Effect of undernutrition on the metabolism of phospholipids and gangliosides in developing rat brain

BY P. V. REDDY AND P. S. SASTRY

Department of Biochemistry, Indian Institute of Science, Bangalore-560 012, *India*

(*Received* 26 *October* 1977 – *Accepted* 3 *February* 1978)

1. Phospholipid content of brains of 3- or 8-week-old undernourished rats was 7–9% less than that for the corresponding control animals and this deficit could not be made up by rehabilitation. Phosphatidyl ethanolamine and plasmalogen were the components most affected in brains of undernourished rats.

2. Incorporation of ^{32}P into phospholipids by brain homogenates was 28% higher in 3-week-old undernourished rats. It is suggested that enhanced phospholipid metabolism in undernourished animals may be related to behavioural alterations noted previously (Sobotka, Cook & Brodie, 1974).

3. Ganglioside concentrations in 3- and 8-week-old undernourished animals were 14% and 11·5% less respectively than those of the control animals and this difference could be made up by rehabilitation. [^{14}C]Glucosamine incorporation in vivo into brain gangliosides was not affected by undernutrition.

It is well recognized that undernutrition during the vulnerable period of growth impairs brain maturation and might lead to an irreversible deficit in higher mental function (Dobbing, 1971). Morphological studies have shown that migration of cells is retarded, proliferation of neuronal fibres decreased and formation of synapses and myelin reduced in rats undernourished from birth to 35 d of age by increasing the litter size and also by limiting the suckling time to 16 h/d (Bass, Netsky & Young, 1970*a*). Parallel biochemical investigations showed significantly lower levels of myelin lipid components in undernourished rat brains. Thus undernourishment produced by increasing the litter size during the suckling period (Benton, Moser, Dodge & Carr, 1966; Davison & Dobbing, 1966; Bass, Netsky & Young, 1970*b*; Geison & Waisman, 1970) or by limiting the food intake from day 5 to day 60 (Culley & Mertz, 1965; Culley & Lineberger, 1968) resulted in lowered levels of total lipid, cerebrosides, cholesterol and proteolipids in rat brains. In contrast, Culley & Mertz (1965), Culley & Lineberger (1968), Geison & Waisman (1970) and Ahmad & Rahman (1975) reported that the brain phospholipid levels are unaltered in postnatally undernourished rats. However, Rajalakshmi, Nakhasi & Ramakrishnan (1974) as well as Ghittoni & Faryana de Raveglia (1972) noted decreased levels of phospholipids in rats undernourished during the suckling period by increasing the litter size. Similarly, while Geison & Waisman (1970) found no change in the ganglioside content, Bass *et al.* (1970*a*), Ghittoni & Faryana de Raveglia (1972) and Krigman & Hogan (1976) observed considerably lowered levels of gangliosides in brains of rats undernourished postnatally. Thus the reports on the effect of postnatal undernutrition on phospholipids and gangliosides are not unequivocal. It is known that phospholipids preponderate in neuronal and synaptic membranes and the latter are particularly rich in gangliosides (Tettamanti, 1971). Furthermore, these lipid components may be involved in membrane phenomena and thus their metabolism in undernourished brains would be of interest. We have therefore investigated the effect of undernutrition on the quantitative changes and metabolism of phospholipids and gangliosides in developing brain.

EXPERIMENTAL

Animals and diet. Albino rats of the strain bred in this Institute were used in these investigations and were fed on a stock diet (Hindustan Lever Research Ltd, Bombay, India) which

contained 250 g protein/kg. Undernutrition was induced by increasing the litter size from six pups in the control group to eighteen pups in the undernourished group (Widdowson & McCance, 1960). The pups were weaned on the 21st day after birth. At this stage, rats were either killed or continued to be undernourished or rehabilitated up to 8 weeks of age. During this period, rats were kept three per cage. Rats in the undernourished group were fed restricted amounts of diet (one-third the amount consumed by the control rats). Rats in the control and rehabilitated groups received food *ad lib.* All the rats were given water *ad lib.*

Killing of animals and removal of brains. Weanling rats were killed by decapitation and 8-week-old rats were killed by cervical dislocation. The whole brains including cerebellum but excluding olfactory lobes were removed to prechilled beakers kept in ice and processed immediately.

Analytical methods. Brain lipids were extracted according to the method of Folch, Lees & Sloane-Stanley (1957). Gangliosides were extracted as described by Suzuki (1964). Total lipids were determined gravimetrically. Lipid-phosphorus was estimated according to the method of Bartlett (1959). Lipid-galactose was hydrolyzed as described by Mallov, McKibbin & Robb (1953) and the sugar content measured by the phenol–sulphuric acid method (Roughan & Batt, 1968) with galactose as the standard. Cholesterol was determined by the method of Hanel & Dam (1955). Phospholipids were separated on thin-layer plates coated with 0·25 mm thick silica gel H (Acme's Laboratory Chemicals, Bombay, India) with the solvent system, chloroform–methanol–acetone–acetic acid–water (50:25:18:2:1·6, by vol.). After separation, phospholipids were located by exposure to iodine vapour and P content was determined after removal of areas of silica gel corresponding to the individual phospholipids. The recovery of phospholipid components from thin-layer plates was 95 ± 3 %. Total plasmalogen content was determined by the I_2-uptake method of Williams, Anderson & Jasik (1962). Total gangliosides were estimated by measuring lipid-bound N-acetylneuraminic acid (NANA) according to Svennerholm (1957). Gangliosides were separated by thin-layer chromatography using silica gel C (Acme's Laboratory chemicals) with chloroform–methanol–2·5 M-ammonia (60:35:8, by vol.) as the solvent system. Gangliosides were located by exposure to I_2 vapour and quantitated by measuring the NANA content as described by Suzuki (1964).

Incorporation of ^{32}P into phospholipids by brain homogenates. Brains were homogenized manually in a loose-fitting homogenizer with (/g tissue) 14 ml 0·02 M-Tris-HCl buffer, pH 7·4, containing 0·2 M-sucrose and 0·00044 M-EDTA. Usually ten 'strokes' up and down were given to obtain a uniform homogenate. The homogenates were incubated as described by McMurray, Strickland, Berry & Rossiter (1957). The incubation mixture in a total volume of 4 ml was taken into a 25 ml flask which contained (M): sodium pyruvate 0·013, sodium malate 0·002, $MgCl_2$ 0·008, NAD 0·0005, cytochrome *c* $8{\cdot}3 \times 10^{-6}$, AMP 0·0025, NaF 0·012, NaH_2PO_4 0·005, CTP 0·0005, Tris-HCl buffer (pH 7·4) 0·015, [^{32}P]orthophosphoric acid (Bhabha Atomic Research Centre, Bombay, India; 300 μCi, neutralized with sodium bicarbonate to pH 7·0) and 3 ml of the homogenate. All solutions were made in water and pH was adjusted to 7·4. The flasks were shaken in a Dubnoff metabolic incubator at 37°. After incubation, lipids were extracted and phospholipids were separated using silicic acid-impregnated paper chromatography (Hokin & Hokin, 1958). Individual phospholipids on the chromatogram were located by staining with Rhodamine 6 G and by autoradiography. Radioactivity in the phospholipid components was determined with an 'end-window' counter (Nuclear-Chicago Corporation, Desplaines, Illinois, USA) with 18 % efficiency.

In vivo incorporation of [^{14}C]glucosamine into gangliosides. D-[1-^{14}C]glucosamine hydrochloride (specific activity 52 mCi/mmol; Radiochemical Centre, Amersham, Bucks., UK) was injected intraperitoneally at the rate of 1 μCi/10 g body-weight and the rats were killed

after 8 h. Gangliosides were extracted and separated by thin-layer chromatography. Radioactivity in gangliosides was measured using a liquid-scintillation counter (Model LS-100; Beckman Instruments, Inc., Fullerton, California 92634, USA) with 90 % efficiency using 5 ml 2,5-diphenyloxazole in toluene (5 g/l) as the scintillation fluid.

RESULTS

Body- and brain-weights. With the experimental design used, undernutrition during the suckling period drastically retarded the growth rate. At 3 weeks of age the undernourished rats showed mean body- and brain-weight deficits of 48 and 16 % respectively. When undernutrition was prolonged until 8 weeks of age, the body-weight deficit increased to 54·6 %, but the brain-weight deficit was only 15 %. These deficits were statistically significant ($P < 0.001$). Rehabilitation of undernourished rats from the 3rd week to the 8th week increased the body- and brain-weights. However, these were still significantly lower than the control values, the deficits in body- and brain-weight being 33 ($P < 0.001$) and 12·1 % ($P < 0.01$) respectively.

Brain lipids. The concentrations of total lipids as well as those of the various lipid classes were significantly lower in undernourished rats at both 3 and 8 weeks of age (Table 1). However, the magnitude of the effect varied among the lipid classes. The effect of undernutrition was most pronounced in the galactolipids which were reduced by 23 % and 24·7 % in 3- and 8-week-old rats respectively. The effect on cholesterol levels was relatively less, the reductions being 5·6 and 11·3 % respectively in 3- and 8-week-old undernourished rats. On the other hand, phospholipid concentration, although statistically significant ($P < 0.05$) was reduced only by 7–8 % in the undernourished rats at both 3 and 8 weeks of age. When the undernourished rats were rehabilitated from the 3rd to the 8th week, as in the experiments of Rajalakshmi *et al.* (1974), there was an increase in the concentration of all lipid classes, but values were still lower than the control values.

When the individual phospholipid components were analysed, phosphatidyl choline, phosphatidyl ethanolamine and plasmalogens were significantly lower in 3-week-old undernourished rats (Table 2). When undernutrition was continued up to 8 weeks of age, the concentration of phosphatidyl serine was also found to be significantly lower. On rehabilitation from 3 to 8 weeks of age, the levels of all the phospholipid components except plasmalogens became comparable to control values.

Incorporation of ^{32}P into phospholipids by normal and undernourished rat brain homogenates. Incorporation of ^{32}P into lipids by brain homogenates was found to be higher when supplemented with pyruvate + malate. This incubation medium showed a 2·7-fold higher incorporation than with glucose and was further enhanced by the addition of CTP. The rate of incorporation of radioactivity was linear at least up to 20 min. Further experiments were therefore conducted using these incubation conditions.

Table 3 shows the effect of undernutrition on ^{32}P incorporation into phospholipids by brain homogenates. More than 90 % of the radioactivity incorporated into phospholipids was found in phosphatidic acid, phosphatidyl inositol and polyphosphoinositide fractions in all age-groups. In 7-d-old rats, incorporation into total phospholipids was not altered significantly with undernourishment. Phosphatidyl choline showed a statistically significant difference ($P < 0.05$) but the radioactivity associated with this lipid component was negligible. In 14-d-old rats also, nutritional deprivation caused no significant change in the incorporation of radioactivity into total phospholipids. There were statistically significant ($P < 0.01$) but small deficits in phosphatidyl choline, sphingomyelin and polyphosphoinositide fractions. Phosphatidic acid and phosphatidyl inositol fractions, on the other hand remained unaltered. Surprisingly, 21-d-old undernourished brains showed statistically

Table 1. *Brain lipid concentrations of normal, undernourished and rehabilitated rats*†

(Mean values with their standard errors for groups of six rats. Results are expressed as mg/g wet brain tissue; values in parentheses represent changes from control values (%))

Age (weeks)	Treatment	Total lipids Mean	Total lipids SE	Phospholipids Mean	Phospholipids SE	Lipid-galactose Mean	Lipid-galactose SE	Cholesterol Mean	Cholesterol SE
3	Control	62·50	1·170	44·80	0·950	1·48	0·047	11·59	0·078
	Undernourished	54·15	1·480	41·00	0·286	1·14	0·031	10·94	0·153
		(−13·3)**		(−8·5)**		(−23·0)***		(−5·6)**	
8	Control	80·60	1·69	49·11	1·040	3·60	0·076	13·30	0·340
	Undernourished	70·56	3·66	45·60	0·975	2·71	0·102	11·80	0·330
		(−12·5)*		(−7·2)*		(−24·7)***		(−11·3)**	
	Rehabilitated	76·40	2·23	46·13	0·820	2·89	0·085	11·92	0·910
		(−5·2)NS		(−6·1)*		(−19·7)***		(−10·4)*	

NS, not significant ($P > 0·05$).
Statistical significance of difference from control values: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of treatments, see p. 404.

Table 2. *Effect of undernutrition and rehabilitation*† *on the concentrations of individual phospholipids in* 3- *and* 8-*week-old rat brains*

(Mean values with their standard errors for groups of six rats. Results are expressed as μmol/g wet brain tissue; values in parentheses represent changes from control values (%))

Age (weeks)	Treatment	Phosphatidyl choline Mean	Phosphatidyl choline SE	Phosphatidyl ethanolamine Mean	Phosphatidyl ethanolamine SE	Phosphatidyl serine Mean	Phosphatidyl serine SE	Sphingomyelin Mean	Sphingomyelin SE	Phosphatidyl inositol Mean	Phosphatidyl inositol SE	Plasmalogens Mean	Plasmalogens SE
3	Control	22·2	0·29	19·3	0·21	6·4	0·14	1·97	0·18	1·99	0·07	7·7	0·02
	Undernourished	20·7	0·23	16·5	0·35	6·3	0·06	1·98	0·09	2·05	0·12	6·8	0·06
		(−6·8)**		(−14·5)***		(−1·6)NS		(0)		(0)		(−11·8)***	
8	Control	21·5	1·20	22·8	0·86	7·6	0·34	2·88	0·26	2·64	0·17	12·6	0·31
	Undernourished	19·7	1·10	19·3	0·79	6·6	0·23	2·73	0·28	2·44	0·23	9·5	0·41
		(−8·6)NS		(−15·5)**		(−13·4)*		(−5·2)NS		(−7·6)NS		(−24·7)***	
	Rehabilitated	20·8	0·94	20·3	1·10	7·14	0·20	3·04	0·22	2·44	0·10	11·66	0·27
		(−3·5)NS		(−11)NS		(−6·3)NS		(0)		(−7·6)NS		(−7·2)*	

NS, not significant ($P > 0·05$).
Statistical significance of difference from control values: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of treatments, see p. 404.

Table 3. *^{32}P incorporation into phospholipids by brain homogenates of control and undernourished rats† during development*

(Mean values with their standard errors for groups of six rats. Radioactivity (counts per min/g brain tissue)

Age (d)	7				14				21			
	Control		Undernourished		Control		Undernourished		Control		Undernourished	
Phospholipids	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total phospholipids	120700	2550	118720	1930	150110	3100	140850	4160	150500	2030	192160	1570**
Polyphospho-inositides	18430	500	19740	680	26430	700	21790	730**	22090	800	28100	650**
Phosphatidyl inositol	62340	1230	59080	1400	77440	1600	81060	1690	67570	870	77100	540**
Phosphatidic acid	18680	480	20000	600	24930	600	24040	370	19980	470	27280	790**
Sphingomyelin	nd	nd	nd	nd	3120	290	2100	70**	3100	130	3500	110**
Phosphatidyl choline	1450	110	2050	100*	4000	270	2980	140**	4050	320	4680	180
Phosphatidyl serine	nd	nd	nd	nd	2200	240	1600	100*	3130	120	4080	190**
Phosphatidyl ethanolamine	1100	180	1280	160	2450	250	1800	100*	3790	140	4160	250

The incubation mixture in a total volume of 4 ml contained (M): sodium pyruvate 0·013, sodium malate 0·002, $MgCl_2$ 0·008, NAD 0·0005, cytochrome *c* $8{\cdot}3 \times 10^{-6}$, AMP 0·0025, NaF 0·012, NaH_2PO_4 0·005, CTP 0·0005, Tris-HCl buffer (pH 7·4) 0·015; ^{32}P approximately 300 μCi, brain homogenate equivalent to 200 mg tissue. Incubation was carried out in a Dubnoff shaker at 37° for 20 min. Radioactivity was corrected for 10^6 counts/min per μmol P in the medium.

nd, not determined.

Statistical significance of difference from control values: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$; other values were not significant ($P > 0{\cdot}05$).

† For details of treatments, see p. 404.

Table 4. *Effect of undernutrition and rehabilitation*† *on ganglioside concentration at* 3 *and* 8 *weeks of age*

(Mean values with their standard errors for groups of six rats; values in parentheses represent changes from control values (%))

Age (weeks)	Treatment	Total gangliosides (mg NANA/g) Mean	SE	Individual gangliosides‡ (nmol/g wet brain tissue) GM_1 Mean	SE	GD_1a Mean	SE	GD_1b Mean	SE	GT_1 Mean	SE
3	Control	827	40	269	15	390	26	196	13	175	10
	Undernourished	711	24	219	12	335	13	159	7	145	6
		(−14)*		(−19)*		(−14)^{NS}		(−19)*		(−17)*	
8	Control	953	19	285	14	382	15	294	16	224	9
	Undernourished	846	17	228	20	306	6	224	13	152	11
		(−11·5)**		(−19·5)*		(−20)***		(−23·5)**		(−32)***	
	Rehabilitated	926	39	267	6	357	15	292	15	213	12
		(−3)^{NS}		(−6)^{NS}		(−6·5)^{NS}		(−0·5)^{NS}		(−4)^{NS}	

NS, not significant ($P > 0.05$); NANA, *N*-acetylneuraminic acid.
Statistical significance of difference from control values: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† For details of treatments, see p. 404.
‡ Nomenclature of gangliosides according to Svennerholm (1963).

significant ($P < 0.01$) and much higher incorporation of radioactivity into total phospholipids. The increase was approximately 28 % more than in the corresponding controls. The increased incorporation was associated mainly with the acidic lipids, i.e. phosphatidic acid, phosphatidyl inositol and polyphosphoinositides and the effects were highly significant ($P < 0.01$).

Gangliosides. Table 4 shows the effect of undernutrition and rehabilitation on ganglioside concentration in rat brain. In 3- and 8-week-old undernourished rats the total ganglioside concentrations were 14 and 11·5 % lower than the corresponding control values and the deficits were significant ($P < 0.05$). On rehabilitation from the 3rd to the 8th week, there was a substantial increase and the brain total ganglioside concentration became comparable with well-fed rats. Analysis of the individual gangliosides (nomenclature according to Svennerholm, 1963) showed significant decreases in the concentrations of GM_1, GD_1b and GT_1 in undernourished brains and the deficits were more pronounced in GD_1b and GT_1 when nutritional deprivation was continued up to 8 weeks. However, when the rats were rehabilitated from the 3rd week, the concentrations of all the gangliosides became comparable to control values at 8 weeks of age.

In vivo incorporation of [^{14}*C*]*glucosamine into brain gangliosides.* Initial experiments showed that when [^{14}C]glucosamine is injected intraperitoneally into weanling rats, maximum incorporation of the radioactivity into brain gangliosides occurs 8 h after the injection. This period was therefore chosen for subsequent experiments. Results given in Table 5, show that the incorporation of [^{14}C]glucosamine into total or individual gangliosides is not altered in brains from undernourished rats when the radioactivity was expressed per g brain. However, the specific activity of total gangliosides expressed as counts/min per μmol ganglioside was higher in undernourished rats, being approximately 21 % higher than control values. This higher specific activity in undernourished rats was statistically significant ($P < 0.05$). Since there was approximately 14 % less ganglioside in brains from undernourished rats, the higher specific activity observed in this group may be largely attributed to decreased pool size. The specific activities of GM_1, GD_1a, GD_1b and GT_1 were also higher in brains from undernourished rats but these changes were not statistically significant.

Table 5. *In vivo incorporation of* [^{14}C]*glucosamine into gangliosides of* 21*-d-old control and undernourished rat brains*†

(Mean values with their standard errors for groups of six rats)

	Radioactivity							
	counts/min per g				counts/min per mol ganglioside			
	Control		Undernourished		Control		Undernourished	
Gangliosides‡	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total gangliosides	8579	497	8809	583^{NS}	3216§	216	3896§	205*
GM_1	855	76	717	78^{NS}	3250	825	3303	785^{NS}
GD_1a	2850	288	2812	252^{NS}	7426	602	8348	441^{NS}
GD_1b	1674	150	1492	180^{NS}	9460	695	10130	265^{NS}
GT_1	2554	237	2180	135^{NS}	14700	1060	15600	692^{NS}

[^{14}C]glucosamine solution in saline (9 g sodium chloride/l) (1·0 μCi/0·1 ml) was injected intraperitoneally at the rate of 1·0 μCi/10 g body-wt. Animals were killed 8 h after injection and radioactivity in total and individual gangliosides was measured as described on p. 405.

NS, not significant ($P > 0{\cdot}05$).

Statistical significance of difference from control values: * $P < 0{\cdot}05$.

† For details of treatments, see p. 404.

‡ Nomenclature according to Svennerholm (1963).

§ Radioactivity expressed as counts/min per μmol *N*-acetylneuraminic acid.

DISCUSSION

Earlier observations that the concentrations of galactolipids and cholesterol are markedly reduced in brains of rats undernourished during the weanling period indicating a deficiency in myelin synthesis was confirmed by the present experiments. The reports on the nutritional effects on phospholipids and gangliosides which preponderate in other neural membranes are, however, equivocal. Culley & Mertz (1965) who induced undernutrition by limiting the food intake from day 5 to day 20 and Geison & Waisman (1970) who induced undernutrition by increasing the litter size found no change in phospholipid concentration in undernourished rat brains. However, Krigman & Hogan (1976) recently reported a substantial reduction of approximately 50 % in the phospholipid concentration in the brains of rats undernourished more severely by increasing the litter size and restricting the suckling time during the weanling period. Our experiments in which undernutrition was brought about entirely by increasing the litter size, clearly showed a significant reduction in brain phospholipid concentration both in 3- and 8-week-old undernourished rats. The magnitude of the deficit was approximately 7–9 % which could not be made up on rehabilitation from the 3rd to the 8th week. Among the phospholipid components, the concentrations of phosphatidyl ethanolamine and plasmalogens were the most significantly reduced components, while phosphatidyl choline was moderately reduced and others were unaffected. These results compare well with the only other reported values for rat cerebral cortex (Ghittoni & Faryana de Raveglia, 1972), except that these authors found a significant reduction in phosphatidyl inositol also. Approximately 51 % of the total lipid of myelin from 20-d-old 'normal' rat brain is phospholipid. Among the phospholipids, 36·6 % is phosphatidyl ethanolamine, 31·6 % is phosphatidyl choline and the rest is accounted for by other phospholipids (Norton & Poduslo, 1973). Plasmalogens constitute approximately 25 % of phospholipids and occur predominantly as ethanolamine plasmalogens. The marked reductions in plasmalogen levels observed in undernourished brains would thus suggest impaired myelination.

The metabolism of phospholipids as studied by the in vitro incorporation of ^{32}P was not

altered in 7- and 14-d-old undernourished rats. But at the weaning stage, undernourished brains showed a significantly higher incorporation of approximately 28 % more than the controls. This enhanced incorporation was mainly in the acidic-phospholipid components. The physiological significance of this observation is difficult to interpret. It has been suggested that the cholinergic receptor may be a proteolipid which is rich in phospholipids (De Robertis, 1971). Sobotka, Cook & Brodie (1974) showed that a state of heightened emotionality is a most characteristic manifestation of perinatal malnutrition at the weaning stage. This has been attributed to an enhanced activation of the brain stem serotonergic system. It is possible that the higher ^{32}P incorporation into phospholipids has relevance to these findings.

Geison & Waisman (1970) found no change in brain ganglioside concentration when rats were undernourished by increasing the litter size during the weaning period. In contrast, Bass *et al.* (1970*a*) and Krigman & Hogan (1976) noted more than 50 % reduction in the total ganglioside content in the brains of weanling rats undernourished by a combination of increased litter size and restricted suckling time. Essentially similar deficits in ganglioside content in undernourished brains were observed by Ghittoni & Faryana de Raveglia (1972) even when undernutrition was brought about only by increasing litter size. Experiments described here showed a significant reduction in brain ganglioside concentration in postnatally undernourished weanling rat brain but the deficit was only approximately 14–15 %. There is now evidence indicating that the nerve-ending membranes are enriched in gangliosides (Lapetina, Soto & De Robertis, 1967). It is therefore likely that undernutrition affects synaptogenesis. This conclusion is supported by the histological studies of Bass *et al.* (1970*a*) and Cragg (1972) which indicated a reduced number of synapses in undernourished rat brains. Experiments with [^{14}C]glucosamine however suggest that the metabolism of gangliosides in brains from undernourished rats is not altered significantly.

The authors gratefully acknowledge financial support from the Indian Council of Medical Research.

REFERENCES

Ahmad, G. & Rahman, M. A. (1975). *J. Nutr.* **105**, 1090.
Bartlett, G. R. (1959). *J. biol. Chem.* **234**, 466.
Bass, N. H., Netsky, M. G. & Young, E. (1970*a*). *Archs Neurol.* **23**, 289.
Bass, N. H., Netsky, M. G. & Young, E. (1970*b*). *Archs Neurol.* **23**, 303.
Benton, J. W., Moser, H. W., Dodge, P. R. & Carr, S. (1966). *Pediatrics, Springfield* **38**, 801.
Cragg, B. G. (1972). *Brain* **95**, 143.
Culley, W. J. & Lineberger, R. O. (1968). *J. Nutr.* **96**, 375.
Culley, W. J. & Mertz, E. T. (1965). *Proc. Soc. exp. Biol. Med.* **118**, 233.
Davison, A. N. & Dobbing, J. (1966). *Br. med. Bull.* **22**, 40.
De Robertis, E. (1971). *Science, N.Y.* **171**, 963.
Dobbing, J. (1971). *Adv. exp. Med. Biol.* **13**, 399.
Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
Geison, R. L., & Waisman, H. A. (1970). *J. Nutr.* **100**, 315.
Ghittoni, N. E. & Faryana de Raveglia, I. (1972). *Neurobiology* **2**, 41.
Hanel, H. K. & Dam, H. (1955). *Acta chim. scand.* **9**, 677.
Hokin, L. E. & Hokin, M. R. (1958). *J. biol. Chem.* **233**, 805.
Krigman, M. R. & Hogan, E. L. (1976). *Brain Res.* **107**, 239.
Lapetina, E. G., Soto, E. F. & De Robertis, E. (1967). *Biochim. biophys. Acta* **135**, 33.
McMurray, W. C., Strickland, K. P., Berry, J. F. & Rossiter, R. J. (1957). *Biochem. J.* **66**, 634.
Mallov, S., McKibbin, J. M. & Robb, J. S. (1953). *J. biol. Chem.* **201**, 825.
Norton, W. T. & Poduslo, S. E. (1973). *J. Neurochem.* **21**, 759.
Rajalakshmi, R., Nakhasi, H. L. & Ramakrishnan, C. V. (1974). *Indian J. Biochem. Biophys.* **11**, 57.
Roughan, P. G. & Batt, R. D. (1968). *Analyt. Biochem.* **22**, 74.
Sobotka, T. J., Cook, M. P. & Brodie, R. E. (1974). *Brain Res.* **65**, 443.

Suzuki, K. (1964). *Life Sci.* **3**, 1227.
Svennerholm, L. (1957). *Biochim. biophys. Acta* **24**, 604.
Svennerholm, L. (1963). *J. Neurochem.* **10**, 613.
Tettamanti, G. (1971). *Adv. exp. Med. Biol.* **13**, 75.
Widdowson, E. M. & McCance, R. A. (1960). *Proc. Roy. Soc.* B **152**, 188.
Williams, J. N., Anderson, C. E. & Jasik, A. D. (1962). *J. Lipid Res.* **3**, 378.

Printed in Great Britain