Growth and development of rats artificially reared on different milk-substitutes

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1. Rat pups were artificially reared (AR) from postnatal day 4 or 5 till day 20 or 21, by fitting them with gastric cannulas through which milk-substitutes could be infused automatically.

2. Three milk-substitutes were compared: milk M, the usual diet for AR studies, which was somewhat low in protein and very high in carbohydrate; milk A, which resembled rats' milk much more closely in composition; and milk isoM, which was based on the high-energy milk M but was made isoenergetic with milk A. Pups given these diets were termed ARM, ARA and ARisoM respectively. Siblings of the AR rats were left with their mothers to form a mother-reared (MR) control group. Rats were autopsied at 20 or 21 d.

3. Growth in body-weight of all groups of AR pups lagged behind that of their MR siblings for about the first week of AR, but the ARM group showed complete catch-up and the ARA group partial catch-up in body-weight during the second week. ARisoM rats were growth-retarded throughout.

4. Inspection of organ weights expressed relative to body-weight revealed disturbances of organ growth in all AR groups compared with MR animals. ARM rats showed excessive epididymal fat pad and liver weights, but deficits in gastrocnemius muscle, heart and adrenal weights. In contrast, ARA rats usually displayed increased spleen and stomach weights, but lower weight of interscapular brown adipose tissue. ARisoM rats had high brain, liver and stomach weights and low muscle and spleen weights relative to body-weight. All AR groups had elongated small intestines.

5. Hence the patterns of abnormal organ growth differed between groups. Those shown by the ARM and ARisoM groups seemed the more seriously abnormal. The diet approximating the composition of rats' milk (milk A) appears, as intended, to be an improved milk-substitute.

A semi-automatic technique for rearing infant rats without their mothers, which involves fitting the pup with a gastric cannula through which a milk-substitute is infused, was developed by Messer *et al.* (1969). Refinements to the method, made by Hall (1975), rendered it much more suitable for general use. It is potentially a powerful technique for developmental studies in a variety of disciplines, including nutrition, pharmacology and psychology, in which precise control of nutritional and other aspects of the early environment is desirable.

However, it has recently been found that rat pups artificially reared (AR) on the diet formulated by Messer *et al.* (1969) differ from mother-reared (MR) pups in several respects. Their intermediary metabolism is characterized by atypical ketone body and carbohydrate metabolism (Sonnenberg *et al.* 1982) and the growth of several of their organs is abnormal at 'weaning' (Diaz *et al.* 1981; Smart *et al.* 1983*a*). Some of the effects on organ growth persist into adulthood, long after the cessation of artificial rearing (Smart *et al.* 1983*a*). It seems likely that many of these abnormalities may be due to the composition of the milk-substitute, which is low in protein compared with rats' milk (about 6% compared with 9%) and high in carbohydrate (about 9% compared with 3%; Messer *et al.* 1969).

There have been many attempts to produce an improved milk-substitute for artificial rearing, but few of these have been sufficiently encouraging to merit publication. Enriching the 'Messer formulation' with protein appears to have little if any beneficial effect on organ growth (Diaz *et al.* 1982). However, a milk substitute has recently been produced which more closely mimics the composition of rats' milk, and this has been found to permit normal levels in AR rat pups of blood metabolites which are derived from the metabolism of fat, carbohydrate and protein (N. S. Auestad, J. D. Bergstrom, Y. H. Ha and J. Edmond, unpublished results).

The present paper reports on the efficacy of this diet for body and organ growth to 'weaning'. Comparisons were made between naturally-reared rats and rats artificially reared on the 'Messer diet' or on the new formula mimicking rats' milk (N. S. Auestad *et al.* unpublished results). In different experiments these diets were given either as originally formulated or in amounts isoenergetic with each other.

METHODS

General

Rats of a black-and-white hooded Lister stock were fitted with gastric cannulas by the method of Hall (1975) on postnatal day 4 or 5. Details of husbandry and rearing conditions are described by Smart *et al.* (1983*a*). From the day of cannulation till the end of the experiments on day 20 or 21, AR rats were given a milk-substitute by intermittent gastric infusion (0·5 h infusion in every 2 h). In addition, from day 18 they had access to about 3 g of a wet mash, which was renewed daily. The mash was made up from powdered Porton mouse diet (Labsure Animal Foods, Castle Street, Poole, Dorset) and water, in the proportions 6 parts by weight Porton mouse diet to 10 parts water.

The constituents of the Messer formula, milk M, were evaporated cows' milk, distilled water and maize oil, with additional vitamins, minerals, methionine, tryptophan and sodium deoxycholate (for details, see Table 1 of Smart *et al.* 1983*a*). The diet which more closely resembled rats' milk in composition is referred to as milk A (N. S. Auestad *et al.* unpublished results). A base was first produced from skimmed milk powder and evaporated cows' milk through dialysis and concentration steps, to which was added maize oil, medium chain triglycerides, vitamins, minerals and essential amino acids (N. S. Auestad *et al.* unpublished results). This milk-substitute closely resembles rats' milk in its gross and detailed composition, including the quantities of triglycerides to give medium-chain-length fatty acids and long-chain-length fatty acids in the ratio 33:67 by weight (Glass *et al.* 1967; Smith *et al.* 1968; Grigor & Warren, 1980). Free lactose is present at 20–25 g/l, while the protein and amino acid content of the milk is formulated to produce an amino acid profile in serum very similar to that of mother-reared pups (N. S. Auestad *et al.* unpublished results). The gross composition and physical properties of rats' milk, milk A and milk M are given in Table 1. Milk A was made up in Los Angeles and flown, deep-frozen, to England.

In each experiment sixteen pups were cannulated for artificial rearing. These were drawn from seven, seven and six litters respectively in Expts 1, 2 and 3, with no more than three pups taken from any one litter. The remaining pups in the litters, reduced to eight per litter, served as MR controls. The mother's pelleted diet (Porton mouse diet) was freely available to her from a wire basket suspended approximately 55 mm above the floor of the cage and was hence accessible to her MR pups when they were physically capable of reaching it.

Only male pups were cannulated and only males (MR and AR) were dissected at 20 or 21 d. AR young were weighed every day and MR young every 4 d. From day 11 all pups were inspected daily for eye-opening.

Table 1. *Gross composition and physical properties of rats' milk and of milk substitutes M, isoM and A*

	Rats' milk*	Milk M†	Milk isoM‡	Milk A§
Protein (g/l)	92	66	52	80
Carbohydrate (g/l)	30	95	75	34
Fat (g/l)	123	133	105	110
Energy content (kJ/l)‖	6595	7549	5972	5974
Osmolarity (mosmol/l)¶	310	690	—	365
pH	6·5**	6·2††	—	6·4

* Values taken from Dymza *et al.* (1964) except where indicated otherwise.
† Values calculated from information supplied by Nestlé Ltd, except where indicated otherwise.
‡ Based on milk M, but made isoenergetic with milk A (for details, see below).
§ Values from N. S. Auestad *et al.* (unpublished results) except where indicated otherwise.
‖ Conversion factors were: 1 g protein = 17 kJ, 1 g carbohydrate = 16 kJ, 1 g fat = 37 kJ.
¶ Determined using an osmometer, model 3L (Advanced Instruments Inc., Massachusetts).
** Value for days 4–17 of lactation taken from Luckey *et al.* (1954).
†† Value taken from Messer *et al.* (1969).

Expt 1

Rats were artificially reared on milk A or mother-reared from 4 to 21 d. The volumes of milk-substitute infused rose in daily increments from 1·75 ml on the first day to 9 ml on the last.

Expt 2

Rats were artificially reared on milk A or milk M or were mother-reared from 4 to 20 d. As far as possible, male litter-mates were represented in all three treatment groups. To compensate for the possibility of small differences between the infusion pumps and water baths used, pups of the two AR groups were equally represented on each infusion pump and in each water bath. Milk A was fortified with an additional 27 mg ferrous sulphate/l diet. Volumes of milk-substitute given rose from 2·5 to 9·25 ml/d.

Expt 3

Rats artificially reared on milk M had heavier epididymal fat pads than either MR pups (Smart *et al.* 1983*a*) or pups given milk A (present Expt 2). To test whether this might be associated with the higher energy density of milk M (Table 1), a version of this diet was prepared which was isoenergetic (v/v) with milk A. This was achieved by adding more water. Vitamins, minerals, methionine, tryptophan and sodium deoxycholate were added in greater amounts than to the usual M formula to return their concentrations to those of the undiluted formula. Rats were artificially reared on milk A or on isoenergetic M formula (isoM), employing the same control measures as in Expt 2, or were mother-reared from 5 to 20 d. Additional $FeSO_4$ was added to milk A, as in Expt 2. The volumes of milk given were increased from 2·5 ml/d at the beginning to 8 ml/d at the end of the experiment.

Artificially-reared rat pups given the A, M and isoM milk-substitutes are referred to as ARA, ARM and ARisoM rats respectively.

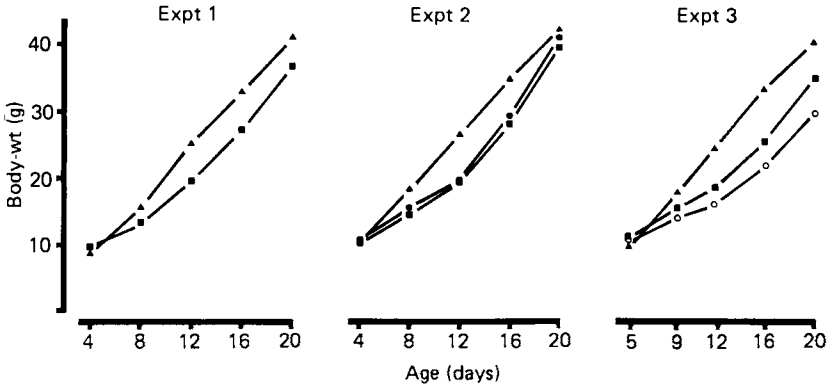

Fig. 1. Mean body-weights (g) from 4 or 5 to 20 d of mother-reared rats (▲) and rats artificially reared on milk A (■), milk M (●) or milk isoM (○). For details of the dietary regimens, see Table 1 and pp. 228–229.

RESULTS

Survival and development

All but one of a total in the three experiments of fifty-three male MR pups survived (98%) till the end of the experiments. Thirty-three out of a total of forty-eight AR pups survived (69%). On rare occasions AR pups pulled their cannulas out and therefore had to be killed. Much more usually deaths were associated with a condition of abdominal distention, colloquially termed 'bloat'. The type of milk-substitute did not influence the incidence of bloating. The numbers of pups which died or were killed because of bloat were as follows: Expt 1, two ARA; Expt 2, four ARA and three ARM; Expt 3, one ARA and three ARisoM. There was a tendency for bloat to occur earlier on the A diet than on either of the M formulas. Four deaths from bloat occurred on or before day 7 on A milk, but none before day 9 on M milk. No such deaths occurred after day 14.

There were no differences in the timing of eye-opening between MR and AR pups or, within the AR group, between pups given the A diet or M diet.

Growth in body-weight

Growth curves for the rats in the three experiments are shown in Fig. 1. The growth of MR rats was virtually linear over the period investigated. However, the growth of all the AR groups was clearly non-linear, reflecting their higher rate of growth in the second half of the AR period than in the first half. All AR groups gained less weight than their MR siblings in the period up to 12 d, but thereafter they put on as much or even more weight, with the exception of the ARisoM group. The growth curve for the ARisoM rats in Expt 3 continued to diverge from those of MR and ARA rats after day 12.

ARA rats weighed less than MR animals at autopsy in Expts 1 and 3, but not in Expt 2 (Tables 4–6, see pp. 232–234). Likewise in Expt 2, the body-weights of ARM and MR rats were similar. However, rats on the ARisoM diet in Expt 3 grew poorly and at autopsy were lighter than both their MR and ARA litter-mates

Body and organ measurements

Mean absolute values (with SE) of body and organ measurements at 20 or 21 d are given in Tables 2 and 3.

ARA v. *MR*. ARA and MR rats were reared in all three experiments. The ARA rats were

Table 2. *Expts 1 and 2. Body and organ measurements at 21 d (Expt 1) and at 20 d (Expt 2) of mother-reared (MR) rats and of rats artificially reared on milk A (ARA) or on milk M (ARM)*

(Mean values with their standard errors; no. of animals in parentheses)

	Expt 1				Expt 2					
	MR(11)		ARA(13)		MR(6)		ARA(4)		ARM(5)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-wt (g)	43·87	1·40	40·20	0·68	42·43	1·36	39·67	0·47	40·95	0·42
Nose–rump length (mm)	117·3	1·6	112·5	1·0	113·3	0·9	109·8	0·3	111·8	0·8
Tibia length (mm)	18·6	0·1	18·2	0·2	18·5	0·2	17·6	0·2	18·1	0·2
Gastrocnemius muscle wt (g)	0·184	0·010	0·168	0·006	0·167	0·004	0·171	0·009	0·146	0·007
Epididymal fat pad wt (g)	0·066	0·004	0·072	0·004	0·049	0·002	0·048	0·006	0·084	0·011
IBAT wt (g)	0·156	0·005	0·127	0·004	0·155	0·009	0·104	0·002	0·140	0·021
Brain wt (g)	1·331	0·026	1·269	0·014	1·404	0·014	1·306	0·020	1·316	0·013
Heart wt (g)	0·238	0·013	0·210	0·006	0·214	0·004	0·192	0·005	0·186	0·003
Kidneys wt (g)	0·417	0·012	0·380	0·005	0·411	0·020	0·405	0·009	0·403	0·012
Adrenals wt (mg)	13·0	0·6	11·2	0·4	14·2	0·6	11·3	0·8	11·6	0·7
Liver wt (g)	2·062	0·081	1·959	0·045	1·752	0·056	1·741	0·025	1·899	0·062
Spleen wt (g)	0·201	0·008	0·193	0·013	0·173	0·011	0·210	0·006	0·150	0·005
Stomach wt (g)	0·307	0·011	0·357	0·007	0·265	0·011	0·341	0·013	0·271	0·004
Small intestine length (mm)	556	20	603	18	542	17	599	10	636	8

IBAT, interscapular brown adipose tissue.

Table 3. *Expt 3. Body and organ measurements at 20 d of mother-reared (MR) rats and of rats artificially reared on milk A (ARA) or milk isoM (ARisoM)*

(Mean values with their standard errors; no. of animals in parentheses)

	MR(6)		ARA(6)		ARisoM(5)	
	Mean	SE	Mean	SE	Mean	SE
Body-wt (g)	40·88	0·87	35·82	0·86	30·45	1·00
Nose–rump length (mm)	113·8	0·8	105·8	1·6	101·8	0·9
Tibia length (mm)	19·2	0·2	18·3	0·2	18·1	0·1
Gastrocnemius muscle wt (g)	0·159	0·008	0·132	0·005	0·097	0·005
Epididymal fat pad wt (g)	0·054	0·005	0·052	0·007	0·042	0·004
IBAT wt (g)	0·193	0·014	0·171	0·014	0·155	0·016
Brain wt (g)	1·323	0·010	1·208	0·007	1·169	0·019
Heart wt (g)	0·205	0·005	0·155	0·005	0·144	0·007
Kidneys wt (g)	0·409	0·017	0·360	0·009	0·295	0·008
Adrenals wt (mg)	13·0	0·4	9·5	0·3	8·7	0·4
Liver wt (g)	1·635	0·050	1·489	0·040	1·345	0·043
Spleen wt (g)	0·154	0·011	0·159	0·007	0·087	0·002
Stomach wt (g)	0·266	0·006	0·316	0·011	0·227	0·004
Small intestine length (mm)	543	9	604	6	602	17

IBAT, interscapular brown adipose tissue.

Table 4. *Expt 1. Body and organ measurements (absolute values and relative values (g/kg body-weight)) at 21 d of rats artificially reared on milk A (ARA) expressed as a percentage of those of mother-reared (MR) rats*

	ARA (% MR)	
	Absolute values	g/kg body-wt
Body-wt	92**	—
Nose–rump length	96*	—
Tibia length	98	—
Gastrocnemius muscle wt	92	101
Epididymal fat pad wt	109	119*
IBAT wt	81***	88*
Brain wt	95*	104
Heart wt	88	96
Kidneys wt	91**	99
Adrenals wt	86*	94
Liver wt	95	104
Spleen wt	96	114*
Stomach wt	116**	127***
Small intestine length	109	—

IBAT, interscapular brown adipose tissue.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's t test).

somewhat smaller than their MR controls. They were shorter in nose–rump length in all experiments and weighed less and had shorter tibias in two experiments (Tables 4–6). They had deficits in whole brain weight and adrenal weight in all experiments and in the weights of heart, kidney and interscapular brown adipose tissue (IBAT) in two experiments. In contrast, they displayed increases in the weight of the stomach and in the length of the small

Table 5. *Expt 2. Body and organ measurements (absolute values and relative values (g/kg body-weight)) at 20 d of rats artificially reared on milk A (ARA) or on milk M (ARM) expressed as a percentage of those of mother-reared (MR) rats, and also ARA values as a percentage of ARM values*

	ARA (% MR)		ARM (% MR)		ARA (% ARM)	
	Absolute values	g/kg body-wt	Absolute values	g/kg body-wt	Absolute values	g/kg body-wt
Body-wt	93	—	97	—	97	—
Nose–rump length	97*	—	99	—	98	—
Tibia length	95*	—	98	—	97	—
Gastrocnemius muscle wt	102	109	87*	90*	117	120*
Epididymal fat pad wt	97	104	171**	178**	57*	58*
IBAT wt	67**	72**	90	93	74	77
Brain wt	93**	99	94**	97	99	102
Heart wt	90*	96	87***	90**	104	107*
Kidneys wt	99	106	98	102	101	104
Adrenals wt	80*	85	82*	84*	97	101
Liver wt	99	106**	108	112**	92	95
Spleen wt	122*	130**	87	90	140***	145***
Stomach wt	129**	138***	102	106	126***	130***
Small intestine length	111*	—	117**	—	94*	—

IBAT, interscapular brown adipose tissue.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's t test).

intestine. Weights of liver, gastrocnemius muscle, spleen and epididymal fat pads were affected in only one experiment or not at all.

Most of these differences in organ weights were in the same direction as the difference in body-weight and, hence, when organ weight was expressed relative to body-weight, several of the differences disappeared. There was no instance of a deficit in ARA rats in relative organ weight which was evident in all experiments, and only IBAT weight was significantly low in two experiments. Relative to body-weight ARA rats had much heavier stomachs (27–38%) in all experiments and heavier spleens in two experiments.

ARM v. *MR*. ARM and MR rats were compared in Expt 2 (Table 5). Despite being normal in whole body-weight, nose–rump length and tibia length, ARM rats had deficits in the weights of gastrocnemius muscle, brain, heart and adrenals. That body-weight was normal in the face of these deficits was probably due to excess fat (71% excess in epididymal fat pad weight) and perhaps also to additional weight through elongation of the small intestine. Expressing organ weights relative to body-weight changed this pattern of differences a little. Relative to body-weight there was no significant difference in brain weight, but the ARM rats had heavier livers.

ARisoM v. *MR*. The ARisoM rats in Expt 3 were markedly growth-retarded (Table 6). Compared with MR controls they had highly significant deficits in whole body-weight, nose–rump and tibia lengths and in the weights of all organs measured, except IBAT and epididymal fat pad. That is, unlike ARM rats in previous experiments, epididymal fat was not increased. Their small intestines were significantly elongated. All but two of the deficits disappeared when body-weight was taken into consideration. Relative to body-weight, only the weights of gastrocnemius muscle and spleen were low, whereas those of brain, liver and stomach were high.

ARA v. *ARM and ARisoM*. The ARA and ARM groups were compared directly in Expt 2

Table 6. *Expt 3. Body and organ measurements (absolute values and relative values (g/kg body-weight)) at 20 d of rats artificially reared on milk A (ARA) or on milk isoM (ARisoM) expressed as a percentage of those of mother-reared (MR) rats, and also ARA values as a percentage of ARisoM values*

	ARA (% MR)		ARisoM (% MR)		ARA (% ARisoM)	
	Absolute values	g/kg body-wt	Absolute values	g/kg body-wt	Absolute values	g/kg body-wt
Body-wt	88**	—	74***	—	118**	—
Nose–rump length	93***	—	89***	—	104	—
Tibia length	95*	—	94***	—	101	—
Gastrocnemius muscle wt	82*	94	61***	82*	135**	115*
Epididymal fat pad wt	95	108	77	104	123	104
IBAT wt	89	101	80	108	110	94
Brain wt	91***	104	88***	119**	103	88**
Heart wt	76***	86*	70***	94	108	91
Kidneys wt	88*	101	72***	97	122***	104*
Adrenals wt	73***	83**	67***	90	109	93
Liver wt	91*	104	82**	110***	111*	94*
Spleen wt	103	118	56***	76*	184***	155***
Stomach wt	119**	135***	85***	115*	139***	118**
Small intestine length	111***	—	111**	—	100	—

IBAT, interscapular brown adipose tissue.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's t test).

(Table 5). ARA rats had much lighter epididymal fat pads than ARM rats and they had shorter small intestines. However, their spleens and stomachs were considerably heavier. These weight differences were still evident when organ weight was expressed relative to body-weight and two more differences emerged. Relative weights of gastrocnemius muscle and heart were higher in ARA rats.

Both ARA and ARisoM rats were reared in Expt 3 (Table 6). The ARA rats were larger in whole body-weight at 20 d, and had heavier gastrocnemius muscles, kidneys, livers, spleens amd stomachs. When the difference in body-weight was taken into account, the ARA rats still had heavier (g/kg body-weight) gastrocnemius muscles, kidneys, spleens and stomachs, but their brains and livers were less heavy.

DISCUSSION

Growth in whole body-weight was generally less satisfactory than we would have wished in these experiments. In Expts 1 and 3 the AR groups had body-weight deficits at autopsy, and even in Expt 2 in which body-weights were normal, they were achieved via abnormal growth curves. Inspection of Fig. 1 reveals that the growth deficiency was confined to the first 7 or 8 d of the experiments, during which AR pups put on conspicuously less weight than their MR siblings. Almost certainly they did not receive sufficient 'milk' during this period to support good growth. ARM rats in a previous experiment (Smart *et al.* 1983a), which had a normal growth curve in body-weight, received about 20% more milk between days 4 and 12 than those in the present experiments. The reason for the lower input of 'milk' was fear of exacerbating bloat. We responded to the first signs of bloat, which sometimes occurred as early as 2 d after the commencement of AR, by making smaller increments in milk input than had been planned. Unfortunately our system, whereby one pump delivers

milk to eight pups at the same rate at any given time, does not permit the milk supply to be tailored to each individual pup's needs. Hence healthy pups may have received less milk than they could have coped with, in order to attempt to protect affected pups.

A detailed discussion of the aetiology of bloat is probably beyond the scope of the present paper and it would, in any case, be wide-ranging and inconclusive. Bloat has been noted since the early days of artificial rearing (Miller & Dymza, 1963; Messer *et al.* 1969) and still appears to be a problem in most laboratories in which artificial rearing is attempted. There is some resemblance between bloat and the condition of human babies (especially premature babies) known as necrotizing enterocolitis (NEC) in both aetiology and symptoms (Diaz *et al.* 1980), but there are also differences. For instance, generalized bleeding, which is a typical sign of human NEC (Brown & Sweet, 1982), appears to be absent from the bloat syndrome. Scientific evidence on bloat is scant. It has been a nuisance rather than an object of study in its own right. However, our distillation of the shared experience of those who have encountered the problem is that its aetiology, like that of NEC, is multifactorial, which makes it difficult to study and to counter.

The principal purpose of the present study was to assess the relative merits of the two milk-substitutes, M and A, in terms of the survival and growth of rat pups. The type of milk-substitute did not influence survival, but there were indications that milk A, which is closer in composition to rats' milk, resulted in more normal organ growth than milk M. The ensuing discussion will be confined to relative organ weights (g/kg body-weight), except where stated otherwise, to avoid complications arising from the presence of body-weight differences between groups in some experiments but not others.

Milk M produced various distortions of body growth, most remarkably in fat deposition, such that ARM rats had excessively heavy (78–90% heavier) epididymal fat pads compared with MR controls (Table 5; Smart *et al.* 1983*a*). They also had heavier livers but lighter gastrocnemius muscles. Two of these differences, i.e. in weights of fat pad and gastrocnemius muscle, are particularly important since they have been found to persist into adulthood, months after the cessation of AR (Smart *et al.* 1983*a*) and, hence, indicate permanent disturbances of growth and not merely ephemeral responses to some aspect of the 'milk' or of the AR situation. ARA rats were much closer to normal in these respects, in that they could not be distinguished statistically from MR animals in two out of three (fat pad) or all three (muscle) of the appropriate comparisons (Tables 4–6). The increased weights of spleen and stomach in ARA, but not in ARM rats, do not seem to us to be particularly worrying consequences in the context of our principal aims, although we should, of course, prefer that they were absent. They are discussed further later. We attribute the apparent improvement of muscle growth and prevention of excessive fat deposition to the greater adequacy of milk A as a nutrient supply, with appropriate amounts of fat, protein, carbohydrate and other known constituents, and with a nutrient impact which is reflected in the appropriate metabolic status for blood substances such as glucose, galactose, individual amino acids, insulin, carnitine and ketone bodies (Sonnenberg *et al.* 1982; N. S. Auestad *et al.* unpublished results).

The possibility that the heavier epididymal fat pads of ARM pups may have been due to the high energy density of milk M compared with either rats' milk or milk A, was tested by giving pups a diluted version of milk M which was isoenergetic with milk A. This speculation was supported by the finding that the epididymal fat pads of ARisoM rats were of similar weight, relative to body-weight, to those of ARA and MR rats (Table 6). The dilution of milk M in the production of isoM milk also had the effect of reducing the protein content to 52 g/l, which is only about 57% of that of rats' milk and 65% of that of milk A (Table 1). This relatively low protein content of isoM milk probably explains why the ARisoM rats had deficits in absolute organ weights compared with MR rats for all the

organs weighed which were not fat depots, and compared with ARA rats for five out of eight non-fat organs.

Weight of spleen relative to body-weight was higher in ARA rats than in either MR or ARM animals (Tables 4–6). The two milk-substitutes, therefore, differed in their effects. Findings from other laboratories do not help to clarify matters, in that relative spleen weights appear to have been normal in the study in which the M milk was first used (Messer *et al.* 1969) but high in a recent experiment with the same milk (Diaz *et al.* 1981). In an early AR study, in which a different milk formula was given by oro-gastric tube, spleen weights were high (Dymza *et al.* 1964). A possible explanation for these disparate findings, including the difference between the two diets used here, is that splenic enlargement reflected infection and that the type or degree of infection varied between studies and between milks. For instance, the relatively enlarged spleens in rats given the A milk in the present investigation may reflect greater colonization by pathogenic bacteria in rats given that milk. Clearly there is scope for microbiological investigation. The spleen is one of the organs which is differentially affected by undernutrition during development (Winick & Noble, 1966) and presumably this is why relative spleen weight was low in rats given the isoM diet, of which the protein content was little more than half that of rats' milk (Table 1).

The gut phenomena are discussed elsewhere (Smart *et al.* 1983*b*; J. Tonkiss, J. L. Smart, N. S. Auestad and J. Edmond, unpublished results) and are currently the subject of further investigation and, hence, will be mentioned only briefly here. There would appear to be a specific effect on the stomach of the type of milk-substitute given, in that stomach weight was greater in ARA but not ARM rats (Tables 4–6; and Smart *et al.* 1983*b*). In contrast, the elongation of the small intestine was found in both AR groups but was more marked in the ARM group. The presence of the effect in both ARA and ARM rats seems to suggest that the cause is some factor common to the two milks, such as their derivation from a cows' milk base, or some non-nutritive aspect of the AR procedure. The greater elongation of the small intestine in ARM rats presumably implicates some specific aspect of the M diet. What evidence there is suggests that these effects of AR on the gut may not be permanent. ARM rats, fed normally on Porton mouse diet from 21 d, showed no differences from MR animals in stomach weight or small intestine length in adulthood (Smart *et al.* 1983*a*). ARA rats have not yet been reared beyond weaning.

It is a cause for concern to those of us who would like to use the AR technique in studies of behavioural development, that all groups of AR rats in the present investigation showed deficits in absolute brain weight, whether body-weight was normal or not. The same has been found in other studies (Diaz *et al.* 1981, 1982; Smart *et al.* 1983*a*). One might question whether the problem is a real one or merely a result of the experimenters' choice of 'normal' reference group. Given that number in the litter influences growth, the largely arbitrary decision regarding what should be a 'normal' litter size becomes important. Perhaps if both groups of workers had chosen ten pups as 'normal' and not eight, we might not have found deficiencies in the brain weights of AR rats compared with 'normal' MR animals. Nevertheless, the finding remains that MR rats had heavier brains than even the best-grown AR groups. The possibility has been considered that there may be effects of the AR situation, perhaps through deprivation of social and other stimulation, which are specific to brain growth, and this cannot be discounted (Diaz *et al.* 1982). The finding in the present study, however, that brain weights of ARA and ARM rats were normal for their body-weights (Tables 4–6) argues against the stimulus deprivation hypothesis and suggests that in whole-body terms the animals were just not 'properly grown'. Assuming that proper growth of the rest of the body could be obtained by 'nutritional improvement', proper brain growth might ensue. Nutritional improvement might include not only change in quality or quantity of diet, but alterations in such factors as meal size or frequency.

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