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Weight Minus Extracellular Fluid as Metabolic Reference Standard in Newborn Baby

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Bhakoo, O. N., and Scopes, J. W. (1971). *Archives of Disease in Childhood*, **46,** 483. **Weight minus extracellular fluid as metabolic reference standard in newborn baby.** In 51 babies of differing size and gestational age, rates of oxygen consumption and corrected bromide space (as a measure of extracellular fluid) were measured. The results are used to examine the concept that weight minus extracellular fluid (ECF) is an appropriate metabolic reference standard in the newborn baby. When the whole group, which included large-for-dates and small-for-dates babies, is considered there is a systematic variation wherein the rate of oxygen consumption thus expressed varies with size. However, when appropriately grown babies only are considered, who varied in birthweight from 1210 g to 3820 g, rates of oxygen consumption thus expressed were constant. The implication is that when unusual rates of oxygen consumption per kilogram body weight are found, they should be interpreted bearing in mind the possibility of an unusual proportion of ECF in the baby. An incidental finding was that small-for-dates babies have a relatively large corrected bromide space.

When minimal rates of oxygen consumption are compared between mammals of differing size, whether within a species or comparing one species with another, a general rule emerges; the resting metabolism expressed per unit weight is *higher* in small animals than in large (Kleiber, 1947). In the human baby there is a 'neonatal violation' of this rule in that the small preterm baby has a *lower* resting metabolic rate than his larger term fellow. Clearly one explanation of this discrepancy is that weight alone is an inappropriate metabolic reference standard. Use of surface area or weight raised to a power of less than unity (such as 0·73) (Kleiber, 1947) merely aggravates the difference in the human newborn (Sinclair, Scopes, and Silverman, 1967). These authors proposed an hypothesis that some account should be taken of the body composition of babies of different size and gestation, and that if absolute values of oxygen consumption were related not to weight but to weight minus extracellular fluid (ECF) (thus, as it were, allowing for the 'wateriness' of pre-term babies) one would find that resting metabolic rates would be the same for preterm small infants and term normal-sized infants. Using measured rates of oxygen consumption and *estimated* ECF values, Sinclair *et al.* (1967) found this to be true. However, the definitive study in which both metabolic rates and ECF were measured in the same infant was needed to confirm or refute this prediction. The main purpose of this study is to report measured resting metabolic rate and bromide space in newborn infants. A by-product of the study is data on ECF spaces in babies of varying weight and gestation including small-for-dates infants.

Materials and Methods

Babies. The 51 babies were asymptomatic infants aged 3 to 7 days in the lying-in wards or the neonatal ward of Hammersmith Hospital, of birthweight between 1210 g and 4420 g and gestational age between 28 weeks and 42¾ weeks. Gestational age was calculated from the first day of the mother's last menstrual period in all but 3 cases. In these 3 cases, all of whom were small-for-dates, the mother was uncertain of her dates and gestational age was assessed by physical and neurological characteristics (Robinson, 1966).

In each case, the procedure was explained to the mother and her permission obtained. Care was taken to exclude infants of mothers who had been given iodides.

The babies have been described in 3 groups: (1) those

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above the 90th centile of weight for gestational age (large-for-dates, LFD), (2) those less than the 10th centile (small-for-dates, SFD), and (3) those between the 10th and 90th centiles (appropriate for dates, AFD). The centile tables used were those devised by the Birthday Trust Survey for English babies (Butler and Alberman, 1969). The SFD group did not include any babies in whom there was clinical suspicion of intrauterine infection or of chromosomal abnormality.

Procedure. Sodium bromide in a solution 20 mg/ml was administered orally, usually by gavage, about 1½ hours after the previous feed. Any baby who failed to take the full dose or who regurgitated or vomited was excluded from the study. The dose given was 80 mg sodium bromide per kg body weight. 3 to 5 hours later 1·5–2 ml of blood was taken by heel-prick into a heparinized tube. Rates of oxygen consumption were measured on the same working day, usually after the following feed.

Measurement of oxygen consumption. Rates of O_2 consumption were measured in postprandial sleep by a closed circuit method (Scopes and Ahmed, 1966a) with the child lying undisturbed on a small stretcher. The environmental temperature chosen was in the neutral range for a baby of his particular size and gestation (Scopes and Ahmed, 1966b), and this was confirmed by ensuring that the skin temperature of the exposed abdominal wall was about 36°C. Measurements were made over a 20–30 minute period when the child was asleep and inactive, and are reported as ml dry gas at 0°C and 760 mmHg.

Bromide estimation. The blood specimen was centrifuged at once, and the plasma separated and stored at —20°C until estimation. The chemical method of Hunter (1953) was used with strict adherence to his procedure. Known standards, including a specimen of the bromide solution given orally were run at the same time. Bromide determinations were performed in duplicate on 0·25 to 0·5 ml plasma, and the mean of the duplicate was used for calculation. In 43 cases, the chemical duplicates were within 5% of each other; in terms of the final calculation of percentage of ECF in the baby the difference of the duplicates was less than ±1% of the mean. In 8 cases it was greater, the variation being ±1·5%, 1·8%, 2%, 2·5%, 3%, 3·5%, and 4% of the mean, respectively. In 7 recovery experiments using 30 µg bromide, added to 66 µg bromide, recovery rates varied from 98–105%.

Bromide space. This was calculated as a corrected bromide space according to the formula:

$$\frac{\text{mg bromide administered}}{\text{plasma bromide (mg/litre)}} \times \frac{0\cdot 9}{0\cdot 95 \times 0\cdot 94}$$

The assumed correction factors are 0·9 for leakage of bromide into RBC, 0·95 for the Donnan factor, and 0·94 for serum water (Cheek, 1961).

Results

Details of the babies together with their corrected bromide spaces and rates of oxygen consumption are given in Table I. All the parameters examined and illustrated in the figures were calculated from the data in this Table. There were no significant differences between studies on days 3, 4, 5, 6, or 7 in any group with respect to Vo_2 or percentage of ECF and so results are considered together. Also there was no significant difference between breast-fed and bottle-fed babies in these respects.

Extracellular fluid (corrected bromide space (CBS)). Table II shows the mean value for the 3 groups of babies. The ECF is a higher proportion of the body weight in small-for-dates infants than in the other two groups (AFD v. SFD diff. = 4·43, P = 0·021 for 40 d. f.). There was no significant difference between appropriate-for-dates infants and large-for-dates infants; however, there were no preterm infants in the large-for-dates group.

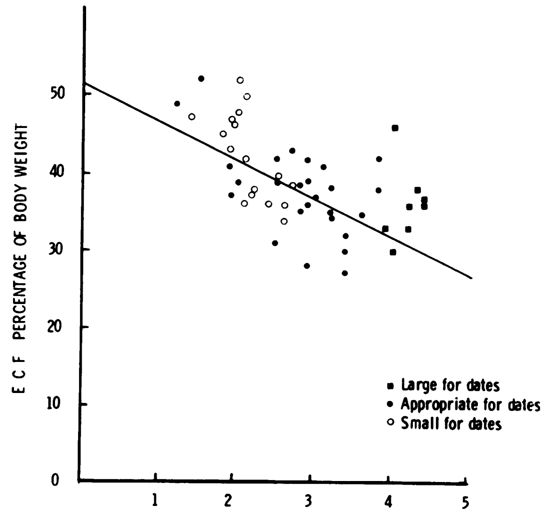

Fig. 1.—*Relation of birthweight to percentage of corrected bromide space. Calculated regression equation (appropriate-for-dates only):* $y - 37\cdot 7 = -4\cdot 9\ (x - 2\cdot 8)$, $r = -0\cdot 56$ $P = 0\cdot 0015$. *Calculated regression equation (all groups):* $y - 38\cdot 9 = -3\cdot 9\ (x - 2\cdot 8)$, $r = 0\cdot 55$ $P < 0\cdot 0001$.

The individual values are shown in more detail in Fig. 1 where the ECF as a percentage of body weight is plotted against body weight. There is a diminishing percentage with increasing body weight

TABLE I

Birthweight, Gestation, Oxygen Consumption, and Extracellular Fluid in Babies Studied

Case No.	Birthweight (g)	Gestational Age Wk	Dy	VO_2 (ml/min)	ECF % body wt	Age (dy) on Study Day	Wt on Study Day (g)
Small-for-dates							
1	1400	33	3	4·2	47·0	5	1230
2	1810	37	2	10·1	45·0	5	1800
3	1890	35	0	10·3	43·3	4	1900
4	1940	35	6	7·4	47·0	5	1960
5	1940	36	1	13·2	47·0	6	1920
6	1970	37	0	15·2	52·0	5	1960
7	2020	39	0	12·8	48·0	6	2120
8	2060	36	2	15·2	36·0	7	2140
9	2060	40	0	17·8	42·0	6	2200
10	2120	42	1	14·2	50·3	4	2180
11	2160	39	0	13·8	37·0	3	2120
12	2180	38	0	11·9	37·0	3	2120
13	2380	38	0	20·4	36·1	3	2340
14	2500	40	3	15·0	39·3	4	2500
15	2560	40	0	15·0	36·0	4	2500
16	2600	39	0	17·4	34·0	4	2500
17	2680	40	2	18·5	38·5	3	2650
Large-for-dates							
1	3940	39	2	20·8	33·0	3	3800
2	3940	37	4	21·8	29·5	3	3600
3	4040	38	6	22·8	46·0	3	3900
4	4180	40	2	25·5	36·0	4	4060
5	4200	41	4	26·2	33·0	5	4100
6	4300	37	3	24·7	38·0	5	4260
7	4380	40	5	27·4	37·0	3	4360
8	4420	38	0	22·0	36·0	6	4220
Appropriate-for-dates							
1	1210	28	0	6·1	49·0	5	1100
2	1520	31	0	8·9	52·0	5	1480
3	1870	31	5	11·3	37·0	6	1730
4	1930	33	5	12·6	40·6	4	1940
5	2040	33	0	8·7	39·0	5	1920
6	2460	35	6	15·6	42·0	5	2420
7	2500	36	5	15·5	39·0	6	2360
8	2540	34	2	17·6	30·6	7	2540
9	2730	38	0	15·0	43·0	7	2340
10	2800	39	4	17·3	38·5	3	2800
11	2820	38	6	14·2	35·4	5	2820
12	2860	40	4	21·3	27·6	6	3120
13	2900	39	3	19·0	42·0	5	2850
14	2940	41	2	16·1	39·0	7	2920
15	2940	38	3	18·3	36·4	3	2840
16	3000	40	0	17·9	37·2	3	2760
17	3120	41	4	21·0	40·8	5	3140
18	3160	38	5	22·7	35·0	5	3000
19	3200	38	2	21·2	38·4	4	3200
20	3200	38	6	20·3	35·0	5	3250
21	3400	41	1	26·0	31·7	5	3230
22	3400	42	3	20·8	29·5	5	3300
23	3420	41	6	18·0	27·2	4	2670
24	3550	40	2	20·7	34·6	5	3320
25	3760	39	3	22·1	42·0	3	3660
26	3820	40	0	23·2	38·0	5	3700

as there is with increasing gestation (Fig. 2). In each case there is a significant correlation between the variables, but the scatter about the line is large.

Fig. 3 shows absolute values of ECF plotted against birthweight where there is a steady rise with increasing weight. Similar plotting of total ECF against gestation merely shows that small-for-dates babies are small (and have a small total ECF) and the reverse is true for large-for-dates babies.

Rates of oxygen consumption. The total rates of oxygen consumption are given in Table I and shown graphically in Fig. 4. Fig. 5 shows rates of oxygen consumption expressed as ml/kg per min against birthweight, and the line drawn is

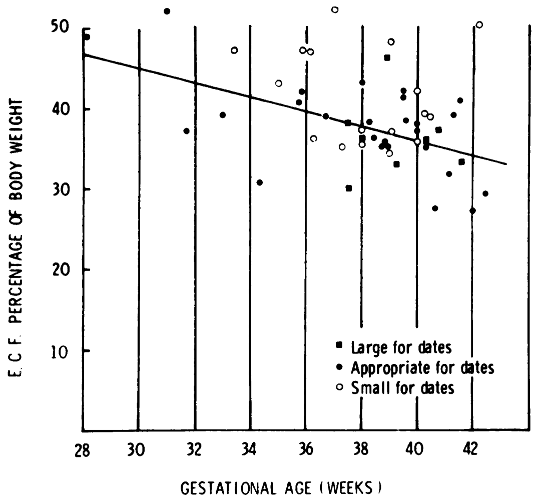

FIG. 2.—*Relation of gestational age to percentage of corrected bromide space. Calculated regression equation (appropriate-for-dates only):* $y-37\cdot7 = -0\cdot92\,(x-38)$, $r = -0\cdot59$ $P < 0\cdot001$. *Calculated regression equation (all groups):* $y-38\cdot9 = -0\cdot84\,(x-38)$, $r = -0\cdot425$ $P < 0\cdot001$.

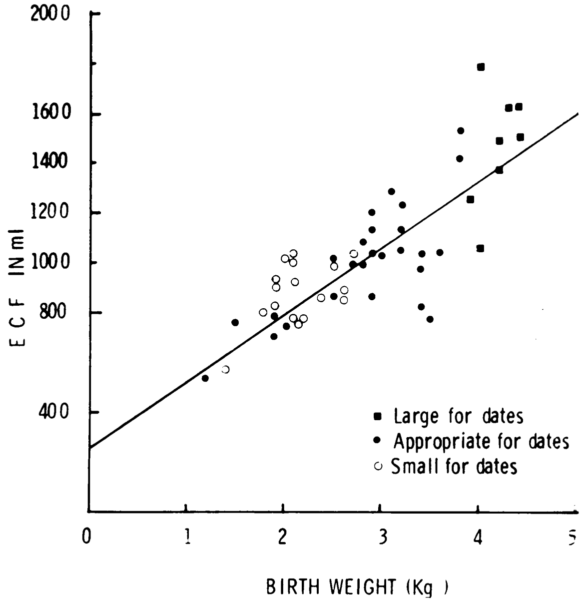

FIG. 3.—*Changes in corrected bromide space related to birthweight. Calculated regression equation (appropriate-for-dates only):* $y-1001 = 266\;(x-2\cdot8)$, $r = 0\cdot79$ $P < 0\cdot0001$. *Calculated regression equation (all groups):* $y-1033 = 275\,(x-2\cdot8)$, $r = 0\cdot85$ $P < 0\cdot0001$

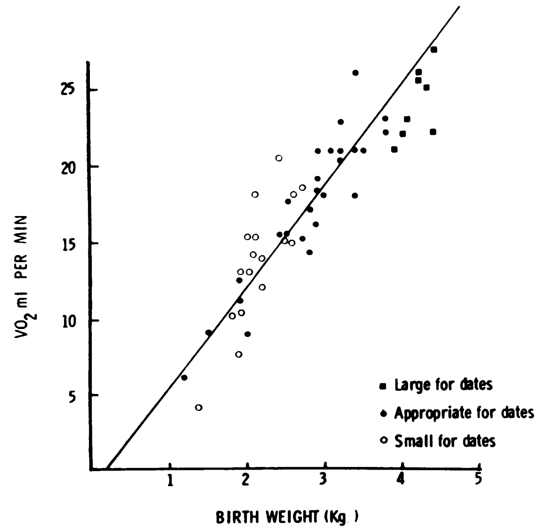

FIG. 4.—*Relation of rates of oxygen consumption to birthweight. Calculated regression equation:* $y-17\cdot4 = 6\cdot71\;(x-2\cdot81)$, $r = 0\cdot91$ $P < 0\cdot0001$.

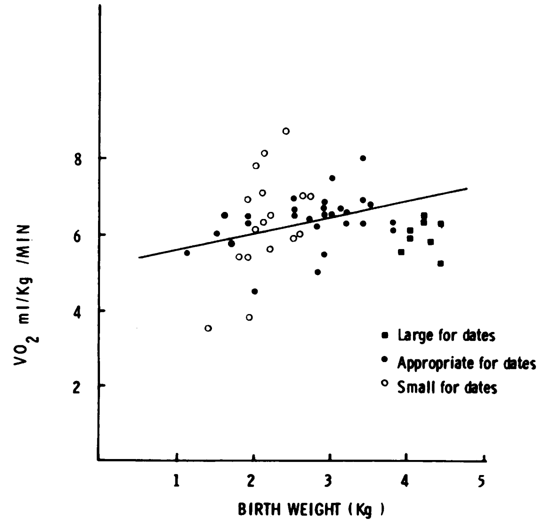

FIG. 5.—*Minimal rates of oxygen consumption expressed per unit body weight related to birthweight. Calculated regression equation:* $y-6\cdot38 = 0\cdot44\;(x-2\cdot8)$, $r = 0\cdot41$ $P = 0\cdot0187$.

TABLE II

Gestational Ages and Corrected Bromide Spaces (as a percentage of body weight) in Three Groups of Babies

Gestation Period (wk)			Group (No.)	Corrected Bromide Space % of Birthweight		
SEM	SD	Mean		Mean	SD	SEM
0·7	3·7	37·8	AFD (26)	37·7	5·8	1·1
0·5	1·5	39·2	LFD (8)	36·1	4·9	1·7
0·5	2·3	38·1	SFD (17)	42·1	5·7	1·4

Abbreviations: AFD = appropriate-for-dates.
LFD = large-for-dates.
SFD = small for dates.
SD = standard deviation.
SEM = standard error of the mean.

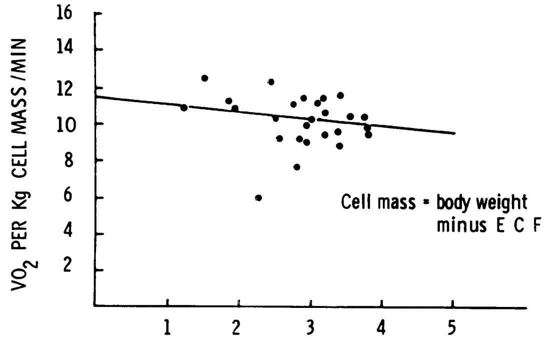

FIG. 6.—*Rates of oxygen consumption expressed per unit (weight minus CBS) related to body weight in appropriate-for-dates babies. Calculated regression equation:* $y - 10{\cdot}4 = -0{\cdot}36\,(x - 2{\cdot}8)$, $r = -0{\cdot}19$ $P = 0{\cdot}18$.

the calculated regression line for appropriate-for-dates babies. There is a significant correlation ($r = 0{\cdot}41$, $P < 0{\cdot}02$) whereby smaller babies have a lower rate of oxygen consumption when expressed thus.

When rates of oxygen consumption are expressed per unit of 'cell mass', i.e. body weight minus ECF, as proposed by Sinclair *et al.* (1967), there is no significant correlation with weight if appropriate-for-dates babies only are used (Fig. 6) ($r = -0{\cdot}19$, $P = 0{\cdot}18$). However, when all babies are included (Fig. 7) there is a small but significant negative correlation ($r = -0{\cdot}24$, $P = 0{\cdot}0444$). It is noteworthy that the variation around the line is not materially reduced by using this reference standard rather than weight (compare with Fig. 5).

When rates of oxygen consumption are plotted (Fig. 8) against weight minus ECF and compared with Fig. 4, it is perhaps noteworthy that the small for-dates babies are more evenly distributed about

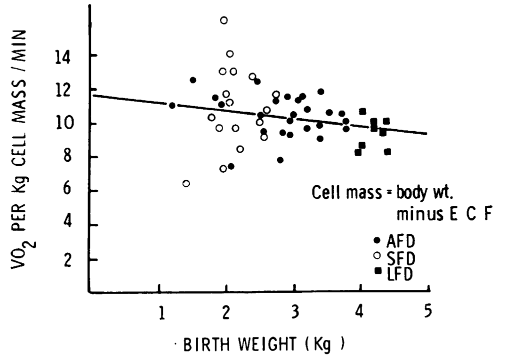

FIG. 7.—*Rates of oxygen consumption expressed per unit (weight minus CBS) related to body weight (all groups). Calculated regression equation:* $y - 10{\cdot}3 = 0{\cdot}5\,(x - 2{\cdot}8)$, $r = -0{\cdot}24$ $P = 0{\cdot}044$.

the line in Fig. 8 where the correlation coefficient is fractionally higher; but the scatter about the line is not significantly different, a finding that is not surprising since total weight and cell mass are closely correlated ($r = 0{\cdot}925$, Fig. 9).

Discussion

The conclusions to be drawn from a study such as this depend on the validity of the assumption that corrected bromide space represents ECF.

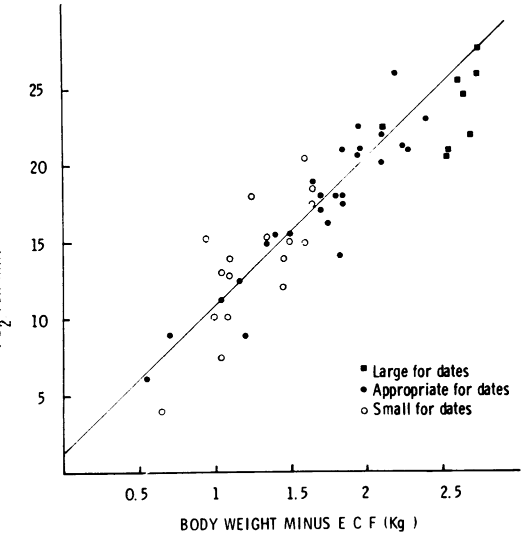

FIG. 8.—*Relation of rates of oxygen consumption to cell mass (i.e. body weight minus CBS). Calculated regression equation:* $y - 17{\cdot}4 = 0{\cdot}0094\,(x - 1702)$, $r = 0{\cdot}92$, $P < 0{\cdot}0001$.

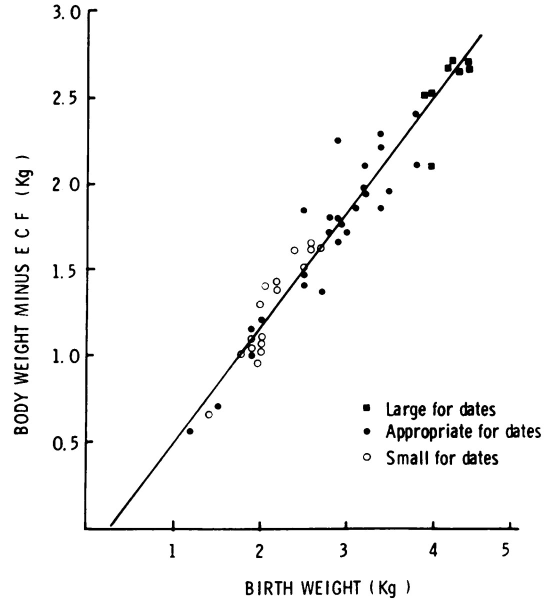

FIG. 9.—*The growth of cell mass (i.e. body weight minus CBS) with increasing weight. Calculated regression equation:* $y—1693 = 659(x—2\cdot 8)$ $r = 0\cdot 96$ $P < 0\cdot 0001$.

This assertion is made and defended by a number of distinguished workers (Cheek, 1961; MacLaurin, 1966; O'Brien, Ibbott, and Rodgerson, 1968) but there is no absolute standard whereby it can be finally substantiated. For instance, the thiocyanate space is slightly but significantly larger in babies (compare Friis-Hansen, 1956) and the estimates made by Sinclair *et al.* (1967) of chloride space (from known figures of carcass analysis) are very much higher than the figures from the present study. Indeed, the major discrepancy between their predictions and our findings are related to the size of the ECF pool which they derived from an uncorrected chloride space. It may be that the serum water of small-for-dates babies would be different from 0·94 and the correction factor of 10% for bromide entering red blood cells is quite inappropriate for small-for-dates babies who are known to have a high haematocrit (Humbert *et al.*, 1969).

If the appropriate factor were larger, it would vitiate both our findings and Cassady's (1970) that small-for-dates babies have a relatively large ECF space.

None the less, corrected bromide space was chosen in this study as a measure of ECF: (1) because it is widely accepted and theoretically as sound as possible, and (2) because oral administration of bromide in this dosage is totally innocuous and is followed by stable plasma bromide levels at 3 to 6 hours after administration. The ages 3 to 7 days were chosen because over this period there is no material change in rates of oxygen consumption (Scopes and Ahmed, 1966a; Bhakoo and Scopes, 1970) and because preliminary experiments had shown no significant change in ECF in any group over this period (see above). In addition, this dose of bromide had no effect on minimal rates of oxygen consumption.

Our findings for corrected bromide space are similar to those of other workers (Cheek, 1961; MacLaurin, 1966; Friis-Hansen, 1956) and especially those of Cassady (1970). Like him we found that in small-for-dates babies a significantly larger proportion of body weight was represented by 'extracellular fluid'. The similarity of our findings is particularly interesting in that his cases were studied in the first 12–24 hours of life, whereas in the present series babies were 3–7 days old, i.e. after feeding. There was no difference between breast-fed and formula-fed babies, despite the fact that the latter were inevitably given a rather higher sodium chloride intake.

The percentage of body weight occupied by ECF measured as corrected bromide space falls with increasing birthweight (Fig. 1) and with increasing gestation (Fig. 2) which is in keeping with the general concept that small preterm infants are more 'watery' than their term fellows. However, though the numbers are small it is noticeable that the points representing large-for-dates infants tends to fall above the mean line in Fig. 1 but about the line in Fig. 2. Thus there is a suggestion not statistically supported on these numbers, that large-for-dates infants have an ECF appropriate to gestation rather than to birthweight. Similarly, inspection of Fig. 1 and 2 leads to the suggestion that small-for-dates infants have an ECF appropriate to weight rather than to gestation (with the proviso, discussed above, that the correction for bromide in red cells is appropriate). Cassady (1970) comments on the relatively low corrected bromide space (CBS) in large but normally grown infants. It is noteworthy that in Cassady's series no baby was heavier than 3540 g and large-for-dates babies were not considered. This series shows a tendency to a *high* corrected bromide space in large-for-dates babies.

The major objective of this study was to confirm or refute the hypothesis of Sinclair *et al.* (1970), of which one of us is co-author. When weight minus ECF is used as a reference standard for rates of oxygen consumption and when appropriate-for-dates babies only are considered, our data show, as Sinclair *et al.* (1970) had predicted, that there is no longer a significant relation between metabolic rate thus expressed and body size. To this extent the hypothesis is supported. However, the hypothesis should not exclude small-for-dates or large-for-dates babies, and when all babies are considered together there is a significant negative correlation; furthermore, use of this reference standard does not materially reduce variation. Thus our data do not support the hypothesis that weight minus ECF is a good representation of the theoretical compartment of 'active cell mass'. It is clearly a better approximation than weight or surface area since it serves in appropriate-for-dates babies, but it is far from perfect. A corollary of the hypothesis was the prediction that small-for-dates babies should have a low ECF: neither our data nor that of Cassady (1970) substantiate that prediction, though it may be noted from inspection of the figures that the overall group labelled small-for-dates is clearly heterogeneous. The compartment described by weight minus ECF shows a difference in quality in which small-for-dates babies have a high rate of oxygen consumption and large-for-dates a low rate. Thus it is in keeping with the general biological rule mentioned in the introduction but not acceptable as a description of 'active cell mass'.

None the less, the concept that metabolic rate is related in a simple proportional way to some fraction of body weight which might be defined in terms of body composition still seems more meaningful than using weight raised to an empirically determined power. Weight raised to the power of say, 1·22, is physiologically meaningless, whereas if a reference standard defined in body composition terms is found to hold true it has important implications. For instance, if weight minus ECF were an accepted reference standard, and one were considering a baby whose rate of oxygen consumption per kilogram was low, one must consider whether the baby was truly hypometabolic or whether the low rate is spurious because of a high ECF. This, we suggest, is in fact the case with the appropriately grown preterm baby, and this sort of reference standard would be useful in interpreting rates of oxygen consumption in malnourished oedematous infants (though, of course, these particular data do not apply to the latter group).

The finding that weight minus ECF appears to be an appropriate reference standard for appropriate-for-dates babies is interesting because of the implications mentioned above, but these empirical findings do not constitute proof of a physiological truth. The possibility remains that the empirical fit could be coincidental. None the less, for any reference standard there should be a theoretical reason for expecting it to be appropriate, and it should hold up to empirical testing in subjects of different size. The further requirement that it should be expected to reduce variation has not been substantiated in these data.

In conclusion, we should emphasize that we do not advocate that ECF need be estimated in every baby in order to decide whether a rate of oxygen consumption is within normal limits. Using weight as a standard and a regression line for reference remains a reasonable practical way to assess a metabolic rate. Our data, and the theoretical background, however, suggest that for any meaningful interpretation of unusual findings, the possibility of an unusual proportion of ECF should be considered.

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