiving deionized water (○) or 0.1% lead acetate (547 mg Pb l^{-1}) (△). Values are the means (± SE) for 14 control and 15 lead-treated litters. There was no significant difference at any time point ($P > 0.05$, t-test).

dams when they were first exposed to lead, but providing the taste aversion was overcome within 2–3 days the body weights of the dams and the growth rate of their litters (Fig. 2) were comparable to controls. Litters of dams showing a persistent reduction in fluid intake were not used in the study.

Blood lead concentration

Table 1 shows whole blood and plasma lead concentrations in the suckling rats at different times during the 3-week exposure period. Lead concentrations in the plasma were below the detection limit (2 μg dl^{-1}) in all groups except the lead-treated animals at 19–21 days post-partum. Whole blood lead concentrations in the treated group had, by 7 days, reached levels comparable to those found at 19–21 days. These values were not substantially different from those reported by others using a similar low-level lead exposure regime [30, 32].

Blood–brain barrier integrity

Cerebrovascular permeability to D-[^{14}C]mannitol was not significantly different from control in forebrain regions of lead-treated suckling rats (Table 2). PS values in the hind-brain were also similar in the two groups, e.g. mean (± SE) in the cerebellum was 1.92 ± 0.07 and 2.07 ± 0.10, and in the brain-stem 1.90 ± 0.07 and 1.93 ± 0.05 × 10^{-5} sec^{-1} in control and lead-treated rats, respectively. The regional PS values for the control 19–21-day-old rats are comparable to those previously reported for the adult rat [36, 40].

Regional cerebral perfusion flow and intravascular volume

Table 2 shows mean regional values for cerebral perfusion fluid flow and intravascular volume during *in situ* brain perfusion with bicarbonate-buffered physiological saline solution. Both parameters were similar in lead-treated and control rats. The regional values for intravascular volume in the untreated 19–21-day-old rats agree closely with those reported previously for the adult rat [36].

Blood–brain barrier permeability to metabolic substrates

Table 3 shows regional cerebrovascular PS values for seven tracer substrates determined by *in situ* brain perfusion in lead-treated and control rats. Also shown are regional blood-to-brain transfer rates for glucose determined by steady-state programmed infusion. A small adjustment was made to the control transfer values for glucose to allow for a slightly

Table 1. Blood and plasma lead concentrations in control rats and rats exposed to lead via milk throughout suckling

	Age (days)	Control		Lead-treated	
		N	(μg Pb dl^{-1})	N	(μg Pb dl^{-1})
Whole blood	7	5	<2	10	54 ± 4
	14	11	2.5 ± 1.7	17	43 ± 1
	19–21	60	6.0 ± 0.6	70	51 ± 2
Plasma	7	4	<2	7	<2
	14	11	<2	17	<2
	19–21	12	<2	13	5.6 ± 1.4

Values are means ± SE for the number (N) of animals shown.

Table 2. Regional cerebrovascular permeability-surface area (PS) products for D-[^{14}C] mannitol, and regional cerebral perfusion fluid flow and intravascular volume in 21-day-old control rats and rats exposed to lead via milk throughout suckling

	Treatment group	N	Frontal cortex	Parietal cortex	Occipital cortex	Caudate-putamen	Hippocampus	Thalamus
PS values for Mannitol ($sec^{-1} \times 10^5$)	Control	6	2.10 ± 0.12	1.95 ± 0.09	2.13 ± 0.14	1.63 ± 0.02	1.65 ± 0.08	1.78 ± 0.07
	Lead	6	2.32 ± 0.10	2.05 ± 0.05	2.25 ± 0.12	1.65 ± 0.03	1.72 ± 0.05	1.73 ± 0.03
Cerebral perfusion fluid flow (F_{pf}) ($ml\ sec^{-1}\ g^{-1} \times 10^2$)	Control	9	5.40 ± 0.41	7.44 ± 0.53	5.52 ± 0.36	6.04 ± 0.45	7.07 ± 0.54	6.57 ± 0.60
	Lead	9	5.00 ± 0.51	6.68 ± 0.25	5.60 ± 0.14	5.38 ± 0.63	5.99 ± 0.28	6.21 ± 0.35
Intravascular volume ($ml\ g^{-1} \times 10^3$)	Control	38	9.24 ± 0.38	8.24 ± 0.28	8.60 ± 0.30	6.50 ± 0.35	8.12 ± 0.39	7.02 ± 0.50
	Lead	35	8.64 ± 0.30	8.29 ± 0.29	8.79 ± 0.43	6.34 ± 0.36	8.03 ± 0.28	6.71 ± 0.35

Values are means ± SE for the number (N) of animals indicated.
Cerebral perfusion fluid flow and intravascular volume were determined during perfusion of the right cerebral hemisphere with HCO_3-buffered physiological saline.
PS values for D-[^{14}C] mannitol were determined by programmed i.v. infusion (see Materials and Methods).

higher mean plasma glucose concentration in the treated animals. The adjustment was made using regional kinetic constants for blood-brain glucose transfer determined in coetaneous suckling rats.*

Analysis of variance showed that for thiamine and L-lysine mean PS values were significantly greater ($P < 0.05$) across brain regions in lead-treated rats compared with controls, and that for L-histidine there was a significant ($P = 0.05$) treatment/region interaction (Table 4). Taking lead-treated and control animals together, region to region differences were highly significant with all substrates except L-lysine. Testing for differences at a regional level using least significant difference criteria showed that for L-lysine, L-histidine and thiamine, the differences in PS-product between lead-treated and control rats were greatest in the cerebral cortex (Table 3). With thiamine, and to a lesser extent L-lysine, the differences in PS product were also apparent in other brain regions.

The uptake of glucose into regions of the cerebral hemisphere was not significantly altered by chronic low-level lead treatment (Table 3). Similar results were obtained for the hind-brain, e.g. mean uptake (± SE) in cerebellum was 0.85 ± 0.05 and 0.82 ± 0.04, and in the brain-stem 1.03 ± 0.05 and 1.00 ± 0.05 $\mu mol\ min^{-1}\ g^{-1}$ wet tissue in control and lead-treated rats, respectively. The average control value is similar to the glucose uptake estimate for whole brain obtained by Daniel *et al.* [37] in 3-week-old rats.

DISCUSSION

In neonatal rats, the neurological signs and vascular encephalopathic changes that are associated with acute lead poisoning generally occur at blood lead levels > 200–500 $\mu g\ dl^{-1}$ [41], whereas in children, the effective threshold for symptomatic intoxication is lower, starting at approximately 80–100 $\mu g\ dl^{-1}$ [4]. Nevertheless, in the rat, exposure protocols that produce blood lead levels as low as 20–50 $\mu g\ dl^{-1}$ have been associated with neurobehavioural deficits [13, 42], impaired or delayed synaptogenesis [43, 44], and alterations in neurotransmitter systems and mechanisms of energy metabolism [12, 45–47]. Thus, although the rat appears to have a greater resistance to frank lead poisoning, neurobehavioural and neurochemical changes have been produced in this species at blood lead levels not uncommon in children from many urban communities.

The treatment regime used in the present study produced blood lead concentrations in the neonates within the range associated with subclinical effects in children (20–80 $\mu g\ dl^{-1}$) and these levels were maintained throughout the period of maximal postnatal brain growth and development [23]. Moreover, there was no effect of treatment on the growth rate of the neonates during the nursing period. The occurrence of undernutrition in experimental animals has been a common confounding feature of studies of lead neurotoxicity, and it has been argued that where growth retardation is evident, any changes in indices

* Moorhouse, unpublished data.

Table 3. Regional brain uptake of glucose and cerebrovascular PS products for seven other metabolites in 19 to 21-day-old control rats and rats exposed to lead via milk throughout suckling

	Treatment Group	N	Frontal cortex	Parietal cortex	Occipital cortex	Caudate-putamen	Hippocampus	Thalamus
Thiamine	Control	6	1.16 ± 0.21	1.46 ± 0.95	1.19 ± 0.13	0.85 ± 0.20	1.00 ± 0.19	0.92 ± 0.22
($sec^{-1} \times 10^4$)	Lead	6	1.75 ± 0.22**	1.55 ± 0.09	1.68 ± 0.06*	1.36 ± 0.06*	1.42 ± 0.12*	1.38 ± 0.09*
Adenine	Control	6	1.92 ± 0.10	2.15 ± 0.10	1.94 ± 0.10	1.82 ± 0.06	1.96 ± 0.09	1.96 ± 0.12
($sec^{-1} \times 10^3$)	Lead	6	2.20 ± 0.06	2.24 ± 0.06	2.37 ± 0.28	1.95 ± 0.05	1.90 ± 0.04	2.20 ± 0.11
L-Lysine	Control	6	0.64 ± 0.01	0.86 ± 0.04	0.84 ± 0.04	0.78 ± 0.05	0.86 ± 0.06	0.83 ± 0.05
($sec^{-1} \times 10^2$)	Lead	6	1.00 ± 0.08***	1.05 ± 0.04*	0.94 ± 0.07	1.00 ± 0.04*	0.93 ± 0.06	0.97 ± 0.06
L-Histidine	Control	6	0.66 ± 0.07	1.11 ± 0.06	0.97 ± 0.16	0.94 ± 0.07	1.04 ± 0.06	1.03 ± 0.11
($sec^{-1} \times 10^2$)	Lead	7	0.98 ± 0.05**	1.08 ± 0.07	1.09 ± 0.06	1.00 ± 0.04	1.03 ± 0.03	1.04 ± 0.06
Choline	Control	7	0.18 ± 0.03	0.21 ± 0.02	0.18 ± 0.01	0.22 ± 0.01	0.18 ± 0.01	0.21 ± 0.02
($sec^{-1} \times 10^2$)	Lead	7	0.15 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.21 ± 0.02
Pyruvate	Control	8	1.08 ± 0.21	1.11 ± 0.17	0.96 ± 0.14	0.84 ± 0.19	0.80 ± 0.10	0.93 ± 0.13
($sec^{-1} \times 10^2$)	Lead	8	1.14 ± 0.09	1.31 ± 0.05	1.07 ± 0.08	0.94 ± 0.13	0.93 ± 0.05	1.07 ± 0.06
3-Hydroxybutyrate	Control	8	0.36 ± 0.02	0.39 ± 0.03	0.36 ± 0.03	0.38 ± 0.04	0.33 ± 0.03	0.35 ± 0.04
($sec^{-1} \times 10^2$)	Lead	8	0.31 ± 0.02	0.32 ± 0.01	0.32 ± 0.01	0.30 ± 0.02	0.27 ± 0.01	0.27 ± 0.02
D-Glucose	Control	6	1.08 ± 0.06	1.06 ± 0.05	0.97 ± 0.05	0.96 ± 0.05	0.89 ± 0.05	1.07 ± 0.05
($\mu mol\ min^{-1}\ g^{-1}$)	Lead	7	1.03 ± 0.05	1.03 ± 0.04	0.93 ± 0.04	0.91 ± 0.04	0.86 ± 0.04	1.04 ± 0.05

Values are mean ± SE.

Significance of difference from control (ANOVA): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

PS products were determined by *in situ* perfusion of the right cerebral hemisphere with HCO_3-buffered physiological saline. Regional brain uptake of glucose was determined *in vivo* by programmed i.v. infusion. Control glucose uptake rates were adjusted (see methods) to values corresponding to the mean arterial plasma glucose concentration in the lead-treated rats (8.7 mM).

Table 4. Summary of F-ratio probability from ANOVA applied to regional brain PS-product for various substrates in lead-treated and control 19–21-day-old rats

Substrate	Source of variation		
	Treatment	Region	Treatment/region
Lysine	0.009†	0.288	0.091
Histidine	0.142	<0.001‡	0.050*
Adenine	0.052	0.032*	0.288
Thiamine	0.014*	<0.001‡	0.263
Choline	0.259	<0.001‡	0.755
Pyruvate	0.451	<0.001‡	0.927
3-hydroxybutyrate	0.061	<0.001‡	0.587

ANOVA was carried out using the GENSTAT statistical program [39]: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$.

of brain development and function could as easily be attributed to malnutrition as to ingested lead [31]. The depressed weight gain of neonates is probably due in part at least to diminished milk production by undernourished dams as a consequence of the aversion to food or fluid containing lead salts [48]. Bornschein *et al.* [49] and Carmichael *et al.* [32] studied the relationships between dose, blood lead concentration and general somatic effects in rodents and found that concentrations of lead in maternal drinking water above 0.2% (1000 ppm Pb) reduced weight, fluid and food intake of dams and produced growth retardation in the nursing young. Carmichael *et al.* [32] further showed that the blood lead levels at which these changes occurred were in excess of 180 $\mu g\,dl^{-1}$ in the dams and 90 $\mu g\,dl^{-1}$ in the offspring. In the present study, fluid and food intake were depressed in some dams at the start of treatment, but in most cases the reaction to the apparent unpalatability of the drinking solution was short-lived. However, our findings suggest that the threshold lead dose at which non-specific nutritional effects may become significant is probably lower than that determined by Bornschein *et al.* [49] and Carmichael *et al.* [32].

Cerebrovascular permeability to the diffusion-limited non-electrolyte mannitol provided a quantitative regional index of BBB integrity in this study. There was no change in BBB permeability to mannitol in the brain regions examined after low-dose treatment; increased "leakiness" did not appear to explain the increased uptake of some metabolic substrates in certain brain regions. As the observations were made at the end of a 3-week exposure period, the possibility of a transient alteration in barrier integrity at an earlier stage of treatment cannot be ruled out. Such a brief, reversible change in cerebrovascular integrity has been reported previously [50], albeit at higher exposure levels (blood lead > 300 $\mu g\,dl^{-1}$) than that used in the present study.

The close correspondence of the regional PS values for mannitol obtained for untreated suckling rats in the present study, and those reported previously for the adult rat [36, 40], shows that the restrictive properties of the BBB are present at an early stage of development. This accords with previous observations in whole brain [25, 51]. The data further indicate that there are no significant regional differences in BBB permeability to mannitol in the three-week-old developing rat. This confirms and extends previous work by Johanson [52], who showed that BBB permeability to mannitol was similar in the cortex and cerebellum of 3-week-old rats, although significantly greater in the cerebellum than in the cortex at earlier stages of postnatal development.

The transfer of metabolic substrates across the BBB was not impaired by low-dose lead treatment in any of the brain regions examined. These findings support previous observations in whole brain [29, 30] using the intracarotid bolus injection technique [53]. Inhibition of glucose transport following lead treatment has been demonstrated *in vitro*, using isolated rat brain microvessels [54] and cultured endothelial cell monolayers [55]. However, these findings have not been substantiated *in vivo*, either by the present study, or by previous work [29]. The reason for this disparity in results is at present unknown.

The instances of increased uptake of metabolites in some brain regions may be due to selective alterations in cerebral metabolism induced by the lead treatment. Evidence from studies on some essential substrates (ketone bodies, glucose, amino acids) indicates a close coupling between cerebral metabolism and transport at the BBB [21 for review]. Thus the observed increases in PS-product may reflect lead-induced differences in region-specific demand for these substances. Cortical regions, notably the frontal cortex, showed the most significant differences. The changes may indicate a biochemical lesion or altered substrate utilization associated with repair processes, although to our knowledge, the frontal cortex has not been identified histopathologically as a target for lead. If not attributable to these processes, it is possible that the lead treatment delayed maturation of the developing brain by a mechanism unrelated to the nutritional status of the animals [43]. Brain uptake rates of many amino acids and other essential substrates are high soon after birth and fall progressively during the first few weeks of life [27, 56–58]. These transport modulations are thought to signify a fall-off in tissue demand as maturation proceeds. Thus a lead-induced delay in maturation might, in susceptible regions, extend the

period during which tissue substrate requirements remain high.

Finally this study shows that with appropriate modifications, the *in situ* brain perfusion technique [36] can be used to investigate developmental aspects of BBB function.

Acknowledgements—This work was supported by the Commission of the European Communities, Environmental Research Programme, Contract No. ENV-781-UK, and by the Wellcome Trust.

REFERENCES

1. Blackman SS, The lesions of lead encephalitis in children. *Bull Johns Hopkins Hospital* **61**: 1–61, 1937.
2. Byers R and Lord E, Late effects of lead poisoning on mental development. *Am J Dis Child* **66**: 471–494, 1943.
3. Pentschew A, Morphology and morphogenesis of lead encephalopathy. *Acta Neuropathol* **5**: 133–160, 1965.
4. U.S. Environmental Protection Agency, Air quality criteria for lead. Research Triangle Park, NC: Health Effects Research Lab, Criteria and Special Studies Office; EPA report No. EPA-600/8-77-017. Available from: NTIS, Springfield, VA; PB-280411, 1977.
5. Needleman HL, Gunnoe CG, Leviton A, Reed RR, Peresie H, Maher C and Barrett P, Deficits in psychologic and classroom performance of children with elevated dentine lead levels. *N Eng J Med* **300**: 584–695, 1979.
6. Yule W, Lansdown R, Millar IB and Urbanowicz M, The relationship between blood lead concentrations, intelligence and attainment in a school population: A pilot study. *Dev Med Child Neurol* **23**: 567–576, 1981.
7. Winneke G, Kramer U, Brockhaus A, Ewers U, Kujanek G, Lechner H and Janke W, Neuropsychological studies in children with elevated tooth-lead concentrations. Part II Extended study. *Int Arch Occup Environ Health* **51**: 231–252, 1983.
8. Hunter J, Ubanowicz MA, Yule W and Lansdown R, Automated testing of reaction time and its association with lead in children. *Int Arch Occup Environ Health* **57**: 27–34, 1985.
9. Yule W, Methodological and statistical issues. In: *The Lead Debate: The Environment, Toxicology and Child Health* (Eds. Lansdown R and Yule W), pp. 193–216. Croom Helm, London and Sydney, 1986.
10. Needleman HL, Low level lead exposure in the foetus and young child. *Neurotoxicology* **8**: 389–394, 1987.
11. Pentschew A and Garro F, Lead encephalo-myelopathy of the suckling rat and its implications on the porphyrinopathic nervous diseases. *Acta Neuropathol* **6**: 266–278, 1966.
12. Winder C, *The Developmental Neurotoxicity of Lead.* MTP Press Ltd, Lancaster, Boston, The Hague, Dordrecht, 1984.
13. Winneke, G, Animal Studies. In: *The Lead Debate: The Environment, Toxicology and Child Health* (Eds. Lansdown R and Yule W), pp. 217–234. Croom Helm, London and Sydney, 1986.
14. Winder C, Garten LL and Lewis PD, The morphological effects of lead on the developing central nervous system. *Neuropath Appl Neurobiol* **9**: 87–108, 1983.
15. Thomas JA, Dallenbach FD and Thomas M, The distribution of radioactive lead (^{210}Pb) in the cerebellum of developing rats. *J Pathol* **109**: 45–50, 1973.
16. Toews AD, Kolber A, Hayward J, Krigman MR and Morrell P, Experimental lead encephalopathy in the suckling rat: concentration of lead in cellular fractions enriched in brain capillaries. *Brain Res* **147**: 131–138, 1978.
17. Silbergeld EK, Wolinsky JS and Goldstein GW, Electron probe microanalysis of isolated brain capillaries poisoned with lead. *Brain Res* **189**: 369–376, 1980.
18. Stumpf WE, Sar M and Grant LD, Autoradiographic localization of [^{210}]Pb and its decay products in rat forebrain. *Neurotoxicology* **1**: 593–606, 1980.
19. van Deurs B, Structural aspects of brain barriers, with special reference to the permeability of the cerebral endothelium and choroidal epithelium. *Int Rev Cytol* **65**: 117–191, 1980.
20. Bradbury MWB, *The Concept of a Blood–Brain Barrier.* Wiley, Chichester, U.K., 1979.
21. Pardridge WM, Brain metabolism: a perspective from the blood–brain barrier. *Physiol Rev* **63**: 1481–1535, 1983.
22. Campbell JB, Woolley DE, Vijayan VK and Overmann SR, Morphometric effects of postnatal lead exposure on hippocampal development of the 15-day-old rat. *Dev Brain Res* **3**: 595–612, 1982.
23. Dobbing J, The later development of the central nervous system and its vulnerability. In: *Scientific Foundations in Pediatrics* (Eds. Davis J A and Dobbing J), pp. 565–577. Heinemann Medical Books, London, 1973.
24. Pratt OE, Adequate nutrition of the developing brain. In: *Advances in Perinatal Neurology* I (Eds. Korobkin R and Guilleminault C), pp. 21–55. Spectrum Publications, New York, 1979.
25. Braun LD, Cornford EM and Oldendorf WH, Newborn rabbit blood–brain barrier is selectively permeable and differs substantially from the adult. *J Neurochem* **34**: 147–152, 1980.
26. Cremer JE and Cunningham VJ, Properties of transport processes of the blood–brain barrier during development. In: *Advances in Physiological Science*, Vol. 7. *Cardiovascular Physiology, Microcirculation and Capillary Exchange* (Eds. Kovach AGB, Hamar J and Szabo L), pp. 299–306. Pergamon Press, London, 1981.
27. Cornford EM, Braun LD and Oldendorf WH, Developmental modulations of blood–brain barrier permeability as an indicator of changing nutritional requirements in the brain. *Pediat Res* **16**: 324–328, 1982.
28. Lorenzo AV and Gewirtz M, Inhibition of [^{14}C]tryptophan transport into brain of lead exposed neonatal rabbits. *Brain Res* **132**: 386–392, 1977.
29. Lefauconnier JM, Lavielle E, Terrien N, Bernard G and Fournier E, Effect of various lead doses on some cerebral capillary functions in the suckling rat. *Toxicol Appl Pharmacol* **55**: 467–476, 1980.
30. Michaelson IA and Bradbury M, Effect of early inorganic lead exposure on rat blood–brain barrier permeability to tyrosine and choline. *Biochem Pharmacol* **31**: 1881–1885, 1982.
31. Michaelson IA, An appraisal of rodent studies on the behavioural toxicity of lead: the role of nutritional status. In: *Lead Toxicity* (Eds. Singhal RL and Thomas JA), pp. 301–365. Urban and Schwarzenborg, Baltimore, 1980.
32. Carmichael NG, Winder C and Lewis PD, Dose response relationships during perinatal lead administration in the rat. A model for the study of lead effects on brain development. *Toxicology* **21**: 117–128, 1981.
33. Fernandez FJ and Hilligoss D, An improved graphite furnace method for the determination of lead in blood using matrix modification and the L'vov platform. *Atom Spectros* **3**: 130–131, 1982.
34. Deane BR, Greenwood J, Lantos PL and Pratt OE, The vasculature of experimental brain tumours. 4. The

quantification of vascular permeability. *J Neurol Sci* **65**: 59–68, 1984.
35. Daniel PM, Love ER, Moorhouse SR, Pratt OE and Wilson P, A method for rapidly washing the blood out of an organ or tissue of the anaesthetized living animal. *J Physiol* **237**: 11–12, 1974.
36. Takasato Y, Rapoport SI and Smith QR, An *in situ* brain perfusion technique to study cerebrovascular transport in the rat. *Am J Physiol* **247**: H484–H493, 1984.
37. Daniel PM, Love ER and Pratt OE, The effect of age upon the influx of glucose into the brain. *J Physiol* **274**: 141–148, 1978.
38. Cunningham VJ, Hargreaves RJ, Pelling D and Moorhouse SR, Regional blood–brain glucose transfer in the rat: a novel double-membrane kinetic analysis. *J Cereb Blood Flow Metab* **6**: 305–314, 1986.
39. Alvey N, Galway N and Lane P, *An Introduction to GENSTAT*. Academic Press, London, 1982.
40. Ohno K, Pettigrew KD and Rapoport SI, Lower limits of cerebrovascular permeability to nonelectrolytes in the conscious rat. *Am J Physiol* **235**: H299–H307, 1978.
41. Winder C and Carmichael NG, The experimental administration of lead to rats in neuropathological and behavioural studies. *J Appl Toxicol* **5**: 140–147, 1985.
42. Cory-Slechta DA, Weiss B and Cox C, Performance and exposure indices of rats exposed to low concentrations of lead. *Toxicol Appl Pharmacol* **78**: 291–299, 1985.
43. Bull RJ, McCauley PT, Taylor DH and Croften KM, The effects of lead on the developing central nervous system of the rat. *Neurotoxicology* **4**: 1–18, 1983.
44. Cookman GR, King W and Regan CM, Chronic low-level lead exposure impairs embryonic to adult conversion of the neural cell adhesion molecule. *J Neurochem* **49**: 399–403, 1987.
45. McCauley PT, Bull RJ and Lutkenhoff SD, Association of alterations in energy metabolism with lead-induced delays in rat cerebral cortical development. *Neuropharmacology* **18**: 93–101, 1979.
46. Sterling GH, O'Neill KJ, McCafferty MR and O'Neill JJ, Effects of chronic lead ingestion by rats on glucose metabolism and acetylcholine synthesis in cerebral cortex slices. *J Neurochem* **39**: 592–596, 1982.
47. Rossouw J, Offermeier J and van Rooyen JM, Apparent central neurotransmitter receptor changes induced by low level lead exposure during different developmental phases in the rat. *Toxicol Appl Pharmacol* **91**: 132–139, 1987.
48. Michaelson IA and Sauerhoff MW, Animal models of human disease: Severe and mild lead encephalopathy in the neonatal rat. *Environ Health Perspect* **7**: 201–225, 1974.
49. Bornschein RL, Fox DA and Michaelson IA, Estimation of daily exposure in neonatal rats receiving lead via dams milk. *Toxicol Appl Pharmacol* **40**: 577–587, 1977.
50. Sundström R, Müntzing K, Kalimo H and Sourander P, Changes in the integrity of the blood–brain barrier in suckling rats with low dose lead encephalopathy. *Acta Neuropathol* **68**: 1–9, 1985.
51. Saunders NR, Ontogeny of the blood–brain barrier. *Exp Eye Res (Suppl)* 523–550, 1977.
52. Johanson CE, Permeability and vascularity of the developing brain: cerebellum vs cerebral cortex. *Brain Res* **190**: 3–16, 1980.
53. Oldendorf WH, Brain uptake of radio-labelled amino acids, amines and hexoses after arterial injection. *Am J Physiol* **221**: 1629–1639, 1971.
54. Kolber AR, Krigman MR and Morell P, The effect of *in vitro* and *in vivo* lead intoxication on monosaccharide transport in isolated rat brain microvessels. *Brain Res* **192**: 513–521, 1980.
55. Maxwell K, Vinters HV, Berliner JA, Bready JV and Cancilla PA, Effect of inorganic lead on some functions of the cerebral microvessel endothelium. *Toxicol Appl Pharmacol* **84**: 389–399, 1986.
56. Baños G, Daniel PM, Moorhouse SR and Pratt OE, The entry of amino acids into the brain of the rat during the post-natal period. *J Physiol* **213**: 45–46, 1971.
57. Baños G, Daniel PM and Pratt OE, The effect of age upon the entry of some amino acids into the brain, and their incorporation into cerebral protein. *Dev Med Child Neurol* **20**: 335–346, 1978.
58. Lefauconnier J-M and Trouvé R, Developmental changes in the pattern of amino acid transport at the blood–brain barrier in rats. *Dev Brain Res* **6**: 175–182, 1983.