An attempt to estimate total body fat and protein in malnourished children

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1. An attempt was made to measure body volume and body fat in malnourished children using the same closed-circuit apparatus for both determinations. If these measurements were sufficiently accurate, they would enable total body protein to be calculated.

2. A helium dilution technique was used to estimate the volume of the child. Although highly reproducible measurements were obtained for the volume of inanimate objects by this technique, the results with children were erratic.

3. The fat stores of the child were determined by measuring the amount of cyclopropane (C_3H_6) which would dissolve in the body. This technique gave acceptable answers with marasmic children who have little subcutaneous fat, but with obese children equilibrium could not be obtained in an acceptably short period of time. It is not practicable to calculate the equilibrium concentration by extrapolation of the early part of the uptake curve in obese children.

Chemical analysis of the whole body (Garrow, Fletcher & Halliday, 1965; Halliday, 1967) or of certain organs (Alleyne, Halliday, Waterlow & Nichols, 1969) from children who have died from severe malnutrition has shown that protein depletion, in the sense postulated by Waterlow (1955), is an important feature common to the syndrome of both marasmus and kwashiorkor. At present, however, there is no method of finding out if the children who die of malnutrition are more severely depleted than those who survive, because there is no satisfactory method of measuring total protein in a living malnourished child. Measurements which provide an estimate of lean body mass in normal children, such as total body water and potassium, creatinine excretion, and anthropometric and skinfold measurements, are invalid when applied to malnourished children. The proportion and distribution of water, fat and protein in the body is grossly distorted, and severe impairment of renal function (Alleyne, 1967) and depletion of total body potassium (Garrow, 1965) alter the normal relationship of creatinine excretion and total potassium to total body protein.

This paper describes an attempt to develop a non-traumatic, clinically useful method which would estimate the amount of body fat and protein in a severely malnourished child, without applying any 'normal' relationship between the main body constituents: water, protein, fat and bone mineral.

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EXPERIMENTAL

Children

The children were in-patients in the MRC Tropical Metabolism Research Unit, Jamaica. The clinical features of malnourished children in this unit have been described elsewhere (Waterlow, 1948; Garrow & Pike, 1967). Measurements of body volume by helium dilution were made on six children. Three were severely malnourished and two were recovering. These children, at the time of measurement, were aged from 6 to 12 months and weighed 3·5–6·5 kg which was approximately 65 % of the weight of well-nourished children of the same height. The final child was 1 year old and was considered to have recovered from malnutrition, having attained his expected weight for height. Another six children were used to obtain cyclopropane uptake curves for fat estimation. Three of these, R.W., P.A.L. and A.M.B., were classically marasmic, aged 7–11 months and weighing 3·2–5·1 kg. Subject A.F. was recovering and H.F. had recovered. The final child, S.P., aged 14 months and weighing 8·1 kg, was retained in hospital after recovery for a dietary study.

Apparatus

A closed-circuit apparatus, illustrated in Fig. 1, was constructed to enable measurements of body volume (and hence density) and cyclopropane to be made. Details of construction, operation and calibration of the apparatus have been published previously (Halliday, 1966).

Determination of body volume

The volume of the child was estimated from the dilution of a known amount of helium in the closed-circuit apparatus surrounding the child. Helium concentration was measured with a katharometer. Equilibrium distribution of helium within the apparatus was achieved 10 min after injection of the gas. From the calculated volume and the body-weight of the child, the density was obtained.

Determination of body fat

An attempt was made to measure the fat content of malnourished children directly from the uptake of the fat-soluble gas, cyclopropane (Lesser, Perl & Steele, 1960) using the apparatus described above. Small gas samples were removed from the circulating gas mixture every 5–10 min and analysed on a mass spectrometer, using the 43 m/e peak to measure cyclopropane and the 28 m/e (nitrogen) peak as a reference.

Calculation of body protein

Total body water in malnourished children can be measured by dilution of deuterium oxide and total mineral by X-ray densitometry (Garrow & Fletcher, 1964). Using the value obtained for fat by cyclopropane uptake and knowing the body-weight of the child, protein can be calculated by difference.
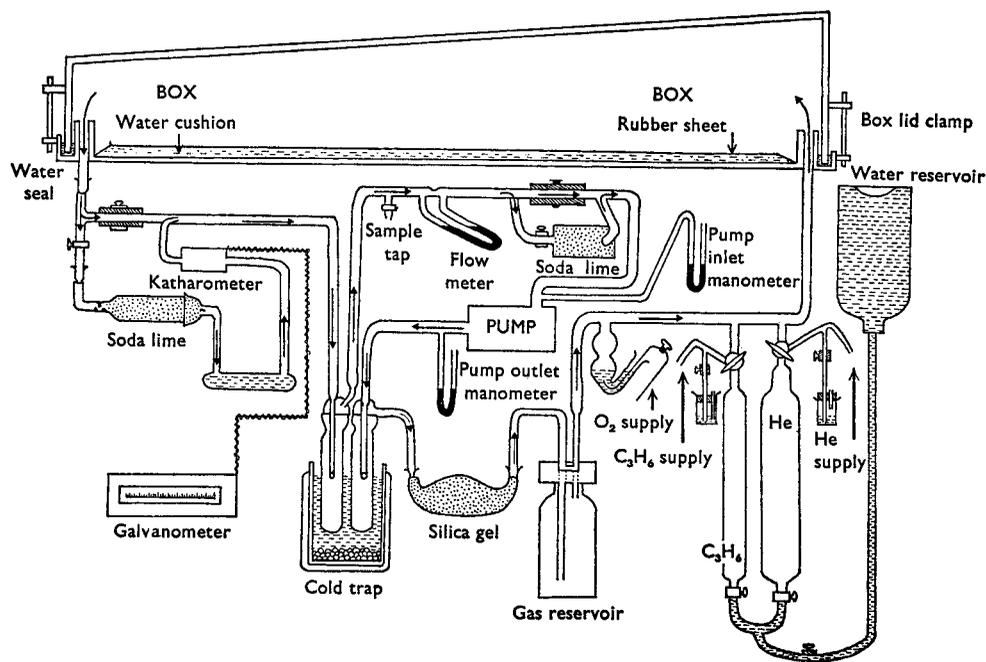
Fig. 1. Diagram of closed-circuit apparatus for measuring body volume.

RESULTS

Volume and hence density by helium dilution

The rate of diffusion of helium from the system, as measured by the katharometer, was found to be 1·2%/h.

Four inanimate objects of known volume were used to investigate the technical errors of the helium dilution technique. The volumes of these objects ranged from 2 to 8 l, and the helium dilution technique gave answers agreeing within ±15 ml (Halliday, 1966).

To estimate the reproducibility of the method when used for the study of living infants, ninety-one measurements of body density were made on six children. In one child, six measurements done on the same day gave densities ranging from 1·056 to 1·114 and, in another, five measurements ranged from 1·029 to 1·094. In order to achieve more reproducible results, measurements were then made at a constant time of day; but neither this, nor sedation achieved satisfactory agreement between repeated measurements.

Cyclopropane uptake measurements

The rate of diffusion of cyclopropane from the circuit was 4·6%/h with a standard deviation of ±0·05%.

At body temperature, 1 g fat will take up 11·26 ml cyclopropane (Lesser *et al.* 1960), so each g fat in the child represents a potential 'cyclopropane space' of 11·26 ml when in equilibrium. If the amount of cyclopropane added to the circuit and the cyclo-
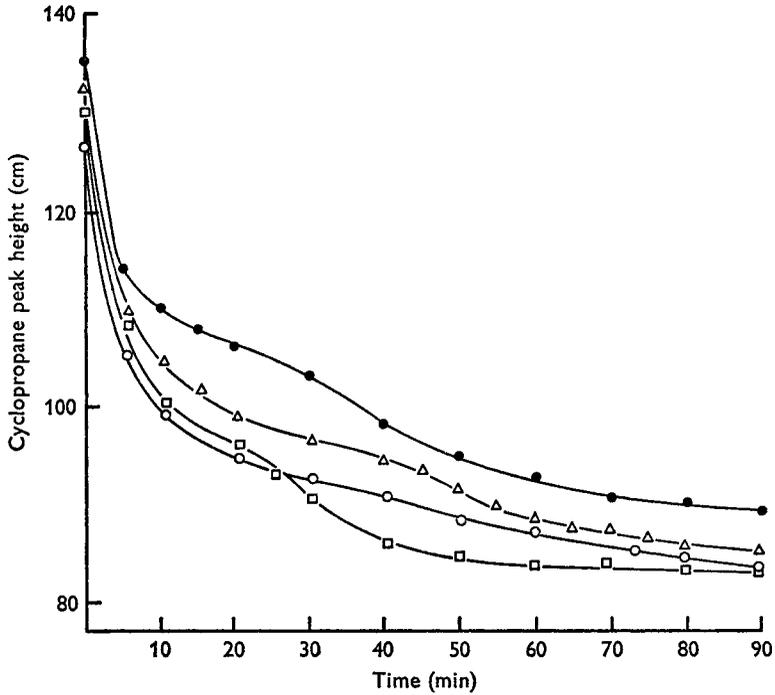
Fig. 2. Four cyclopropane uptake curves measured serially on malnourished child A.F. at 16 d (○), 23 d (□), 30 d (△) and 37 d (●) after admission to hospital.
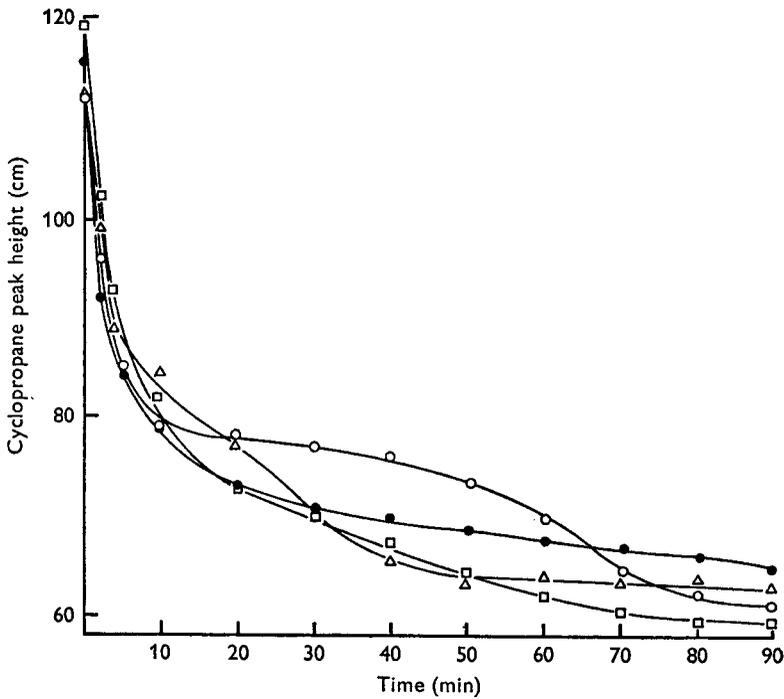
Fig. 3. Four cyclopropane uptake curves measured serially on recovered child H.G. at 85 d (○), 87 d (△), 88 d (●) and 94 d (□) after admission to hospital.

propane concentration at equilibrium (approx. 3 %) is known, the apparent volume of distribution can be calculated. The gas space in the circuit is known (see p. 149), so the amount taken up by the child, and hence his fat stores, can be calculated.

The fat content of subject A.F. was measured on four occasions at weekly intervals, making the assumption that by the end of the experiment the cyclopropane had equilibrated with total body fat. The results are represented in Fig. 2. Fat apparently contributed a smaller percentage of body-weight in consecutive weeks, being 9·9, 9·8, 9·0 and 7·0 % of body-weight at 16, 23, 30 and 37 d after admission to hospital.
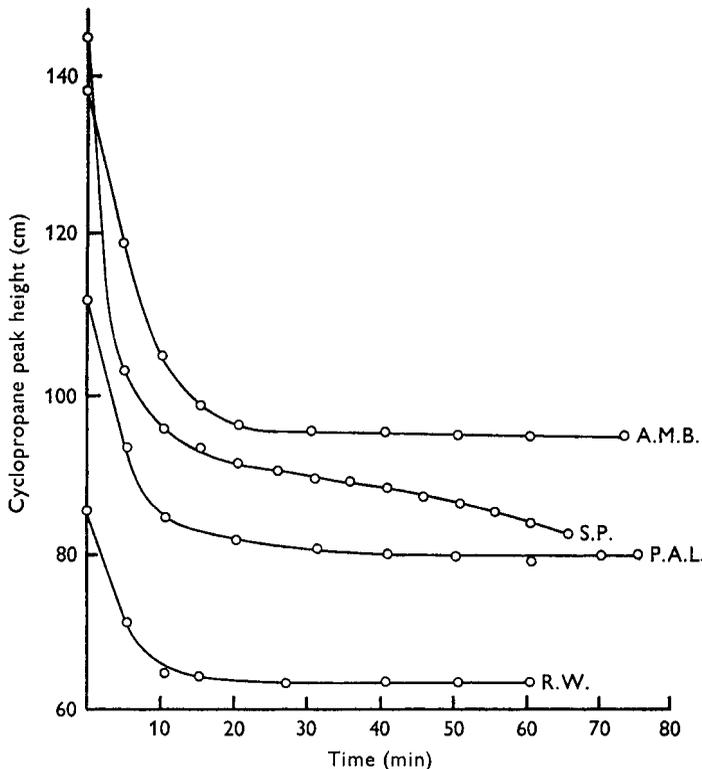

Fig. 4. Cyclopropane uptake curves for three malnourished children, A.M.B., P.A.L. and R.W., and one recovered child (S.P.) measured once on each subject. For clinical details see p. 148.

Subject H.G., originally malnourished, had been in the ward for nearly 3 months, and had recovered. Four measurements were made on this subject over a 10 d period. Each experiment was continued for 90 min. The results are shown in Fig. 3, and fat was calculated to contribute 7·4, 6·9, 6·6 and 7·9 % of body-weight at 85, 87, 88 and 94 d after admission to hospital. Fig. 4 shows the results obtained for four infants for whom only a single fat determination was made. For R.W., P.A.L. and A.M.B. it was made within the 1st month of their admission to hospital. The final subject, S.P., had been in the ward for 6 months and had been on a special dietary study for 3 months before determination was made.

In each determination the fat content of the child was calculated from the lowest

measured value of cyclopropane remaining in the circuit. The uptake curves of subjects R.W., P.A.L. and A.M.B. demonstrate that equilibrium was achieved after 60 min. Inspection of the uptake curves of subjects A.F., H.G. and S.P. shows that there was a very rapid initial uptake of cyclopropane, followed by a period of much slower uptake into certain fat pools. Even by plotting the values semi-logarithmically, it was not found possible in the latter part of the experiment to obtain a convincing straight line which could be extrapolated to infinite time. In fact, some of the curves exhibit inflexions which suggest that changes in perfusion rates of certain body fat depots must have occurred during the cyclopropane uptake studies.

DISCUSSION

The apparent density of children, measured by helium dilution, varied by about 0·06 units in a single day, equivalent to a variation of 6 % in body-weight or volume. Body-weight can be measured to ± 0·1 %, so this variability must be due to error in apparent volume. From values obtained by chemical analysis of the whole body (Halliday, 1967), the expected density of a marasmic child is about 1·04. The apparent densities of subject N.McN., who was clinically marasmic, ranged from 0·992 to 1·093. The very low apparent density can reasonably be accounted for by gas trapped in the gut, which would not equilibrate with the helium mixture in the box. Although it is not practicable to measure intestinal gas routinely, X-rays of children show that, in proportion to body-weight, they may have very large gas bubbles in the stomach. On one occasion, the estimated volume of gas within the stomach of N.McN. was 80 ml. In order to bring a true density of 1·04 down to 0·992, a volume of about 200 ml would be necessary; this is not impossible.

No reasonable explanation for the unduly high apparent densities can be offered. The possibility was considered that the presence of the child in the box in the absence of helium might affect the katharometer zero. This was tested, but found not to be so. It was therefore concluded that density measurements by helium dilution were unsatisfactory. S. J. Fomon (personal communication) has intimated that workers in his laboratory have been unable to reproduce the good agreement shown by their earlier results (Fomon, Jensen & Owen, 1963) on volume determination on two normal infants.

Lesser et al. (1960), using adult human subjects for determining body fat with cyclopropane, found that small amounts of cyclopropane were still being absorbed after 8 h. A mathematical procedure was adopted to extrapolate for the equilibrium value. Successful extrapolation depends on the rate coefficients governing the uptake of cyclopropane by the fatty tissues remaining constant throughout the experimental period. This involves the assumptions that the cyclopropane is delivered to the alveolar surface at a constant rate and that diffusion across the pulmonary surface is also constant. Two other necessary assumptions are constancy of cardiac output and constant perfusion of the adipose tissue. There seems to be very little information in the literature about the relative perfusion rates of different fat compartments within the body.

In the light of this discussion it is of interest to consider the cyclopropane uptake curves obtained in the series of experiments described here. The three subjects, R.W., P.A.L. and A.M.B. (Fig. 4), were all thin and wasted with little or no subcutaneous fat. They also had enlarged livers and minimal oedema and were considered clinically to be marasmic. It is possible that the great majority of the total body fat in these infants was located in the liver, brain, and the remainder of the central nervous system – three sites well-perfused by the circulatory system, which would therefore be expected to constitute rapidly equilibrating pools for cyclopropane. In these three marasmic children, the cyclopropane uptake suggests a fat content of 6·0, 11·3, and 6·3 %, which is of the same order as that of marasmic children analysed *post mortem* (Halliday, 1966).

In marked contrast to these subjects was S.P., in whom the cyclopropane uptake indicated that only 4·4 % of the body-weight was fat. At the time of the estimation, the infant was visibly obese. Clinical opinion was that fat probably constituted at least 25 % of his body-weight. Approximate calculation from the observed curve showed that with this amount of fat, saturation with cyclopropane would take 4–5 h. It would obviously be undesirable and impractical to keep infants of this age in the apparatus for such a long time.

It is therefore concluded that the cyclopropane uptake method is invalid for children with much adipose tissue, since in these children it would take too long to reach a true equilibrium.

In terms of incidence, mortality and morbidity, protein deficiency in children is one of the most serious diseases in the world. Chemical analysis of children who have died from malnutrition has shown that there is severe protein depletion, and that indirect methods of estimating body solids, mentioned on p. 147, are likely to be invalid. The work described here shows that two possible methods of assessing directly the stores of fat and protein in a malnourished child are also unreliable. The situation is therefore very unsatisfactory, because there is no objective measurement by which, of two malnourished living children, one can be shown to be more severely protein-depleted than the other.

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