MEASUREMENT OF TOTAL BODY FAT IN LOW BIRTH WEIGHT INFANTS

PROEFSCHRIFT

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What are little boys made of?
Slug and snails and puppy-dogs' tails;
That's what little boys are made of.
What are little girls made of?
Sugar and spice and all things nice;
That's what little girls are made of.

Nursery Rhymes, ed. J.O. Halliwell (1844)

For he knoweth whereof we are made;
he remembereth that we are but dust.

Psalm 103 : 14

CONTENTS

LIST OF ABBREVIATIONS

B. wt.	= birth weight
CBS	= corrected bromide space
ECV	= extracellular volume
FFM	= fat free mass
G.a.	= gestational age
Post. a.	= postconceptional age
TBF	= total body fat
TBP	= total body protein
TBW	= total body water

Chapter I

INTRODUCTION AND AIMS

Growth is one of the most important phenomena of childhood, particularly in infancy. Growth velocity in the human is greater during the first year than in subsequent years. A healthy, full term, West-European baby trebles his birth weight in the first year, while in the same period his body length increases by approximately a half. This rapid increase in weight and length is however surpassed by the intra-uterine growth velocity. From the beginning of the pregnancy, the growth velocity, expressed in grams per day gradually increases. Growth velocity is greatest in the eighth month of pregnancy, being approximately 35 grams per day. The weight increase is however proportionally greatest in the first trimester of pregnancy, being approximately 6% per day and gradually decreasing to about 1.5% per day by 38 weeks of gestation.

Growth of the body as a whole, as well as that of the various individual organs, takes place through an increase in the number of cells and through an increase in their size. It was shown in animals by Winick and Noble (1966), that growth follows a definable pattern. This is probably the same in all mammals, including man, although the time scale varies between species. Three consecutive stages of growth can be defined according to Winick and Nobel (1966).

1. Growth by cell multiplication. Directly after fertilization, the embryo grows exclusively by increase in the number of cells.

2. Growth by multiplication and increase in volume of the cells. This stage of growth is characteristic of the period during which the various organs develop and the cells differentiate.

3. Growth exclusively by increase in the volume of the cells. This stage of growth is entered as soon as the "adult" number of cells is reached. Not all organs reach this stage at the same time.

The differentiation between an increase in cell numbers and an increase in cell volume, as two partially overlapping aspects of growth, is not without meaning in the study of situations which lead to growth retardation. Widdowson and McCance (1960; 1963) have done interesting studies on this subject in young rats. They showed that diet restriction, during the first three weeks directly after birth, led to irreversible growth retardation. Young rats who were initially normally fed but who then received a restricted diet from the 9th until the 12th week, also developed growth retardation. However, after increasing the amount of food in the diet, complete "catch-up" growth occurred. It is logical to suppose that early underfeeding, occurring during a period of cell multiplication, leads to a permanent reduction in cell numbers. Undernutrition occurring later, in a stage when the definitive number of cells has already been reached, will lead to a reduction in size of the cells but their number remains normal. These can reach normal size on resumption of a normal diet. Their conclusion was that the earlier that underfeeding occurs and the longer it continues, the greater the chance of serious and irreversible growth retardation. This research is especially interesting because as far as growth of various organs is concerned, the newborn rat is approximately comparable to the 18 week human foetus. It is tempting to use the conclusions from this research on young rats,

to explain intra-uterine growth retardation in man. However, great care is necessary here. Through the development of echography it has recently become possible to follow intra-uterine growth accurately and to define the time when intra-uterine growth retardation appears. However, a long term follow up study of low birth weight children will be necessary to establish whether early intra-uterine growth retardation in man is irreversible, whereas later intra-uterine growth retardation is reversible.

Meanwhile children with a low birth weight form an important problem in health care. In spite of optimal antenatal care, about 7% of all live born children in many West-European countries and in the United States weigh 2500 grams or less at birth. This applies to 12000 - 13000 children per year in the Netherlands. Two thirds of this group are children who are born prematurely (preterm), that is after a pregnancy of 28 to 37 weeks duration. One third are children who have experienced a reduction in intra-uterine growth and as a result are born with a weight which is significantly lower than that which is usual for this length of gestation. These children are called "small-for-dates" (S.F.D.) or "small-for-gestational age" (S.G.A.). To distinguish between low birth weight children one can use the so-called "intra-uterine growth" curves. These are curves compiled by plotting the birth weight of a large number of children against the duration of the gestation. Percentile lines can be obtained by arranging the birth weight per week of duration of gestation. Kloosterman (1969) has defined intra-uterine growth curves for the Netherlands. S.F.D. is defined as a birth weight which is at or below the 2.3 percentile line on this curve. The intra-uterine growth curves of Usher and McLean (1969) have the advantage that, as well as weight, the length and occipito-frontal head circumference are defined so that these parameters can also be used in the judgement of intra-uterine growth. In severe intra-uterine growth retar-

dation the body length is also retarded whereas head growth is frequently relatively spared. If growth in head circumference may be considered as a measure of cerebral growth, then it may be suggested that the central nervous system is the last to be affected in cases of intra-uterine growth retardation.

Clinically there are differences between S.F.D. and preterm babies. The preterm baby has a thin, shiny, sometimes oedematous, red skin that is covered with lanugo hair. There is less subcutaneous fat tissue than in the full term baby. The soles of the feet are smooth. The cartilage in the ears is absent or present in only small amounts, as is the glandular tissue in the breasts. The external genitalia have an immature aspect with large labia minora in girls and undescended testes in boys. The S.F.D. baby appears more mature as far as the above features are concerned, provided that he is not also preterm. The skin is often ample and wrinkled, the subcutaneous fat tissue is often even less than in the preterm baby of a similar gestational age. The head is relatively large. The S.F.D. baby frequently gives the impression of being more active and alert than the preterm baby. Various scoring systems have been developed for the more accurate and systematic judgement of the degree of maturity. One of the best known is that developed by Dubowitz (1970).

There are differences in body composition between S.F.D. and preterm babies. Important changes occur between the various body compartment during the normal intra-uterine development of the human foetus. The very young foetus consists mostly of water with a small amount of protein (in cells) and almost no fat. During intra-uterine development there is a gradual increase in the protein compartment while the fat clearly increases in relation to the total body water. S.F.D. babies contain less fat than preterm babies of a similar weight, they have a higher percentage of total body water (Cassady and Milstead, 1971) and a

higher percentage of extracellular water (Cassady, 1970) in comparison with normally grown babies of a similar gestational age.

Intra-uterine growth retardation can be caused by maternal factors such as insufficient uterine blood flow as a result of pre-existent vascular disease, hypertension during pregnancy, smoking or uterine anomalies. Placental factors are also important, for example placental insufficiency as a result of placental infarcts or "abruptio placentae", or relative insufficiency as in multiple pregnancy. Foetal factors such as chromosomal abnormalities and intra-uterine infections may also lead to intra-uterine growth retardation.

The aims of treatment in the two groups of low birth weight children are different. For the premature baby, who has a normal weight for gestational age, the aim is to provide for an undisturbed continuation of his intra-uterine growth curve. Whereas for the S.F.D. baby, the aim is to provide for growth to enable him to catch up with the original growth curve which he was following before growth retardation began. In order to achieve this, some authorities have advised feeding regimes of 200 ml/kg body weight per day giving 140 to 160 kilocalories/kg body weight/day. In many cases it is impossible to feed these babies orally because of complicating disease, particularly respiratory distress. Intravenous feeding is then indicated. In most cases this consists of intravenous glucose solutions sometimes with added amino-acid mixtures and fat emulsions. It is clear that such intensive treatment, particularly in very small patients (below 1500 g), should take place in a specialized centre.

Moreover it should be noted that even though a satisfactory growth curve is achieved for weight and length it does not necessarily mean that the fluid and feeding regime which is being followed is optimal. Weight and length are only the results of the changes which take place in

the various body compartments during growth. The question may be asked whether the presently accepted regimes indeed achieve either maintenance or restitution of the normal relationship for age between the various body compartments. An increase of 100 grams in body weight could mean for example an increase of 90 grams in body fat and 10 grams in body water. But it could also mean an increase of 90 grams in body water and 10 grams in body fat. In order better to be able to judge the treatment of these low birth weight babies it is necessary to have an extra parameter of growth. Therefore we would like to know the body composition before and at various times during treatment. Several questions form the foundation for the research described in this thesis.

a. Are there differences in body composition between preterm and S.F.D. babies of similar body weight?
b. How does the body composition of these children change during growth in the first few weeks after birth?
c. Are there differences in body composition between S.F.D. and preterm babies when they have reached the postconceptional age of 40 weeks?

Present evidence in the literature on body composition in the newborn has been obtained from carcass analysis by (among others) Widdowson and Spray (1951), Widdowson and Dickerson (1964) and Widdowson (1967, 1974). In order to do a longitudinal study of body composition it is necessary to have an "in vivo" method. A review of the usual methods for measuring body composition "in vivo" is given in Chapter II. However, these methods are in general unsuitable for use in young babies.
The methods that we have chosen for the measurement of total body fat, total body water and extracellular volume are described in Chapter III. These methods were first used on experimental animals (guinea-pigs) and the results obtained were checked by subsequent carcass analysis.

Thereafter measurements were done on a number of low birth weight babies.
Chapter IV describes the patient groups, the therapy which they had received and in particular the fluid and feeding regimes which were followed. Subsequently the results of the measurements on these children are given.
In Chapter V we give the fat and water percentages which we found and these are compared with the figures obtained from carcass analysis given in the literature . Later in the chapter we compare the results of the measurements in preterm and S.F.D. babies. Thereafter the longitudinal studies which were done on a few patients are discussed. The results for total body fat measured by the skinfold thickness technique (Dauncey et al. 1977) and our method are compared.
A summary in Dutch is given at the end.

Chapter II

METHODS FOR MEASURING BODY COMPOSITION
A SHORT SUMMARY OF THE LITERATURE

II.1. Carcass analysis

Chemical analysis of the dead body is the oldest method used to obtain information on the body composition of man. The first figures from carcass analysis in adults were published over a century ago by Moleschott in his book "Physiologie der Nahrungsmittel : ein Handbuch der Diätetik" (1859). Here, figures were given for the amount of protein, fat, extractable material, salt and water in the human body, as well as an analysis of the composition of a large number of dietary items. In 1863 Bischoff published more detailed results on the body composition of man. The method which he used was careful dissection of the body, followed by separate weighing of the fat tissue and various organs. The water content was measured by weighing before and after drying. The studies of Moleschott and Bischoff are of greater historical interest than of scientific value. Moleschott, for instance, does not give the origin of his data. Hereafter data was published by Mitchell (1945), Widdowson et al. (1951), R.M. Forbes et al. (1953, 1956), G.B. Forbes et al. (1956) on the carcass analysis of seven adults. This small number is explained by the fact that adult cadavers are difficult to obtain for this purpose. Moreover the processing of the large amount of material puts great demands on the laboratory. The American researchers (Mitchell, 1945, R.M. Forbes et al. 1953, 1956) followed the technique of careful dissection, whereby not only the internal organs but also the skin.

striated muscle, skeleton and fat tissue were analysed separately. The amount of body water was determined by weighing before and after drying. Widdowson (1951) divided the body into a smaller number of parts - the skin, muscle and skeleton were not taken separately - thereafter the material was steeped in strong hydrochloric acid and after heating was homogenized and analysed chemically. The quantity of body water was calculated from the difference between the body weight and the dry weight of the analysed material. Fat was measured by ether-extraction following the Soxhlet principle. Total nitrogen was measured by the Kjeldahl method. It was possible to measure a number of minerals quantitatively during chemical analysis. Mitchell et al. (1945) measured calcium (Ca) and phosphorus (P); R.M. Forbes et al. (1953, 1956) measured at the same time sodium (Na), potassium (K), chloride (Cl), boron (B) and cobalt (Co). G.B. Forbes (1956) measured Na, K, Cl, Ca, P, B, Co and magnesium (Mg) while Widdowson (1951) measured Na, K, Ca, P, Mg, Ca, iron (Fe) and zinc (Zn) although not Cl because of the pretreatment of the material with hydrochloric acid.

The number of carcass analyses performed on foetuses and newborn babies is greater. Those that deserve mention are the studies of Von Bezold (1857) who was the first to analyse a human foetus (of 523 grams); Fehling (1877) who measured total body water, total nitrogen (N) and fat in 21 foetuses; Givens and Macy (1933) who measured water, Ca and Mg in 25 foetuses; Iob and Swanson (1934) who measured water, fat, N, Na, K, Ca, Mg, P, Fe, Cu, Zn and iodine (I) in 27 foetuses. We shall use these data for total body water and fat in a comparison with our own findings in Chapter V. There are a number of much smaller studies which will not be considered.

The above summary shows the advantage of carcass analysis over other methods i.e. that very complete information can be obtained on body composition. Chemical analysis is

still the only method to measure the total amount of most compounds in the body. In contrast, this method can only be used after the death of the subject so that no information can be obtained on the changes which the various body compartments undergo during life. For this it would be necessary to study a large number of cadavers of people who had died at various ages. This is difficult to realize because of the enormous amount of work entailed by this method and the limited availability of material.

II.2. "In vivo" methods

II.2.1. Dilution methods

The principle is that the volume (V_2) of a fluid compartment can be calculated if a substance of known concentration (C_1) and volume (V_1) is dissolved in it, and after equilibration, the new concentration of the substance (C_2) can be measured. The following holds true for static equilibrium:

$$C_1 . V_1 = C_2 . V_2$$

from this it follows that:

$$V_2 = \frac{C_1 . V_1}{C_2} \quad \ldots\ldots 1$$

where C_1V_1 is the amount of substance dissolved.

Application of this method to a non-static system, such as the living organism, requires a number of assumptions to be made:

1. the given substance must be rapidly and fully dispersed in the compartment to be measured (equilibration);

2. the compartment which is the subject of the study must be the only compartment in which the substance is dispersed;
3. the compartment which is the subject of the study must not undergo any volume changes during the study period.

Dilution methods are used for the measurement of:

a. Total body water (TBW)

For this deuterium oxide (D_2O), tritiated water, antipyrine or urea may be used. Comparison of these methods shows that they give different results. D_2O and tritiated water give a result which is approximately 2% higher than that obtained with antipyrine because, as well as mixing with the water compartment, there is an exchange with hydroxyl groups in other chemical compounds (Foy and Schnieden, 1960; Bradbury, 1961; Tisavipat et al., 1974).

In our study we used the D_2O-dilution method for the measurement of total body water because of the following considerations:

- Antipyrine is rapidly metabolized in the body. Therefore in using antipyrine it is necessary to measure the concentration in the plasma at various intervals after administration and thereby to construct a curve which can be extrapolated to time 0. This gives the initial concentration after equilibration in the fluid compartment. Thus, using antipyrine, it is necessary to take repeated blood samples which can be taxing for the patient.
- Tritiated water is less suitable because it contains a radioactive isotope.
- Urea is less suitable for use in young babies for the practical reason that multiple plasma concentrations must be measured: first the initial concentration of endogenously produced urea must be measured and thereafter the process of equilibration must be followed by repeated plasma estimations (McCance and Widdowson, 1951[b]). The excretion of urea in the urine must also be accurately

measured at the same time. Accurate urine collection in young babies is frequently unsuccessful.
- $\underline{D_2O}$ has the advantage that only one blood sample is necessary, at the end of a three hour equilibration period. In view of the relatively long biological half life (10 days) of D_2O, the amount excreted during the equilibration period is insignificant. The giving of a small quantity (3gms per Kg body weight) of the non-radio-active D_2O entails no risks to the patient.

b. Extracellular Volume (ECV)

This comprises plasma, interstitial fluid and transcellular fluid by which is understood: cerebrospinal fluid, synovial fluid, tear fluid, fluid in exocrine glands, bile, urine and the fluid in the bowel.

The ECV can be measured with the help of inulin, mannitol, sodium thiosulphate, sodium thiocyanate, sodium bromide, radioactive chlorine (Cl^{38}) and sodium (Na^{24}). The results obtained are not identical. Inulin gives results which are lower than those found using other substances, because it does not penetrate two of the most important transcellular water compartment i.e. the cerebrospinal fluid and the fluid in the bowel (Finkenstaedt, 1953; Morrison, 1959). This is probably because of the size of the molecule (Swan et al. 1954). Measurements with mannitol give comparable results to those obtained in man with inulin. Mannitol is metabolised during the equilibration period and this must be corrected for (Levitt and Gaudino , 1950). Thiosulphate also gives comparable results to inulin (Ikkos, 1956). Thiocyanate penetrates the erythrocyte and the mucosa of the stomach and this leads to overestimation of the ECV (McCance and Widdowson, 1951^{b}). Overestimation of the ECV also occurs with the use of sodium bromide, because exchange takes place with chloride ions in the tissues. Thus it is usual to speak of the "bromide space" instead of the ECV in this

case.

Exchange with the Na and Cl in the cells also occurs when using Na^{24} and Cl^{38}. Therefore it is usual, when discussing results from such isotope dilution methods, not to speak of a specific fluid compartment but to speak of "total exchangeable" ion e.g. Na or Cl.

We used sodium bromide in our measurement of the ECV because of the following considerations:

- inulin and mannitol have the disadvantage that repeated blood samples are necessary in order to follow the equilibration process and moreover it is also necessary to collect the urine accurately in order to measure the excretion. The latter is also true for thiosulphate and thiocyanate.
- the use of radioactive isotopes was felt undesirable.
- there was a good bromide measurement technique readily available using a selective Br^-electrode (Degenhart, 1972). The technical details of this measurement are discussed in Chapter III.3. The amount of sodium bromide given was far below the pharmacological dose.

c. Plasma Volume

The dye T1824 (Evans Blue) or I^{131} may be used for this measurement. The measurement of plasma volume is used in studying a number of clinical conditions such as dehydration, heart failure, shock, blood loss and burns. Knowledge of the plasma volume is less important in the study of body composition because it is a relatively small body compartment i.e. about 4-5% of total body volume.

d. Total exchangeable potassium

The total exchangeable potassium may be taken as an estimate of the total cell mass of the body and can be measured using the radioactive isotope K^{42}. Because equilibration occurs slowly (about 24 hours) it is important that the amount excreted in the urine is taken into

consideration. Body potassium determination using K^{42} measurement is 3-10% lower than the same estimation using K^{40} measurement (see II.2.2.).

A further comparison of substances used to measure fluid compartments of the body is given in fig. 1.
Table I gives some results of dilution techniques derived from the literature.

II.2.2. K^{40} Measurement

The potassium (K) in the body is predominantly intracellular. Measurement of the total amount of K in the body can therefore give information on the total cell mass. In order to measure the total amount of K in the body, use can be made of the fact that 0,012% of K naturally exists in the form of K^{40}, an isotope with a very long half life (10^9 years), that gives off a gamma-radiation of 1.46 MeV.

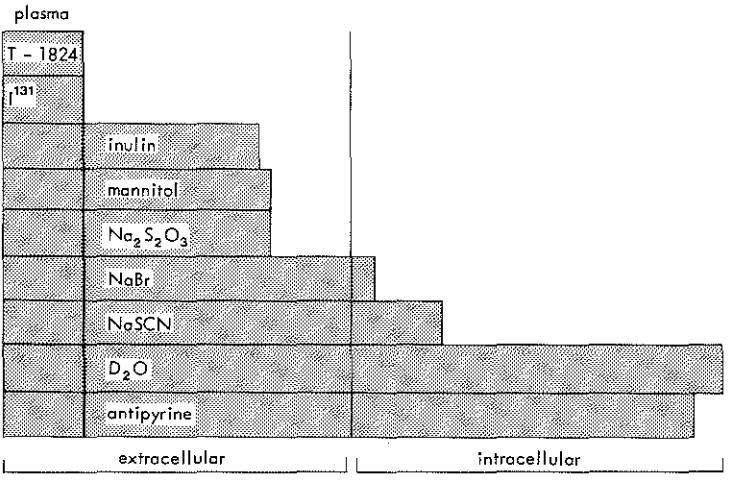


Fig. 1. Comparison of substances used to measure fluid compartments of the body.

Compartment	Volume		References
Plasma (ml/kg body wt.)			
Evans blue	42-46	(adults)	Gibson and Evans (1937)
I^{131}	40	"	Storaasli (1950)
Extracellular volume (% body wt.)			
Inulin	15	(adults)	Berger et al. (1949)
	16	"	Schwartz et al. (1950)
Mannitol	16	"	Schwartz et al. (1950)
	23	"	Elkinton (1947)
Na-thiosulfate	17	"	Cardozo and Edelman (1952)
Na-thiocyanate	22	"	Lavietes et al. (1936)
	27	"	Moore (1946)
Na-bromide	27	"	Schwartz et al. (1949)
	29	"	Brodie et al. (1939)
	38	(infants)	Cassady (1970)
Total body water (% body wt.)			
D_2O	62	(adults)	Schloerb et al. (1950)
	77	(infants)	Friis-Hansen (1957)
Antipyrine	52	(adults)	Soberman et al. (1949)
	69	(infants)	Cassady and Milstead (1971)

Table I Some results of dilution techniques in man.

The gamma radiation can be measured in the so called "total body counter". For the detection of gamma radiation use is made of the fact that in certain materials small flashes of light are produced whenever energy from gamma radiation is absorbed. This phenomenon is called scintillation. The flashes of light are converted into electrical pulses and these are then amplified in the so called "photomultiplier tube". The size of the electrical pulse is proportional to the amount of gamma energy absorbed. With the help of electronic analysis apparatus the electrical pulses are selected for size and counted. This results in the gamma spectrum. The total amount of potassium in the body can be calculated from the size of the K^{40} peak in the spectrum which is formed from the number of pulses representing a gamma energy of 1.46 MeV.

There are two types of scintillation counters:

1. counters in which the detection system is formed by one to four detectors each containing a large sodium iodide crystal. These are placed in the proximity of the experimental subject.
2. counters in which the detection system comprises a large, hollow, double walled cylinder. The space between the two walls of the cylinder is filled with liquid scintillation material. The experimental subject is placed within the cylinder.

Scintillation counters must be placed in a room which is protected from extraneous gamma radiation (e.g. cosmic radiation) by lead or iron walls.

Experience is still very limited with K^{40} measurement in babies and young infants (Forbes et al., 1963; Maresh et al. 1966; Novak et al. 1970, 1973). Buying and installing a total body counter demands a considerable investment and calibration is difficult.

On the other hand the method is non-invasive and safe.

II.2.3. Densitometry

Starting with the assumption that the body consists of two compartments, fat and lean body mass, each with its own constant specific gravity (s.g.), it is possible to calculate the proportional relationship between the two compartments by measurement of the specific gravity of the whole body. The specific gravity can be defined as:

$$\text{s.g.} = \frac{\text{Body weight}}{\text{Body volume}}$$

The body weight is measured by weighing in air and the body volume by weighing under water. A correction must be made for the air in the lungs. This can be measured by the N_2 washout technique.

Siri (1955) derived the following formula for percentage of fat in man: assuming that fat has a specific gravity of 0,9 and lean body mass a specific gravity of 1,1:

$$\text{Percentage fat by weight} = \frac{4.95}{\text{s.g.}} - 4.5$$

Assuming slightly different specific gravities for fat and lean body mass Keys and Brozek (1953) deduced the following formula:

$$\text{Percentage fat by weight} = \frac{4.201}{\text{s.g.}} - 3.813$$

and Rathbun and Pace (1945):

$$\text{Percentage fat by weight} = \frac{5.548}{\text{s.g.}} - 5.044$$

Under-water weighing for the determination of specific gravity is for obvious reasons unsuitable for use in babies and young infants.

The method developed by Pearse et al. (1976 a, 1976 b) for measuring body volume (and hence specific gravity) is worthy of special attention. Here the baby was placed in one of two identical airtight boxes and an identical volume of air was pumped in sinusoidal fashion in and out of each box simultaneously. The fluctuation in the differential pressure between the two boxes allowed for the calculation of the body volume. No correction is necessary for air in the lungs or bowel with this technique.

II.2.4. Radiography

This method depends upon the measurement of bone, muscle and subcutaneous fat on X-ray photographs. An impression can be obtained of the size of these compartments in the whole body by these measurements. The hand X-ray is chosen for preference, because the thickness of the cortex of the second metacarpal can be used as an index for the total skeletal mass (Garn, 1970). Tanner (1962) has followed radiologically the changes which occur during growth. The growth velocity of bone and muscle tissue seems to follow a curve which closely resembles the growth velocity curve for body length: it begins high in the first year after birth and then drops rapidly to a plateau until puberty. When the puberty growth spurt occurs it forms a peak. Skin plus subcutaneous fat decrease from the age of 9 months until about 6 years and thereafter, particularly in girls, the subcutaneous fat increases.

From the practical viewpoint the method does not appear to be useful for detecting changes in body composition which take place within a period of days or weeks. However the radiological method can offer useful information on changes which take place over a longer period. The irradiation involved is an obvious disadvantage of this method.

II.2.5. Anthropometric methods

The measurement of the subcutaneous fat layer in various places on the body is a much used method for obtaining an impression of the extent of the fat compartment. This method is also frequently used in young infants. The technique is simple and uses a skinfold caliper (e.g. Harpenden). The results are easily reproduceable provided that it is done by the same operator using the same caliper. Measurement is usually done in two places, namely on the extensor side of the upper arm (triceps) and below the tip of the scapula (subscapular), always on the left side. Tanner and Whitehouse (1962, 1975) have produced percentile curves for triceps and subscapular skinfold thickness for children from 0-16½ years of age. Oakley et al. (1977) did the same for newborn babies with a birth weight between 2,250 and 4,500 grams and a gestational age of 37-42 weeks.

Efforts have been made in various ways to get more information about body composition employing skinfold measurement. It is a well known observation that after application of the skinfold caliper to the skin it takes some time before a stable value can be read off. This is very probably due to squeezing interstitial water out of the skinfold. Brans et al. (1974) quantified this phenomenon by taking readings at 15 and at 60 seconds after application of the skinfold caliper. According to these authors these so-called "dynamic skinfold measurements" can give an impression of the subcutaneous interstitial fluid and therefore of the ECV.

Dauncey et al. (1977) have developed a method for calculating the total amount of subcutaneous fat using nine measurements: the circumference of the head, chest, upper arm, thigh and calf, the length of the whole body, arm and leg, crown-rump length and two skinfolds (triceps and subscapular). They found that, for babies, this gave reasonable agreement with the percentages for total body fat known

from carcass analysis. We have calculated the amount of total body fat using the method of Dauncey et al. (1977) in a number of our patients and these results are discussed later (Chapter V.2.).

II.2.6. Neutron activation

In this method the body is irradiated with neutrons causing a number of elements to change partially into isotopes which then emit γ-rays. This gamma radiation can then be measured in the total body counter. In this way the Ca, P, Na, Cl and N in the body can be measured. (Anderson et al., 1964, Nelp et al. 1970; Cohn and Dombrowski, 1971; Harvey et al. 1973; Dombrowski et al., 1971; Harvey et al. 1973; Dombrowski et al., 1973). Although the amount of neutron irradiation is quite small (about 1 rem) it makes this method less suitable for children.

II.2.7. Creatinine excretion in the urine

The amount of creatinine which is excreted in the urine is generally considered to be a reflection of the muscle mass of the body. Various authors (Muldowney et al., 1957; Chinn, 1967; Young et al., 1968; Cheek, 1968; Boileau, 1972; Turner and Cohn, 1975; Forbes and Bruining, 1976) have developed formulae for the calculation of lean body mass in man using the 24 hour excretion of creatinine and comparing this with other measurement techniques for lean body mass such as K^{40} measurement, K^{42} dilution, densitometry, total body water and carcass analysis.
These authors give data for adults. Only Cheek (1968) has calculated for a group of babies the relationship between creatinine excretion in the urine and intracellular water. Using this relationship plus that between lean body mass and total body water (lean body mass in Kg = total body water x $\frac{1}{0.73}$) given by Malina (1969), Forbes and Bruining (1976) have derived the following formula for the

relationship between lean body mass and creatinine excretion:
Lean body mass (Kg) = 2,78 + 0,0491 x creatinine in mg/day.
Accurate urine collection is a condition for the use of this method. This should preferably be done over several consecutive days. The fact that the creatinine excretion in the urine can vary from day to day (Paterson 1967, Zorab et al. 1969) must be taken into account. The collection of urine for example over 3 consecutive days in young babies can lead to practical problems.

II.2.8. Measurement of fat by the absorption of fat soluble gases

This method has been used successfully by a number of authors (Lesser et al., 1960, 1971; Hytten et al. 1966) for the measurement of total body fat in experimental animals and adults. The method has the disadvantage that it requires complicated apparatus and that the measurement takes a long time, which can be unpleasant for the subject. Its greatest advantage is that it is a direct method for measuring total body fat in longitudinal studies. Further it is non-invasive. For these reasons we chose the gas absorption method adapted for use in young babies. The method which we used is described in detail in the next chapter.

Chapter III

METHODS FOR MEASURING BODY COMPOSITION USED IN THIS STUDY

III.1. The Xenon absorption method for the measurement of total body fat

III.1.1. Theoretical considerations

A closed system was chosen for the measurement of total body fat by the gas absorption technique. (Mettau et al., 1977). A known amount of the gas for absorption was injected into the circuit and the subject breathed the gas mixture in the system for a given time. During this period the gas for absorption diffuses through the lungs and is distributed to the various body tissues, in particular the fat. The difference between the concentration of the gas in the circuit measured at the beginning and end of the experiment gives the volume of the gas which has been absorbed by the organism. It is necessary to keep the volume of the system as small as possible in order to obtain distinct concentration differences and thus accurate estimation of the amount of gas absorbed. This has been done in adult subjects by using a helmet which covers the head or with a snug fitting anaesthetic mask (Lesser and Zak, 1963). These methods are unpleasant for the subject, particularly in longer lasting experiments and are not suitable for newborn babies or infants. Furthermore, possible gas loss through transcutaneous diffusion is not taken into account. (Klocke et al., 1963).

These objections are answered by placing the whole patient in a closed incubator which allows good observation of the patient and, just as in the usual incubator for

newborn babies, provides for maintenance of a suitable environment for the baby with regard to temperature, relative humidity and oxygen concentration. The disadvantage of this method is that it requires a relatively large circuit volume. In our apparatus this is 6 to 16 times larger than the volume of the patient. As a result, the difference in the concentration of the gas before and after absorption is, at best, very small. Using gas chromatographic analysis of the gas mixture in the circuit, the error in measurement was 0.5 - 1.0%. This was due to instability of the gas chromatograph and to sampling error. Thus the method for measuring the drop in concentration of the gas, (which was of the order of 0.5% during the whole absorption period) was not accurate enough to give any useful information. However, this information can be obtained when the absorption phase is followed by a second period, during which the gas which has been absorbed by the organism is allowed to diffuse back into the circuit from which the circulating gas has previously been removed. The process of desorption can be followed accurately by repeated gas chromatographic analysis of the gas/air mixture in the circuit. The resultant washout curve can be used, as in our experiments, for the calculation of the total amount of inert gas which has been absorbed by the organism and subsequently desorbed. This is explained below (III.1.6.).

The absorption coefficients

The Bunsen absorption coefficient (α) was used for comparison of the solubility of the various gases which were considered as measurement gases. This may be defined as the volume of gas (Vgas) at $0^{o}C$ (T_o) and at a partial pressure of 760 mm Hg (P_o) which dissolves in a unit volume of liquid (V) at the temperature of the experiment and at a partial pressure of 760 mm Hg:

$$\alpha = \frac{V_{gas}(P_0, T_0)}{V} \quad \ldots\ldots 2$$

If the partial pressure is not 760 mm Hg, then, assuming proportionality, it follows that

$$\alpha = \frac{V_{gas}(P_0, T_0)}{V} \cdot \frac{P_0}{P_1} \quad \ldots\ldots 3$$

where P_1 = the partial pressure of the gas
and P_o = 760 mm Hg

The Oswald solubility coefficient (L) has also been used. This may be defined as the relationship between the volume of the absorbed gas and the volume of the absorbing liquid (V) under conditions P and T.

$$L = \frac{V_{gas}(P,T)}{V} \quad \ldots\ldots 4$$

This is an equilibrium constant which is independent of the partial pressure of the gas. The temperature T and the total pressure P must be defined in order to define this coefficient.

The Oswald solubility coefficient at a pressure of 760 mm Hg (P_o) is defined as:

$$\beta = \frac{V_{gas}(P_0, T)}{V} \quad \ldots\ldots 5$$

From the law of Boyle/Gay-Lussac ($\frac{VP}{T}$ = constant) it follows that:

$$V(P_0,T_0) \cdot \frac{P_0}{T_0} = V(P,T) \cdot \frac{P}{T} = V(P_0,T) \cdot \frac{P_0}{T} \quad \ldots\ldots 6$$

Therefore:

$$\alpha \cdot \frac{P_0}{T_0} = L \cdot \frac{P}{T} = \beta \cdot \frac{P_0}{T} \quad \ldots\ldots 7$$

and:

$$\alpha = L \cdot \frac{P}{P_0} \cdot \frac{T_0}{T} \quad \ldots\ldots 8$$

and:

$$\alpha = \beta \cdot \frac{T_0}{T} \quad \ldots\ldots 9$$

We have used the Bunsen absorption coefficients in water (α_w), fat (α_f) and protein (α_p) given by Shu-Yuan Yeh and Peterson (1963, 1964, 1965) in our calculations. Where Oswald solubility coefficients were given these have been converted to Bunsen coefficients using equation (9) above.

The Bunsen coefficients at 4 different temperatures (45, 37, 30 and 25°C) are given in Table II.

The values for α for temperatures lying between these values were calculated by a quadratic interpolation programme:

$$\alpha(t_i) = C_0 + C_1 t_i + C_2 t_i^2 \quad \ldots\ldots 10$$

where t_i is the rectal temperature less 1°C (Lesser et al., 1960) and C_o, C_1 and C_2 are constants.

t (°C)	α_w	α_p	$\alpha_{f(rat)}$	$\alpha_{f(human)}$
45	0.0584	-	1.4276	1.4748
37	0.0685	0.1315	1.5712	1.6197
30	0.0806	0.1761	1.7197	1.7667
25	0.0894	0.2182	1.8376	1.8677

Table II Bunsen absorption coefficients for water, protein, rat and human fat at 4 temperatures taken from the literature (Shu-Yuan Yeh and Peterson 1963, 1964, 1965).

The choice of inert gas for measuring total body fat.

The gas to be used must, as far as possible, fulfil the following criteria:

a) it must be inert. That is to say that it should not undergo any chemical change or fixation in the body;
b) it must not be toxic and it must not have any physiological effects such as narcosis;
c) it must have a high solubility coefficient in fat so that the measurement results are optimal;
d) its solubility coefficient in the other body compartments, particularly water, must be relatively low;
e) the gas must be easy to measure.

A comparison of the absorption coefficients for oil (fat) and water, of the various gases considered is given in Table III.

	$\alpha(37^{\circ}C)$ oil	$\alpha(37^{\circ}C)$ water	$\frac{\alpha(\text{oil})}{\alpha(\text{water})}$
Carbon dioxide (CO_2)	0.876	0.56	1.6
Xenon (Xe)	1.70	0.085	20.0
Krypton (Kr)	0.43	0.045	9.6
Radon (Ra)	19.00	0.15	126.7
Nitrogen (N_2)	0.067	0.013	5.2
Cyclopropane	11.26	0.46	24.5
Nitrous oxide (N_2O)	1.4	0.44	3.2
Ether	50.00	15.4	3.2
Helium (He)	0.015	0.0085	1.8
Oxygen (O_2)	0.12	0.024	5.0

Table III Bunsen absorption coefficients for oil and water for a number of gases.

Radon has the highest α_{oil}(fat) and also the highest $\alpha_{oil}/\alpha_{water}$ - ratio. However, because of its radioactivity, it is unsuitable. Next to Radon, Xenon is most suitable, both with regard to the α_{oil} and the $\alpha_{oil}/\alpha_{water}$ ratio. Krypton is slightly less suitable. Cyclopropane is also good but its narcotic effect is nevertheless a disadvantage and the same applies to nitrous oxide and ether. After weighing these considerations, Xenon was chosen in a concentration of 5%. This gas also satisfies the other proposed criteria: it is inert, in this concentration it is not narcotic and it is easily measured in a gas chromatograph.

III.1.2. Apparatus*)

The apparatus for measuring total body fat by Xe-absorption consists of a glass bell jar (which is capable of being sealed hermetically) connected to a closed circuit containing automatic control systems for temperature, relative humidity, oxygen concentration, air flow and air pressure. A gas chromatograph is connected to the circuit for the measurement of the Xenon concentration. Fig. 2 shows the apparatus with its various components. Fig. 3 shows the circuit diagrammatically. In the detailed description which follows the numbers in brackets after the various components refer to the numbers given to these components in the figs. 2 and 3.

The closed circuit

Two glass bell jars (one of 15.5 L and one of 39.8 L) (fig. 4) were constructed as follows. For each bell jar a round ended glass cylinder (Schott-Marne, D 50 glass, internal diameter 300 mm, wall thickness 7 mm) of desired length was cut longitudinally at the desired height and

*) The apparatus was designed and constructed by the Central Research Workshop, Medical Faculty, Erasmus University, Rotterdam (Head: Ir. H.A. Bak).

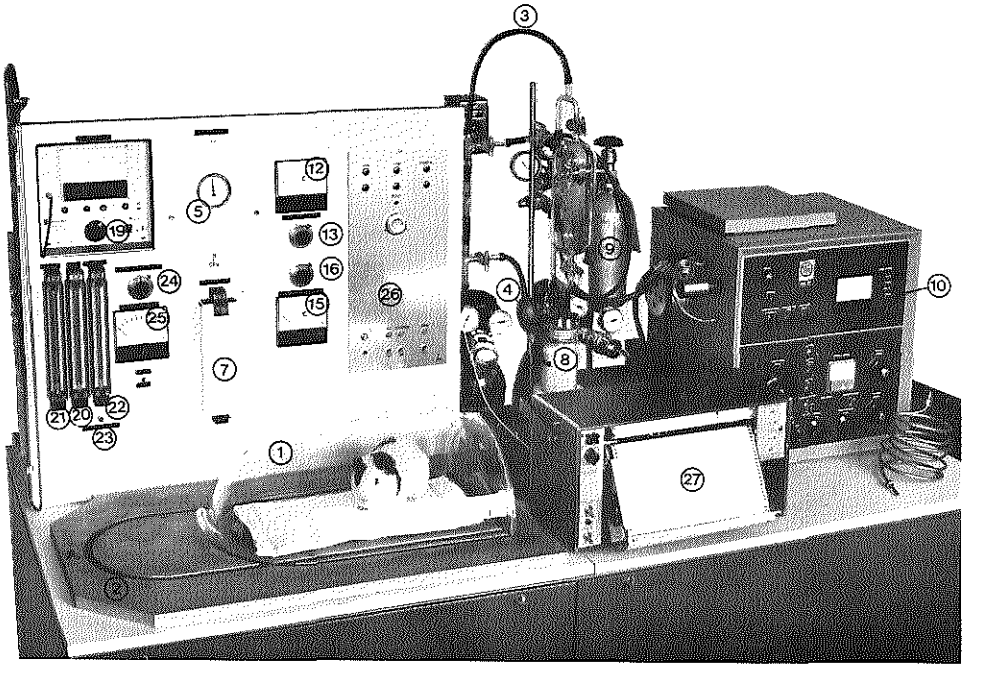


Fig. 2 Apparatus for measuring total body fat by Xe-absorption. Legend see p. 39.

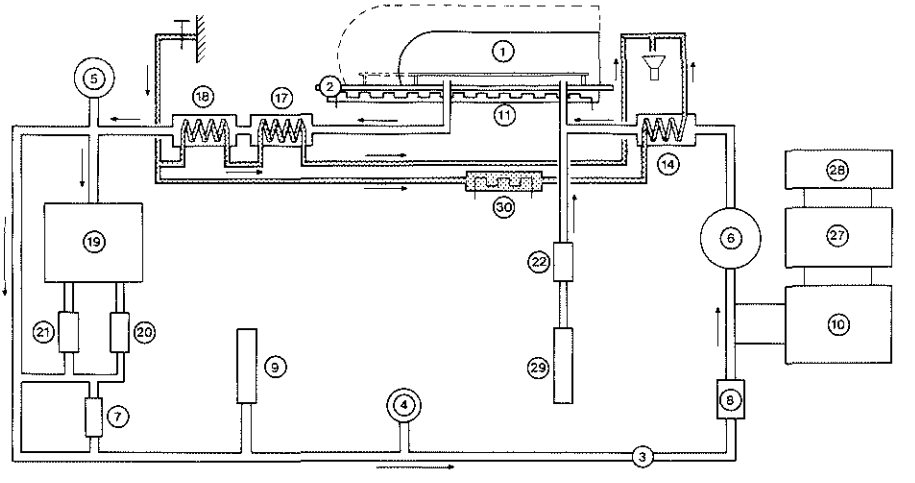


Fig. 3 Apparatus for measuring total body fat by Xe-absorption. Diagram of circuit. Legend see p. 39.

Legend fig. 2 and 3

1. Glass bell jar
2. Aluminium baseplate
3. Neoprene tubes
4. Rubber bulb
5. Manometer
6. Membrane pump
7. Main flowmeter
8. Soda-lime container
9. Xenon cylinder
10. Gas chromatograph
11. Heating element
12. Incubator temperature meter
13. Incubator temperature control dial
14. Main condenser
15. Condenser temperature meter
16. Condenser temperature control dial
17. Condenser
18. Condenser
19. Oxygen concentration meter
20. Oxygen flow meter
21. Shunt flow meter
22. Oxygen injection flow meter
23. Control screw for oxygen injection
24. Control dial for oxygen concentration
25. Oxygen uptake meter
26. Alarm signals
27. Pen recorder
28. Integrator
29. Oxygen cylinder
30. Quartz heater.

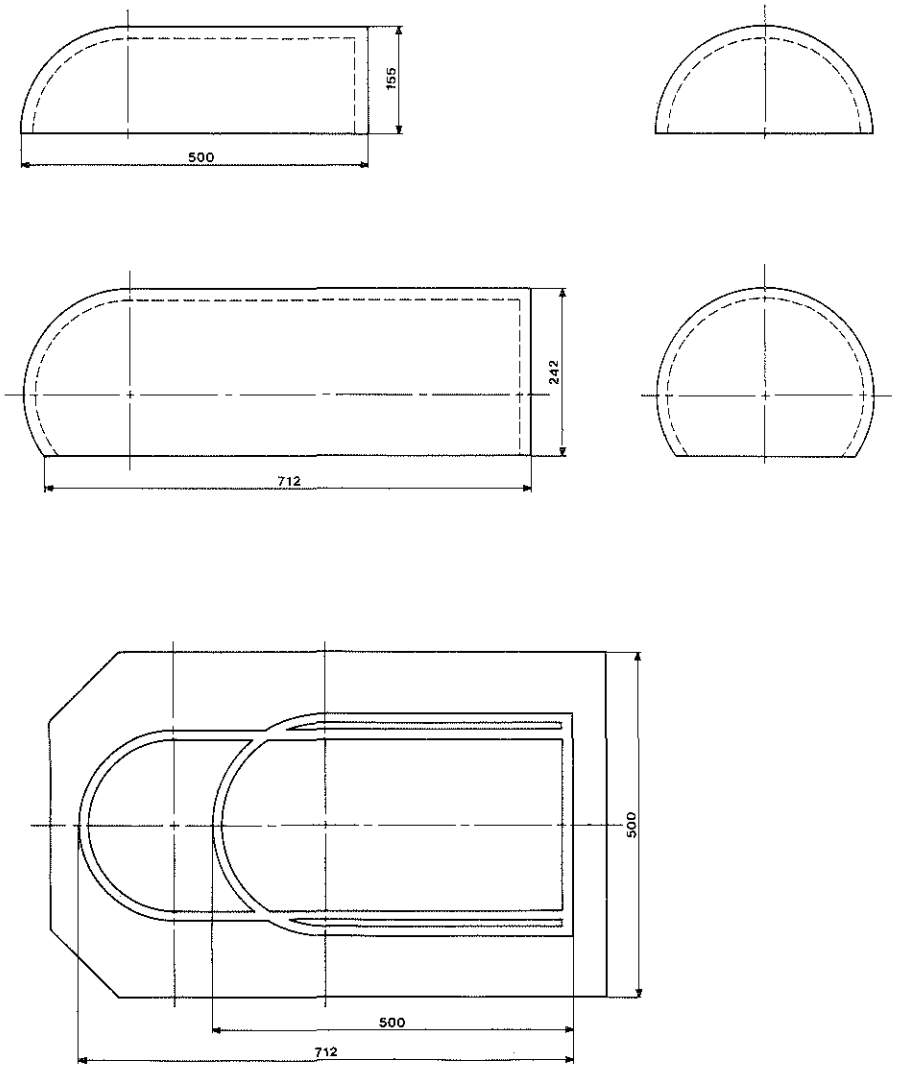


Fig. 4 Dimensions of the two glass bell jars, in mm.

fitted with a plate of glass (thickness 7 mm) at the open end. The two pieces of glass were fixed to each other with a special glue (EC 1294/1295, M3 Company) which is impermeable to fluids and gases. The edges of the glass were ground flat and polished (1). An anodized aluminium plate of 20 mm thickness was chosen as the baseplate for the bell jar (2). Grooves were cut in it corresponding to the shape of the bottom of the glass cover. These grooves were fitted with strips of foam rubber soaked in glycerol. An air tight connecting piece (Leybold-Heraeus, GM. 32/915) was set into the baseplate for transmission of the signals from the patient to the monitoring equipment.
Two openings were made in the aluminium baseplate. The space under the bell jar, when resting on the baseplate, was connected to the rest of the circuit through these openings by neoprene rubber tubing (3) (internal diameter 6 and 9 mm).
Neoprene rubber was chosen because it is almost impermeable to gases.
A hermetic seal between the bell jar and the baseplate can be obtained by sucking air out with the help of a small rubber bulb (4) to give a pressure of approximately minus 30 cms water. A manometer (5) (Econosto, range +10 to -60 cms water) was built into the circuit to measure this negative pressure. Air was pumped through the circuit by a membrane pump (6) (Charles Austen, Mk II, 0-8 L/min) at 8 L/min. This was indicated on a flow meter (7) (Rotameter, Brooks Instruments; 0 to 10 L/min.). A soda-lime container (8) was also built into the circuit for the absorption of carbon dioxide. A cylinder of Xe (9) and a gas chromatograph (10) were also connected to the circuit.

The heating system

A heating element, consisting of five nichrome resistance wire coils connected in parallel, was placed between two layers of asbestos (11). This was fixed to the

undersurface of the baseplate and was covered with a thin aluminium plate. The baseplate and the air in the bell jar can be warmed by this heating element (maximum capacity 100 W with a voltage of 25 $V_{eff.}$ alternating current). The temperature in the bell jar can be measured with a P.T.C. element (platinum resistance sensor, Degussa, PT 100) and read off on a meter (12). The difference between the desired and actual incubator temperature was fed to an electronic control system (13) which controlled the current to the heating element.

The humidity regulation

Watt's law was used in the regulation of humidity in the system. This states that in a closed system in which in various places various temperatures occur, the water vapour pressure will tend towards the saturated water vapour pressure at the lowest occuring temperature.
A condenser (14) was placed in the circuit before the incubator (with regard to the direction of the circulation of air). Tap water was warmed by a quartz heater (30) and fed into the condenser in the opposite direction to the air flow through it. The air in the condenser takes on the temperature of the condenser and the water vapour pressure becomes fully saturated at that temperature. When the incubator temperature is higher than the condenser temperature then the water vapour pressure in the incubator will be the saturation value for the temperature of the condenser. The desired humidity could be set by altering the condenser and incubator temperatures. The condenser temperature could be preset on a dial (15) and the actual condenser temperature read off on a control meter (16). Two condensers (17, 18) were placed in the circuit after the incubator to cool the air coming out of the incubator in order to prevent condensation forming in other parts of the circuit which could be sensitive or difficult to reach.

Regulation of the oxygen concentration

Provisions were made to keep the oxygen concentration in the circuit constant. Oxygen was provided from a cylinder (29) via a pressure reducing valve (Air Liquide, Parvabloc), a solenoid valve (Econosto, ASCO), a flow meter (Rotameter, Brooks Instruments; 0-1,5 L/min.), and a needle valve (Kuhnke). The partial pressure of oxygen was measured by an oxygen analyser (19) (Servomex OA 100). The flow of oxygen through the analyser cell (100 ml/min) was given by a flow meter (20) (Rotameter, Brooks Instruments: 0-150 ml/minute) while the shunt flow passed through a second meter (21) (Rotameter, Brooks Instruments, 0-5 L/min.). Before the air was passed through the analyser it was dried and cooled in a condenser. Regulation of the oxygen concentration was done by intermittent opening and shutting of a solenoid valve in the oxygen supply line with a time cycle of 20 seconds. The length of the oxygen injection period was controlled electronically, based on the oxygen concentration measured in the oxygen analyser. The maximum opening time of the valve was 10 seconds per 20 second cycle. The oxygen flow through the open valve could be read on a flow meter (22) and was adjustable on a dial (23) from 0.4 to 0.8 L/min. The desired oxygen concentration could be set with a dial (24). The average amount of oxygen supplied was given on a control meter (25) which gave the average time of oxygen injection as a percentage of the time cycle.

Safety system

An audible alarm system and warning lights were placed together on a control panel (26) for the following:

Negative pressure

A negative pressure of 50 cms water would be sensed by a photo electric system in the manometer (5) and would lead to the opening of a safety valve and to the pump being

switched off. Warning would be given by a red light and an audible alarm.

Flow

Sufficient flow is necessary for satisfactory dispersion of oxygen and Xe in the circuit and for removal of carbon dioxide by the soda-lime container. A flow of less than 7 L/min. would be signalled by a photo electric cell in the main flow meter (7). After a latent period of 3 minutes this would lead to the opening of a safety-valve; warning would be given by a red light and an audible alarm.

Oxygen concentration

An oxygen concentration below 20.5% as measured in the oxygen analyser would lead, after a latent period of 3 minutes, to the opening of a safety-valve; warning would be given by a red light and an audible alarm.

Power failure

An audible alarm, powered by a rechargeable battery, would be brought into operation in the case of power failure.

The Xenon detection system

The Xenon concentration in the circuit was measured in our experiments by a gas chromatograph (10) (Varian Aerograph 90-P_3) with a manually operated solenoid valve. The stainless steel column was 2 meters long and was filled with an 80/100 mesh 5 Å molecular sieve (Applied Science, catalogue number 05652). The measurements were done with a column temperature of 250°C. The filament current was 200 mA. Helium was used as carrier gas at a pressure of 3.4 atmospheres and a flow of 100 ml/minute. Under these conditions the number of theoretical plates of the column was approximately 1000.
A 10 ml sample was taken every 2 minutes. The signal from

the gas chromatograph was drawn by a pen recorder (Hewlett-Packard 7100 B) and the peaks were simultaneously measured by an electronic integrator (Infotronics 100 A).
Under the above conditions the separation of Xe from oxygen and nitrogen was very satisfactory as can be seen in fig. 5.

III.1.3. General procedure

Before starting any experiment the pressure, flow and temperatures in the column, injector and detector of the gas chromatograph were checked.
Thereafter the patient (or experimental animal) was placed in the incubator. In all experiments the smaller of the two incubators was used. The larger incubator was made for measurements in full term babies. Animals were put into a metal cage, which fitted exactly under the bell jar. Infants were laid on a mattress, consisting of a stainless steel frame with a cloth covering. Electrodes for continuous monitoring of heart rate, respiration rate, E.C.G. and rectal temperature were attached to the infant and connected to the monitoring instruments via the air-tight connecting piece in the aluminium base. Temperature, and oxygen concentration in the incubator and condenser were set at the desired levels. The pump was started and the glass bell jar was closed. Hermetic closure was obtained by withdrawing air out of the circuit, as described above, until a pressure of about minus 30 cms water was obtained.

99,9% Pure Xenon (Xe), (L'Air Liquide), was injected to give a concentration of about 5 percent. The body now started to absorb Xe but the drop in Xe concentration was so small that gas chromatographic analysis at this stage did not allow an accurate determination of the decrease in concentration, which was in the order of 0.02% absolute or 0.5% of the initial concentration. During the absorption phase the temperature of the incubator and condenser and the negative pressure were kept constant. The oxygen

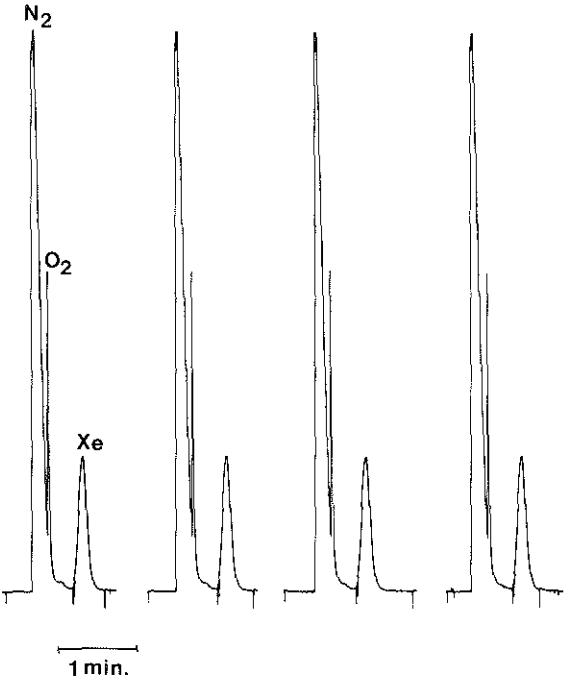


Fig. 5 Gas chromatographic tracing showing separation of the Xe from oxygen and nitrogen.

concentration in the circuit was kept constant by intermittent injection. Carbon dioxide was absorbed in a soda-lime container.

At the end of the absorption phase (120 minutes) the partial pressure of Xe in the circuit was measured by the gas chromatograph and the incubator was immediately opened for two minutes during which time the circuit, the surface of the patient and the incubator were cleared of Xe as completely as possible with a fan, the incubator was reclosed and the negative pressure restored.

Now the second stage of the experiment, the desorption

phase, was begun, during which the Xe which had been absorbed by the body diffused out again into the circuit. In contrast to the absorption phase, the dynamics during the desorption phase could be followed accurately by repeated measurements of the Xe concentration. A 10 ml sample of gas was withdrawn every 2 minutes and analysed in the gas chromatograph. A Xe appearance curve was obtained (fig. 6) by plotting Xe concentration against time. For practical reasons (feeding schedules, nappy changing etc.) the measurement of the desorption phase was stopped after 150 minutes. Analysis of the Xe-appearance curve allowed calculation of the amount of Xenon originally absorbed (see III.1.6.).

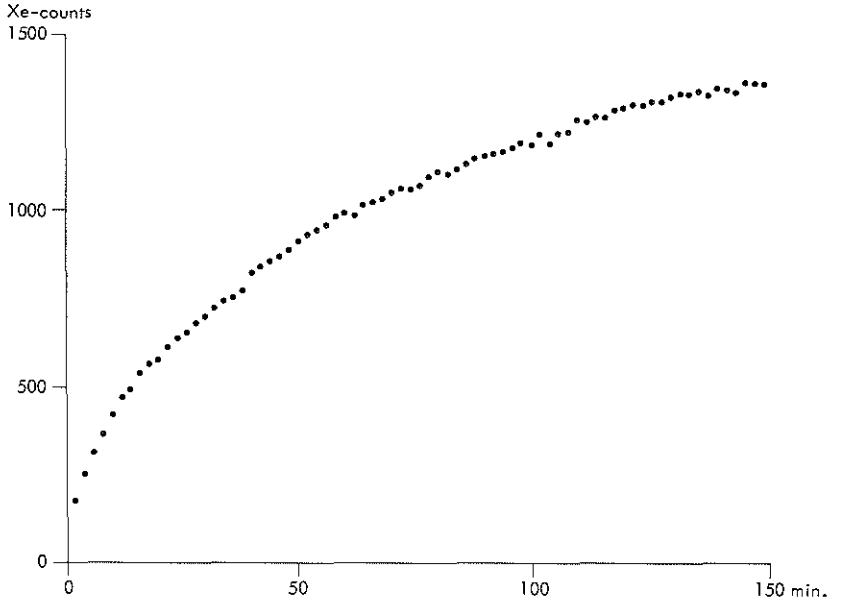


Fig. 6 Xe desorption curve, Xe versus time.

III.1.4. Correction factors

A number of corrections must be made prior to the mathematical processing of the desorption curve.

III.1.4.1. Correction for sampling

Every analysis by the gas chromatograph (GC) removes 10 ml of the Xe/air mixture from the circuit. During a desorption phase of 150 minutes, sampling every 2 minutes, 75 gas samples are withdrawn for GC-analysis and thus 75 x 10 = 750 ml is removed; this is approximately 4% of the volume of the circuit. The calculation of the correction for this loss of Xe is given in Table IV.

Time	Xenon removed in the sample	Measured Xe (concentration)	Corrected Xe measurement
0	–	–	–
2	δXe_2	Xe_2	Xe_2
4	$\delta(Xe_2+Xe_4)$	Xe_4	$Xe_4+\delta Xe_2$
6	$\delta(Xe_2+Xe_4+Xe_6)$	Xe_6	$Xe_6+\delta(Xe_2+Xe_4)$
i	$\delta(\Sigma Xe_i)$	Xe_i	$Xe_i+\delta(\Sigma Xe_{i-1})$

Table IV Calculation of correction for Xe-loss by sampling.

$$\delta = \frac{\text{volume of the gas sample in ml}}{\text{volume of the circuit in ml}} \quad \ldots\ldots 11$$

The calculation of δ is done for each point with the help of a calculator programme (see appendix).

III.1.4.2. Correction for the interval between the absorption and desorption phases (clearing phase).

Some Xe is inevitably retained in the circuit at the end of the 2-minute interval between the absorption and desorption phases; complete elimination of Xe during this short period is impossible. This must be allowed for. Therefore all Xe values measured during the desorption phase have to be reduced by the amount of Xenon still present in the circuit at the end of the 2-minute clearing phase.

It must also be noted that desorption of Xe from the organism starts as soon as the incubator is opened at the beginning of the clearing phase, and some Xe is lost by desorption from the body during this 2-minute interval. This must also be allowed for. The amount of Xe lost during the clearing phase has to be added to all Xe values measured during the desorption phase. The magnitude of both corrections can be calculated after extrapolation of the measured desorption curve to the time minus 2 minutes, i.e. the beginning of the clearing phase.

Figures 7 and 8 are examples of Xe washout curves, during the first 30 minutes of the desorption phase. The best-fitting parabola passing through the first 15 points is calculated with a quadratic least-square fitting formula:

$$Xe_t = b_0 + b_1 t + b_2 t^2 \quad \ldots\ldots 12$$

where Xe_t = the amount of Xe at time t
and b_o, b_1 and b_2 are constants.

The amount of Xe still present in the system at time t=0, i.e. the end of the clearing phase, is called Xe (A). The amount of Xe lost by desorption during the 2-minute clearing phase is called Xe (B).

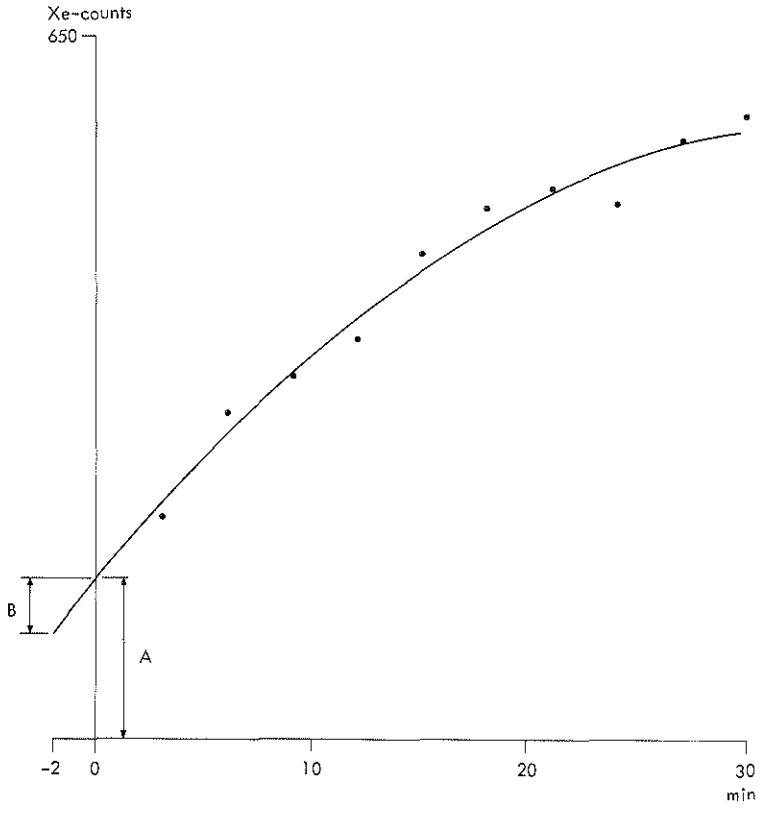


Fig. 7 Beginning of the Xe desorption curve, showing the way in which the correction for the clearing phase is determined.

Xe (A) must be subtracted from all Xe-values obtained during desorption and Xe (B) must be added. The net correction is therefore the subtraction of Xe (A) - Xe (B). This value is obtained by substituting t = -2 in the formula (12). The obtained value is subtracted from each point. This correction is between 0 and 5% depending on how efficiently clearing has been performed between the absorption and desorption phases.

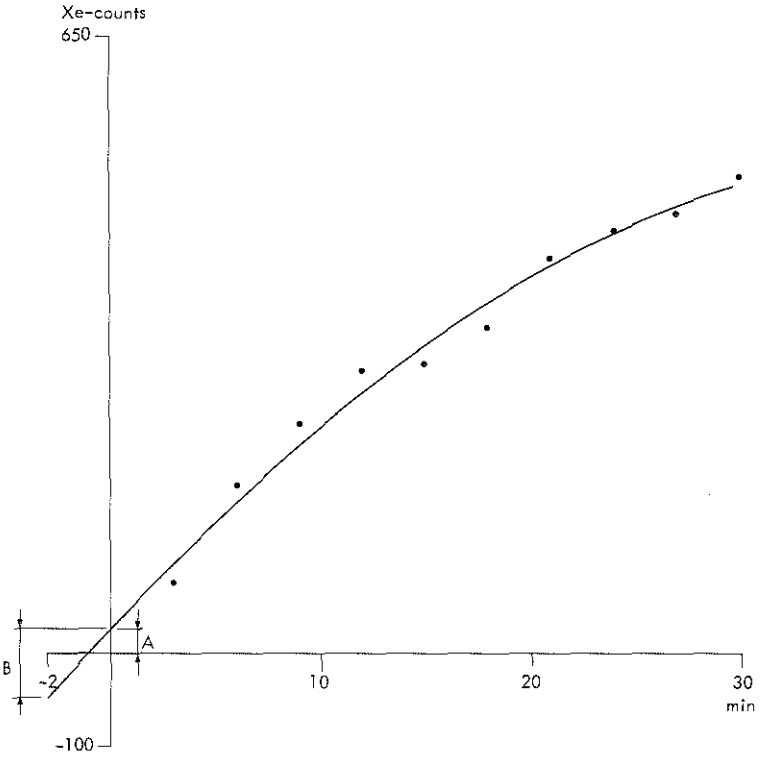


Fig. 8 Beginning of the Xe desorption curve, showing the way in which the correction for the clearing phase is determined.

III.1.4.3. Spurious absorption

Xe is not only absorbed by the living organism during the absorption phase but also by the various parts of the circuit such as the neoprene tubing. Some of the Xe absorbed by the circuit is also released during the desorption phase. Moreover, some Xe (probably a small amount) remains in the dead space in the circuit (e.g. the manometer) after the clearing phase and this is released during the desorption phase. This so called "spurious absorption" and "spurious desorption" can be measured by

doing a number of experiments with the bell jar empty. Xe washout curves with the bell jar empty are obtained in the same manner as described in III.1.3.

Fig. 9 gives an example of such a spurious desorption curve. Depending on the fat volume of the patient, the spurious absorption forms 10-25% of the total absorption. The correction for spurious absorption is not made for each measurement as is done with the other two corrections because of the following considerations:

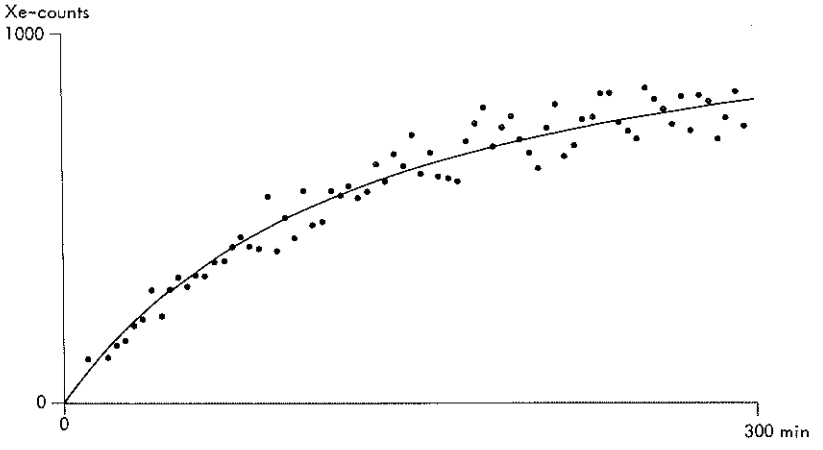


Fig. 9 Xe-desorption curve for "spurious desorption".

a) In contrast to the generally smoothly rising gross desorption curve, the spurious desorption curve is somewhat irregular. This apparently illustrates the very irregular release of Xe from the various parts of the circuit. In the normal experiment this phenomenon is completely masked by mixing with the greater quantity

of Xe which is desorbed from the patient. If the results of the spurious desorption curve were subtracted point by point from the gross desorption curve then an irregular net washout curve would be obtained. It is unlikely that this would give the true picture if it is accepted that the process of desorption gradually tends towards an equilibrium state just like the process of absorption as was shown by the work of Lesser et al. (1960) and Lesser and Zak (1963).

b) Spurious absorption is not derived mathematically from the gross desorption curve but is measured by separate experiments. Thus correction for spurious absorption means in fact that the results of one desorption curve (spurious) must be subtracted from another desorption curve (the gross desorption curve). In no experiment was the Xe concentration identical at the beginning of the absorption phase because of small variations in the amount of Xe injected. Therefore comparison of the desorption curves based on numerical values for Xe concentration during desorption is not possible. Comparison is however possible when the measurement results are expressed as percentages of the concentration at the end of the absorption phase. It is possible in principle to calculate each point of both the gross desorption curve and the spurious desorption curve as percentages of the concentration of Xe at the end of the absorption phase and to subtract one from the other. However this is not done for practical reasons but the final value of spurious absorption curve, obtained by extrapolation (see III.1.6.1.) and expressed as a percentage of the concentration of Xe at the end of the absorption phase, is used as a measure of the spurious absorption.

III.1.5. The net Xe desorption curve

The net desorption curve results, after correction for loss of Xe via the gas chromatograph (III.1.4.1.), and for the interval between the absorption and desorption phase (III.1.4.2.). The results from this curve are used to calculate the amount of Xe absorbed by the patient. Spurious absorption is given separately and used in the final programme for the calculation of the total body fat.

III.1.6. Calculations

III.1.6.1. Extrapolation of the desorption curve

It was found that the desorption phase had still not reached equilibrium after 2 to 3 hours. Even after five hours the curve was still rising slightly. For practical reasons it is not possible to let the desorption phase continue until equilibrium is reached. In practice a desorption time of 150 minutes was chosen. The equilibrium value must then be calculated by extrapolation. It is possible to represent the Xe washout curve with the usual exponential expression: $Xe_t = \sum_i A_i(1-e^{-a_i/t})$ 13

Fitting data to this kind of expression can not be done by simple means. Several numerical approaches are known, requiring considerable computer facilities. When applied to our data (i = 3), we found that small variations in these data, even when obtained from one subject, could lead to extrapolation values differing by an order of magnitude. A different mathematical approach, as far as we know not applied before to washout-curves, was found to give more reliable results.
On transforming the original values (Xe, t) with Xe as the corrected Xe peak area at time t, into (^{1}/Xe, ^{1}/t) one obtains a straight line for t > 40 minutes. This is shown

in figure 10.
Fitting of the curve with an iterative procedure is now relatively simple. The final value of the Xe peak (when $t \rightarrow \infty$) is calculated as follows:

$$\frac{1}{Xe_{\infty}} = A + \frac{B}{t_{\infty}} \rightarrow \frac{1}{Xe_{\infty}} = A \quad \ldots\ldots 14$$

A and B are both known constants and Xe can be found arithmetically.

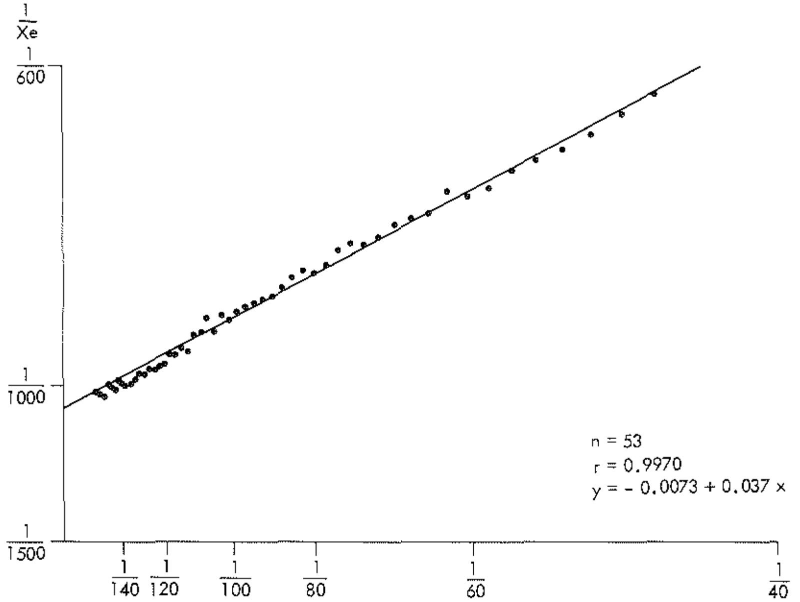


Fig. 10 Xe-desorption curve, $^{1}/Xe$ versus $^{1}/t$.

III.1.6.2. The Xe absorption curve

What has already been described for the desorption phase is also true of the absorption phase. That is to say that an equilibrium is not reached between the partial pressure of Xenon in solution in the body and that in the

circuit within a reasonable time. This was shown in a series of animal experiments, each with the same guinea-pig, whereby under otherwise identical experimental conditions, the amount of Xenon absorbed during various absorption times was measured. The amount of Xenon which had been absorbed was calculated from extrapolation of the desorption curves and was expressed as a percentage (β_t) of the Xenon concentration at the end of the absorption phase.
Table V gives the various values for β_t with the relevant absorption time in minutes.

Absorption time (min.)	β_t
60	1,777
90	2,019
120	2,198
120	2,242
240	2,457
300	2,579
∞	2,882

Table V β_t-values for guinea-pig experiments with corresponding absorption times.

The relationship between β_t and absorption time (t_{abs}) is reproduced graphically in fig. 11. A linear relationship between $^1/\beta_t$ and $^1/t_{abs}$ was found in the animal experiments, and therefore mathematical extrapolation and calculation of $\beta_{t\infty}$ is possible in the manner already described for the desorption curve.

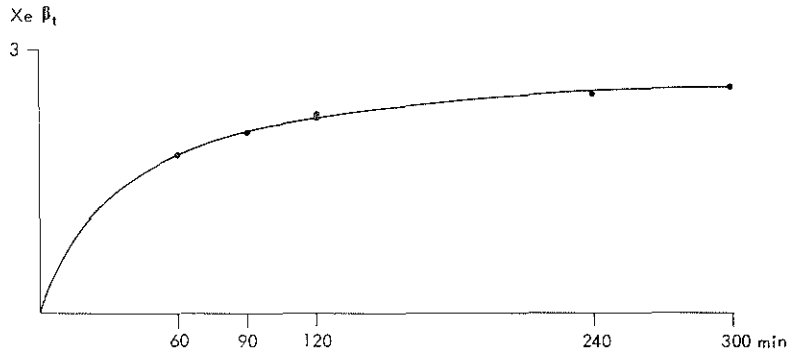


Fig. 11 Graphic presentation of relationship between β_t and absorption time, for guinea-pig experiments.

By $\beta_{t\infty}$ is therefore understood the amount of Xe, absorbed by the organism after infinite absorption time, expressed as a percentage of the Xe concentration at the end of the absorption phase. The absolute amount of Xe that is absorbed after infinite time can be expressed as:

$$Xe\,\frac{abs_\infty}{des_\infty} = \frac{\beta_{t\infty}}{\beta_t} \cdot Xe\,\frac{abs\ t}{des\ \infty} \qquad 15$$

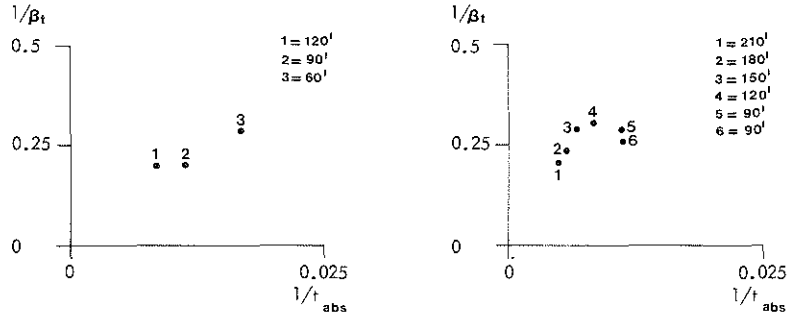
Table VI gives a number of values for $\frac{\beta_{t\infty}}{\beta_t}$ in relation to the duration of the absorption phase.

Absorption time (min.)	$\frac{\beta_{t\infty}}{\beta_t}$
60	1,622
90	1,427
120	1,285
240	1,173
300	1,117

Table VI Relation between $\frac{\beta_{t\infty}}{\beta_t}$ and absorption time.

We have called the expression $\frac{\beta_{t\sim}}{\beta_t}$ the asymmetry factor (A.F.). A linear relationship between $^1/\beta_t$ and $^1/t$ abs, such as was found reproduceably in the animal experiments, could not be found in experiments with patients, as is shown in fig. 12 and fig. 13. The failure to prove a definable relationship between absorption time and the amount of Xe absorbed in patients can be explained as follows:

- The adult laboratory animal is in a steady state with regard to body composition. Total body weight and body composition changed minimally within the 7-10 day period necessary for the series of experiments done on the effect of absorption time.
- In contrast the human newborn baby is in a phase of rapid growth. The changes are obvious even within a period of 7-10 days. There is an increase in weight of 150-250 gms. and in the same period the total body water decreases by 1-2 percent by weight and the amount of fat and protein increases. All of these relatively rapid changes mean that within the period of 7-10 days necessary for serial experiments it was impossible to demonstrate a consistent pattern of Xe absorption in relation to the absorption time.



Figs. 12 and 13 Graphic presentation of the relationship between $^1/\beta_t$ and $^1/t_{abs}$ in two patients.

However, it may be expected that, given a constant body composition, a longer absorption time would lead to a higher percentage of Xe absorption in the newborn baby. Therefore, for practical reasons, it was decided to use the values for $\frac{\beta_{t\sim}}{\beta_t}$ (asymmetry factor) found in the guinea-pig experiments, in the final calculation of the total body fat of babies.

In presenting the results of our measurements (Chapter V) it will be shown that there is, in fact, little difference between the speed of absorption, and therefore the asymmetry factor, in the human baby and the guinea-pig, since the fat percentages of babies calculated with the help of the asymmetry factor derived from the guinea-pig experiments, are found to be in good agreement with figures obtained from carcass analysis.

The relationship between the duration of the absorption phase of Xe and the percentage of Xe absorbed are also considered for the so-called "spurious absorption" and calculated using the asymmetry factor $\frac{\beta_{t\sim}}{\beta_t}$. See Table VII and VIII.

Absorption time (min.)	β_t
60	0,395
90	0,517
120	0,611
180	0,749
240	0,844
300	0,889
$\sim$	1,363

Table VII β_t-values for "spurious" absorption, in relation to absorption time.

absorption time (min.)	$\frac{\beta_{t\infty}}{\beta_t}$
60	3,451
90	2,636
120	2,231
240	1,615
300	1,533

Table VIII $\frac{\beta_{t\infty}}{\beta_t}$ - values for "spurious absorption", in relation to absorption time.

III.1.6.3. Calculation of the total body fat

During the experiment Xenon is present:

a. in the space under the glass bell jar: V_s if the bell jar is empty and $V_s - V_b$ if a baby is in it
b. absorbed in the total body fat of the baby (V_f): amount $\alpha_f V_f$
c. absorbed in the total body protein of the baby (V_p): amount $\alpha_p V_p$
d. absorbed in the total body water of the baby (V_w): amount $\alpha_w V_w$
e. absorbed in the circuit (V_c): amount $\alpha_c V_c$.

P_1 is the partial pressure of Xe in the circuit when equilibrium is reached during the absorption phase. $V_{Xe}(1)$ is the volume of Xe which is absorbed by the body of the organism and in the various parts of the circuit, at the end of the absorption phase.

$$V_{Xe}(1)(STP) = \left[\alpha_f V_f + \alpha_p V_p + \alpha_w V_w + \alpha_c V_c\right] \frac{P_1}{P_0} \quad \text{..... 16}$$

STP = under standard conditions of temperature and pressure.

P_2 is the partial pressure of Xe in the circuit when equilibrium is reached during the desorption phase. $V_{Xe}(2)$ is the volume of Xe which is absorbed by the body of the organism and in the various parts of the circuit, at the

end of the desorption phase.

$$V_{Xe}(2)(STP)=\left[\alpha_f V_f+\alpha_p V_p+\alpha_w V_w+\alpha_c V_c\right]\frac{P_2}{P_0}+(V_s-V_b)\cdot\frac{T_0}{T_s}\cdot\frac{P_2}{P_0} \quad \ldots\ldots 17$$

When all the free circulating Xe is removed in the interval between the absorption and desorption phases and when, during this interval, no Xe has diffused out of the organism, then the following holds:

$$V_{X_e}(1)(STP)=V_{X_e}(2)(STP) \quad \ldots\ldots 18$$

or

$$\left[\alpha_f V_f+\alpha_p V_p+\alpha_w V_w+\alpha_c V_c\right]\frac{P_1}{P_0}=\left[\alpha_f V_f+\alpha_p V_p+\alpha_w V_w+\alpha_c V_c\right]\frac{P_2}{P_0}+(V_s-V_b)\cdot\frac{T_0}{T_s}\cdot\frac{P_2}{P_0} \quad \ldots\ldots 19$$

From this it follows that:

$$\alpha_f V_f+\alpha_c V_c=(V_s-V_b)\cdot\frac{T_0}{T_s}\cdot\frac{x}{1-x}-(\alpha_p V_p+\alpha_w V_w) \quad \ldots\ldots 20$$

where $x=\frac{P_2}{P_1}=\frac{O_{II}}{O_I}$

O_I = The concentration of Xe, measured by the gas chromatograph, when equilibrium has been reached at the end of the absorption phase.

O_{II} = The concentration of Xe, when equilibrium has been reached at the end of the desorption phase, calculated by extrapolation of the Xe washout curve,

$$= Xe\ \frac{abs_t}{des_\infty}$$

Formula (16) must still be corrected for spurious absorption (see III.1.4.3.). P_1' is the partial pressure of Xe in the circuit when there is neither an animal nor a baby in the bell jar and when the equilibrium situation has been reached during the absorption phase.
P_2' is the partial pressure of Xe in the circuit when there

is neither an animal nor a baby in the bell jar and when the equilibrium situation has been reached during the desorption phase.
With the bell jar empty V_b, V_f, V_p and V_w are zero. Therefore Formula (20) becomes:

$$\alpha_c V_c = V_s \cdot \frac{T_0}{T_s} \cdot \frac{x'}{1-x'} \quad \text{..... 21}$$

where $x' = \frac{p'_2}{p'_1} = \frac{O_{II}^{sp}}{O_I^{sp}}$

O_I^{sp} = the Xe concentration, measured with the gas chromatograph, at the end of the absorption phase when equilibrium is attained.

O_{II}^{sp} = the Xe concentration at the end of the desorption phase when equilibrium has been attained, calculated by extrapolation of the Xe washout curve,

$= Xe \frac{abs_t}{des_\infty}$

Formula (21) subtracted from formula (20) gives the formula corrected for spurious absorption:

$$V_f = \frac{(V_s - V_b) \cdot \frac{T_0}{T_s} \cdot \left[\frac{x}{1-x} - \frac{V_s}{(V_s - V_b)} \cdot \frac{x'}{1-x'}\right] - \alpha_p \cdot V_p - \alpha_w \cdot V_w}{\alpha_f} \quad \text{..... 22}$$

or:

$$V_f = \frac{(V_s - V_b) \cdot \frac{T_0}{T_s} \left(\frac{\frac{\beta_{t\infty}}{\beta_t} \cdot \frac{O_{II}}{O_I}}{1 - \frac{\beta_{t\infty}}{\beta_t} \cdot \frac{O_{II}}{O_I}} - \frac{V_s}{(V_s - V_b)} \cdot \frac{\frac{\beta_{t\infty}^{sp}}{\beta_t^{sp}} \cdot \frac{O_{II}^{sp}}{O_I^{sp}}}{1 - \frac{\beta_{t\infty}^{sp}}{\beta_t^{spur}} \cdot \frac{O_{II}^{sp}}{O_I^{sp}}} \right) - \alpha_p V_p - \alpha_w V_w}{\alpha_f} \quad \text{..... 23}$$

where:

V_f = volume of fat in ml.

V_s = volume of circuit + bell jar in ml.

V_b = volume of patient, based on body weight and specific gravity.
The specific gravity was taken from figures given in the literature (Rathbun and Pace, 1945; Pitts, 1963).

T_o = 273.15 ^oK

T_s = temperature of bell jar in ^oK

$\frac{\beta_{t\infty}}{\beta_t}$ = gross asymmetry factor for the patient

O_I = concentration of Xe at the end of the absorption phase

O_{II} = concentration of Xe at the end of the desorption phase, (corrected for Xenon loss via the gas chromatograph and for the Xe lost from the organism during the clearing phase) extrapolated to the value Xe $\frac{abs_t}{des_\infty}$

$\frac{\beta^{sp}_{t\infty}}{\beta^{sp}_t}$ = the asymmetry factor for spurious absorption

O^{sp}_I = the concentration of Xe at the end of the absorption period for spurious absorption

O^{sp}_{II} = the concentration of Xe at the end of the desorption period for spurious desorption (corrected for Xe loss via the gas chromatograph and for Xe loss during the clearing phase), extrapolated to the value Xe $\frac{abs_t}{des_\infty}$.

V_p = volume of protein compartment in ml.

V_w = total body water in ml.

α_p = Bunsen absorption coefficient for protein

α_w = Bunsen absorption coefficient for water

α_f = Bunsen absorption coefficient for fat.

The 95% confidence limits were calculated for the extrapolated desorption curve. The fat volume was calculated three times from each desorption curve; once using the mean value for O_{II} and once each using the upper and lower confidence limits respectively. Assuming proportionality, the fat volumes so obtained were considered to be the mean value and the 95% confidence limits.

III.2. Measurement of total body water

The D_2O dilution method was used in our experiments for the measurement of total body water. The principle of the dilution method is explained in Chapter II section 2.1. The D_2O was measured by infra-red spectrophotometry as described by Turner et al. (1960) with some modifications.

The procedure was as follows: Three hours after giving an accurately measured amount of D_2O (approximately 3 grams per Kg body weight) intravenously, a 0,5 ml blood sample was taken. This was centrifuged and the plasma was pipetted off and placed in a dry glass test tube and frozen immediately. This plasma sample remained in the deep freeze until it could be worked on further. Before measurement the sample of plasma was thawed carefully and distilled under vacuum into a glass test tube which was kept at $-70^{\circ}C$ in carbon dioxide cooled acetone.
Distillation at low temperature prevents other volatile components of the plasma coming over in the distillate. The pure water obtained in this manner consists of a mixture of H_2O and an unknown amount of D_2O. The sample was placed in a cuvette and the optical density at a wavelength of 3,98 μ

was measured in a spectrophotometer (Perkin Elmer, type 257). Zero calibration was done beforehand by filling the cuvette with distilled water (not containing D_2O) and measuring the optical density at the same wavelength. A calibration curve was constructed by measuring the optical density of five standard solutions, containing known amounts of D_2O, at the same wavelength. There is a linear relationship between the D_2O concentration in water and the optical density at a wavelength of 3,98 μ. After measuring the optical density of the unknown sample the D_2O concentration could be read off directly from the calibration curve. Knowing the amount of D_2O which had been given to the patient it was now a simple matter to calculate the volume of the water compartment.

The cuvette was rinsed with acetone between each measurement and blown dry with nitrogen. It was necessary to construct a new calibration curve for each series of measurements because the transparency of the walls of the cuvette gradually decreases.

We applied the previously mentioned correction of minus 2% to our measured percentages for total body water (Foy and Schnieden, 1960; Bradbury, 1961; Tisavipat et al., 1974).

The total body water was measured by this method a total of 68 times in 51 patients.

III.3. Measurement of the extracellular volume (ECV)

The volume of extracellular fluid was measured by the bromide (Br^-) dilution method. An electrode selective for Br^- was used for the measurement of the Br^- concentration as described by Degenhart et al. (1972). The method was as follows: A capillary blood sample of 0,5 ml was taken. After centrifugation 200 μl plasma was mixed with 100 μl of a 2 mM sodium bromide solution (Br^-). The millivoltage (V) was read off in this solution with a Br^- electrode (Beckman).

This measurement was repeated four times, each time after the addition of a further 25 µl of the previously mentioned sodium bromide solution.
A regression line of the following type was calculated from the results:

$$(Br^-)_i = a_0 + a_1 V_i + a_2 (V_i)^2 \quad \ldots\ldots 24$$

a_o, a_1 and a_2 are constants which were measured every time for each patient. Thereafter, NaBr (50 mg/Kg body weight) was given to the patient intravenously. A 0.5 ml capillary blood sample was taken after equilibration (approximately 3 hours). After centrifugation a millivoltage in the plasma was read off. By substitution of the V-value so obtained the Br^- concentration could be calculated. The amount of sodium bromide which had been given was known and the ECV could therefore be calculated.

This method was performed a total of 60 times in 46 patients.

III.4. Total body protein (literature data)

No method for measuring total body protein directly was available to us. We performed carcass analysis on the guinea-pigs as a control for our experiments with Xe in the measurement of total body fat. During this carcass analysis we also measured total body protein and total body water. However this information was not, of course, available for the babies. We used the results to be found in the literature of carcass analysis in babies given by Widdowson (1974). These are given in Table IX.

Gestational age in weeks	weight in grams	total water weight %	total fat weight %	total protein weight %	rest weight %
26	1000	86.0	1.0	8.7	4.3
31	1500	84.7	2.3	10.5	2.5
33	2000	81.0	5.0	12.0	2.0
35	2500	77.6	7.4	12.2	2.8
38	3000	72.7	12.0	11.6	3.7
40	3500	68.6	16.0	11.0	4.4

Table IX Total body water, fat and protein in percent by weight in young babies measured by carcass analysis (Widdowson, 1974).

III.5. Measurement of the amount of subcutaneous fat by skinfold measurement

The method of Dauncey et al. (1977) was used to measure the subcutaneous fat in 4 patients who had also had Xe absorption measurements. In this method the body is assumed to consist of a ball (the head) and 5 cylinders (trunk and limbs). It is assumed that the head has practically no subcutaneous fat. The diameter of the head is taken as the head circumference divided by π. The length of the cylinder of the trunk is the crown-rump length minus the diameter of the head and the circumference of this cylinder is the chest circumference. The arm cylinders are considered to be identical and have a length which is that from acromion to the styloid process of the radius and a circumference which is that of the middle of the upper arm. The leg cylinders are also considered to be identical. Their length is the crown-heel length minus the crown-rump length and their circumference that of the mid-thigh. The surface area can be calculated for the trunk and limb cylinders. The thickness of the subcutaneous fat layer is calculated by subtracting 2 mm (for the thickness of the epidermis)

from the measured skinfold thickness measured below the scapula, over the triceps muscle and over the quadriceps femoris. Multiplication of the calculated subcutaneous fat layer by the area of the cylinder gives the total amount of subcutaneous fat.

III.6. Animal experiments

The validity of the previously described method for the measurement of total body fat using Xe absorption and desorption was checked in a series of animal experiments. Guinea-pigs were chosen as the experimental animal because they are relatively simple to keep in the laboratory, they require little care and they thrive well on a standard laboratory diet (Hope Farms, Holland) and water.
The adult guinea-pig contains a considerable quantity of fat: 10-20 percent by weight (Rathbun and Pace, 1945) and has a body weight which is comparable with that of the birth weight of the smallest viable human babies. For practical reasons it was not possible to use newborn guinea-pigs for the animal experiments. It would have been very interesting to do so because the guinea-pig is, so far as is known, the only mammal, apart from man, which at birth already has well developed white as well as brown fat (McCance and Widdowson, 1977).
Table X gives a comparison of the percentages of fat in the newborn and in the adult of a variety of animal species and man.

Repeated measurements were done in 8 guinea-pigs. The first measurement results are not considered as they were performed before gas chromatography was satisfactorily set up. The retention time of the gas sample in the gas chromatograph was 10 minutes which limited the number of samples which could be taken per experiment. Thus it was not possible to obtain a washout curve which could be well

	newborn	adult
Man	16.0	20-30
Guinea-pig	10.0	10-15
Rabbit	5.8	10.0
Pig	1.1	50.0
Rat	1.1	20.0
Mouse	2.1	5.0

Table X Total body fat in percent by weight in a number of species at birth and in adulthood. (McCance and Widdowson, 1951, 1977).

defined mathematically. Improvements in the setting up of the gas chromatograph, whereby the retention time could be reduced to less than 2 minutes (see III.1.2., p.44), led to an increase in the number of measurements during the desorption phase from 15 to 75. The desorption curve so obtained could be well defined mathematically.

Measurements during absorption and desorption were done in this manner in 3 guinea-pigs. These guinea-pigs were subsequently killed by ether inhalation and were deep frozen and then divided into pieces of approximately 5 cubic centimeters. The pieces were placed in an desiccator in which a vacuum was maintained and which contained a quantity of the extremely hygroscopic substance P_2O_5. The desiccator was placed in a water bath at $70^{\circ}C$. After several weeks the material which had been dried in this manner was ground in a household mixer (Waring Blender). The coarse powder obtained in this manner was then further dried for a number of weeks by the method described above. The total body water was obtained from the difference in weight before and after drying. The total body fat was calculated by weighing samples of the dried material accurately before and after fat extraction in the Soxhlet apparatus. The fat was extracted in the Soxhlet apparatus with hexane for 8 hours and then with a chloroform/methanol mixture (2 parts by volume chloroform to 1 part methanol) also for 8 hours.

Chapter IV

DESCRIPTION OF THE PATIENTS WHO WERE INVESTIGATED AND THE TREATMENT WHICH THEY HAD RECEIVED

IV.1. The Neonatal Unit, Department of Paediatrics, Sophia Children's Hospital

All the patients in whom total body fat, total body water and extracellular volume were measured as described in Chapter III were inpatients on the Neonatal Unit of the Department of Paediatrics in Sophia Children's Hospital.

The Unit is a reference centre for neonatology for the city of Rotterdam and for the region of South West Holland. The catchment area has a total population of 2 million with approximately 26,000 live births per year. Babies who require intensive care during the first few weeks of life are admitted. This generally involves one or more of the following problems:

- birth weight below 1500 grams
- gestation of less than 32 weeks
- serious respiratory problems
- serious blood group antagonism
- congenital abnormalities requiring emergency treatment
- serious infections
- inborn errors of metabolism.

The patients in these categories come from paediatric departments in hospitals which are not able to offer intensive care.

Further, from hospitals which are only able to offer limited neonatal care, patients are admitted with the following problems:

- birth weight between 1,500 and 2,500 grams
- gestation of 32-37 weeks
- observation after a difficult delivery
- birth weight above the 97th centile on the intra-uterine growth chart
- hyperbilirubinaemia
- acquired severe metabolic problems (e.g. hypoglycaemia, hypocalcaemia).

Approximately 400 patients per year are admitted to the Neonatal Unit of the Paediatric Department of Sophia Children's Hospital. Of these approximately 80% are patients in the first group who require intensive care and 20% from the second group requiring medium care. The Unit has 24 beds (with 6 for intensive care).

IV.2. General aspects of treatment

IV.2.1. Climate regulation

It is attempted to nurse the patients at their neutral thermal environment so that the minimum number of calories are needed for basal metabolism and that the maximum possible remain available for growth. This is achieved by keeping the room temperature constant at 30°-32° C. This means that babies with a weight above 2,000 grams can be nursed naked in open cots. Babies below 2,000 grams are nursed in incubators in which the temperature can be regulated from 32°-37° C. The radiant heat loss remains minimal because of the small difference between the temperature inside the incubator and that of the room. Radiant heat loss to the outside is limited by triple glazing in the windows. In contrast the influence of sunlight is limited by external blinds. The air is changed regularly by an air conditioning system and the humidity is kept constant at 50-60%.

IV.2.2. Hygiene

The treatment area is separated by double doors from the rest of the Unit to prevent bacterial contamination from outside. The air-conditioning system in the treatment area gives a slight positive pressure for the same reason. After hand washing in the sluice a gown is put on before entering the treatment area. Personnel who have to remain in the treatment area for long periods (e.g. nurses) wear special light clothing because of the high temperature. A new plastic disposable apron is put on before handling each patient to prevent cross infection. There are strict rules about hand washing.

IV.2.3. Fluid and feeding regimes

It is attempted to reach a caloric intake of 140 kcal per kg body weight per day as quickly as possible with a fluid intake of 180-200 ml per kg body weight per day. Some very dystrophic S.F.D. babies are given up to 160 kcal per kg body weight per day. This is achieved by beginning on the first day with an intravenous glucose solution (10-15%) under blood sugar control. Electrolytes (Na, K, Ca, Mg) are given as necessary. At the same time oral feeding is begun in the form of a continuous gastric drip three to six hours after birth. "Humanised" milk (Almiron M2, Nutricia) or an "adapted" milk especially for low birth weight infants (Nenatal, Nutricia) are given in gradually increasing amounts.

In principle, 60 ml per kg body weight is given on the first day and this is increased by 20 ml per kg body weight per day until 180-200 ml per kg body weight is reached. The quantity of intravenous fluid is reduced by the amount of fluid which is given orally.

Whenever there is other pathology (especially respiratory difficulties needing artificial ventilation) which makes oral feeding impossible for a long period then full intravenous feeding is substituted with amino-acid mixtures (7%

Vamin, Vitrum) and fat emulsions (10% Intralipid, Vitrum) as well as glucose solutions. By this method it is quite possible to supply the caloric needs of the baby and to achieve satisfactory growth. Such a regime requires the regular measurement of the amino-acid spectrum (Visser et al., 1973) and the intralipid concentration (Forget, 1975) in the serum.

IV.3. Description of the patients who were investigated

Total body fat was measured by 22 patients, total body water in 51 patients, extracellular volume in 46 patients. Investigation of body composition was only done when the clinical condition of the patient permitted and when it did not mean alterations in treatment. In general this meant that in most cases it was not possible to do the investigation during the first week after birth, because it was necessary to wait until the administration of intravenous fluids had been stopped.

The methods involved in the investigation were discussed with the parents in general terms. There is no evidence that the investigation carried any risks for the patient. The administration of D_2O and NaBr was done, whenever possible, through an already present intravenous infusion. Blood samples for the determination of D_2O and NaBr were combined as much as possible with blood sampling for clinical reasons. Blood samples are taken regularly in these patients in the period after birth for monitoring the internal milieu.

The group of patients who were investigated can be subdivided as follows:

- 15 preterm (44 measurements)
- 7 S.F.D. (28 measurements)

22 72 measurements of total body fat

Tables XI to XVI give a review of these patients with regard to gestational age, birth weight, clinical problems, treatment and condition on discharge. By "treatment" is

meant every special treatment which the baby received and not the general measures which have been described in IV.2. above.

Figs. 14 and 15 show the growth curves for weight of the two groups of patients.
Figs. 16 to 21 inclusive give the same growth curves divided into smaller groups for a clearer view of the individual growth patterns.

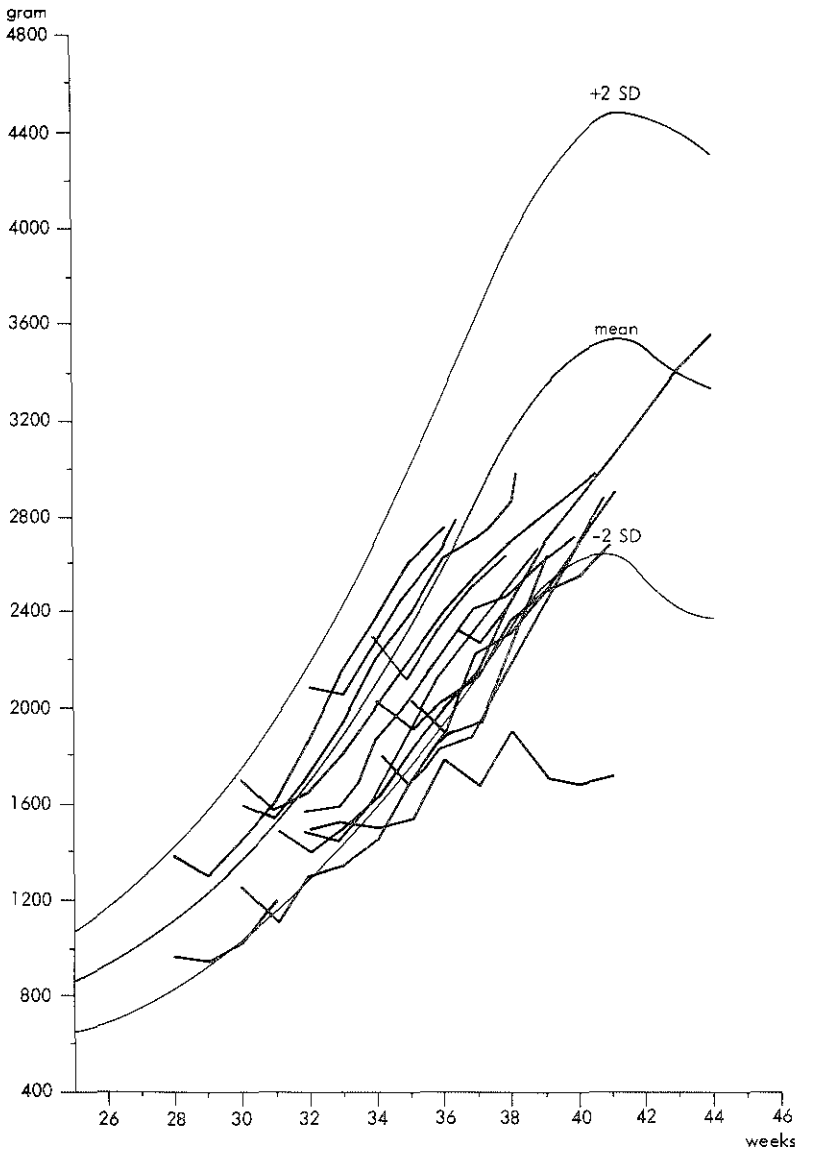


Fig. 14 Growth curves for weight of 15 preterm babies during the period of clinical observation.

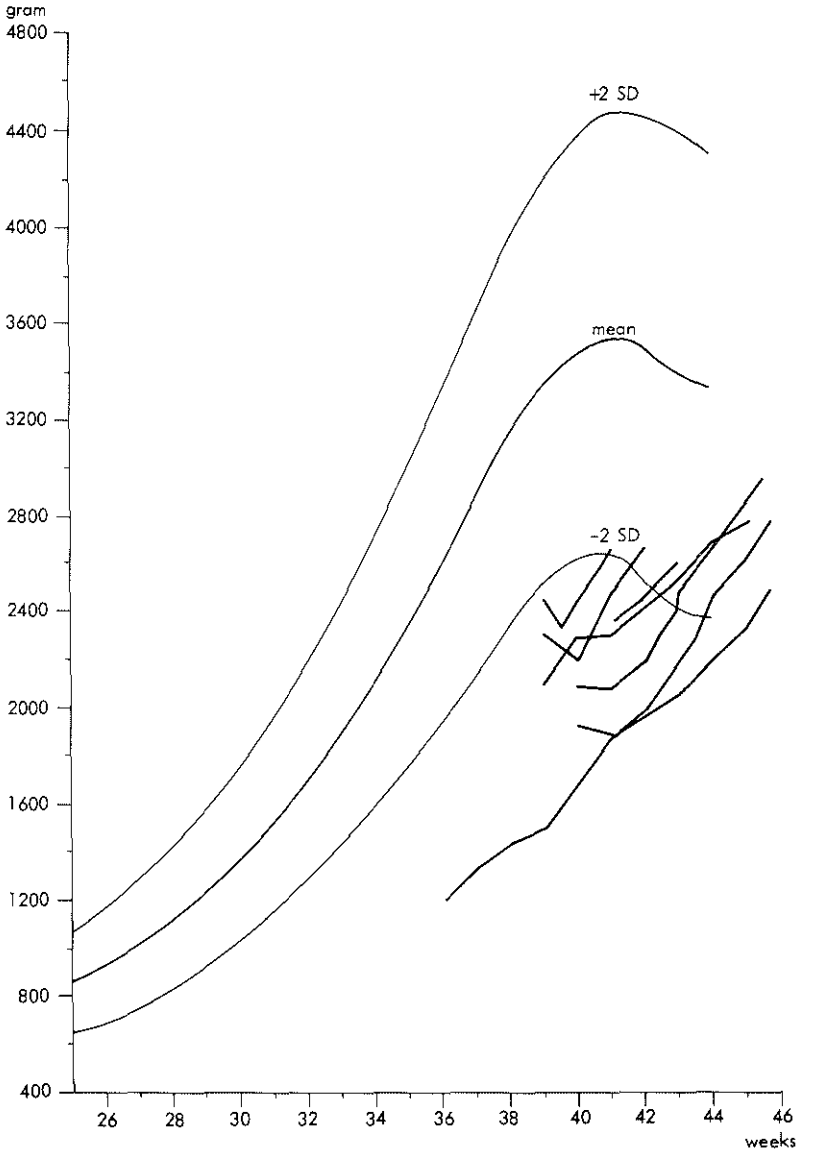


Fig. 15 Growth curves for weight of 7 S.F.D. babies during the period of clinical observation.

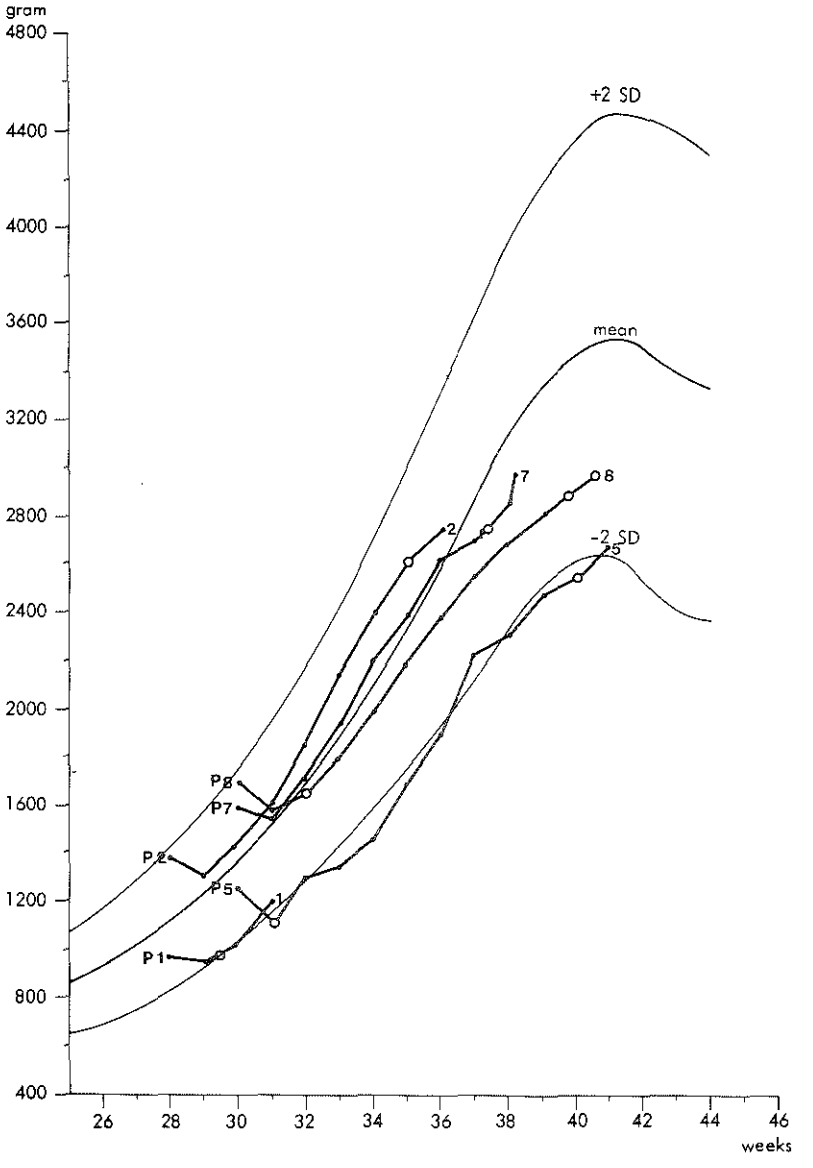


Fig. 16 Growth curves for weight of patients P1, P2, P5, P7 and P8, during their stay in hospital. Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XI.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
P1	F	28/970	mild RDS	O_2 under hood	†day 23; autopsy refused!
			septicaemia	kanamycin/ cefaloridin	
P2	F	28/1360	hyperbilirubinaemia	phototherapy	well, day 58, 2780 g.
P5	M	30/1260	twin transfusion syndrome, donor (Hb 10.5 g%)	blood-transfusion	well, day 58, 2650 g.
			septicaemia	kanamycin/ cefaloridin	
			aspiration pneumonia	ampicillin	
P7	F	30/1630	hyperbilirubinaemia (max. 295 µmol/L)	XCT	well, day 59, 2990 g.
P8	M	30/1690	twin transfusion syndrome, recipient (Hb 25.2 g%)	withdrawal of blood	well, day 46, 3100 g.

Table XI Clinical data of patients P1, P2, P5, P7 and P8.
G.a. = gestational age in weeks
B.wt. = birth weight in grams
XCT = exchange transfusion
RDS = respiratory distress syndrome

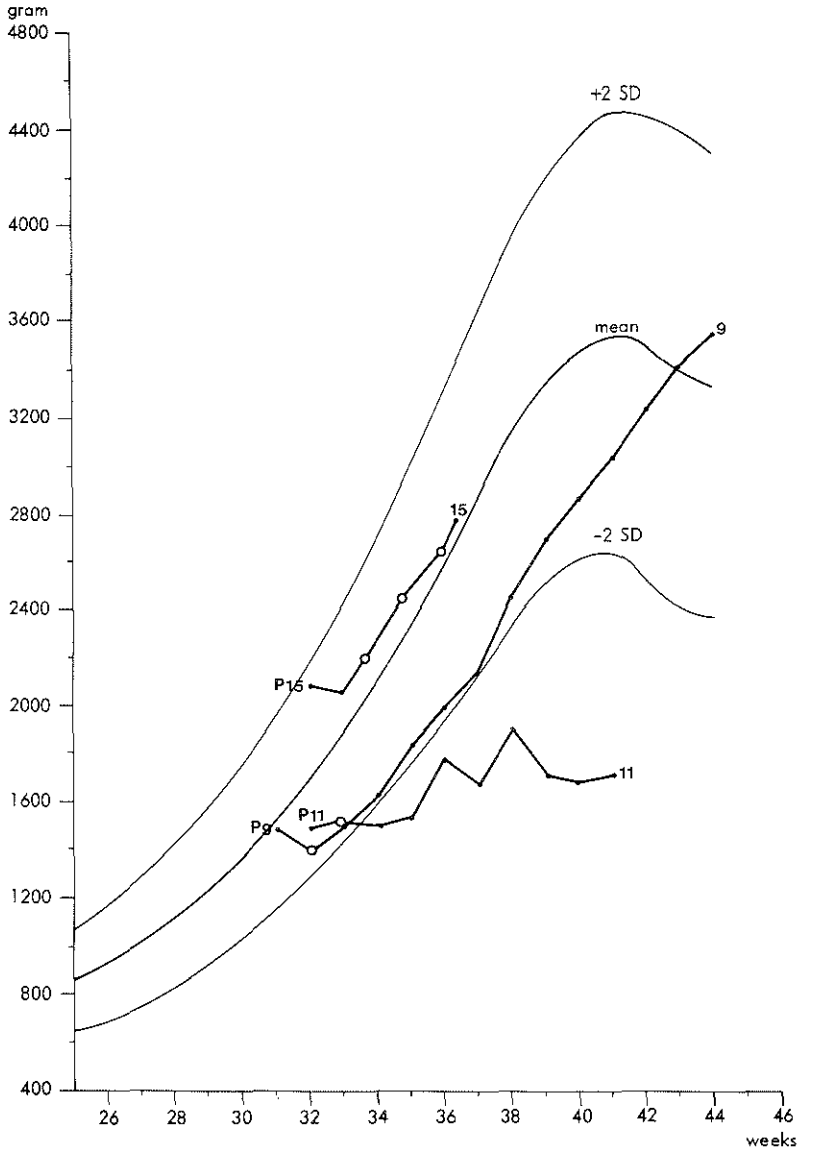


Fig. 17 Growth curves for weight of patients P9, P11 and P15, during their stay in hospital.
Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XII.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
P9	M	31/1490	hyperbilirubinaemia (max. 186 µmol/L)	phototherapy	well, day 97, 3700 g.
			abscess of parotid gland	drainage	
P11	F	32/1480	twin transfusion syndrome, recipient (Hb 21.6 g%)		
			hyperbilirubinaemia (max. 304 µmol/L)	XCT	† day 59
			septicaemia	penicillin/ kanamycin IPPV	autopsy refused
P15	F	32/2100	maternal eclampsia neurological depression because of maternal medication (diazepam)	no special therapeutic measures	well day 31, 2660 g.

Table XII Clinical data of patients P9, P11 and P15
G.a. = gestational age
B.wt. = birth weight in grams
XCT = exchange transfusion
IPPV = intermittent positive pressure ventilation

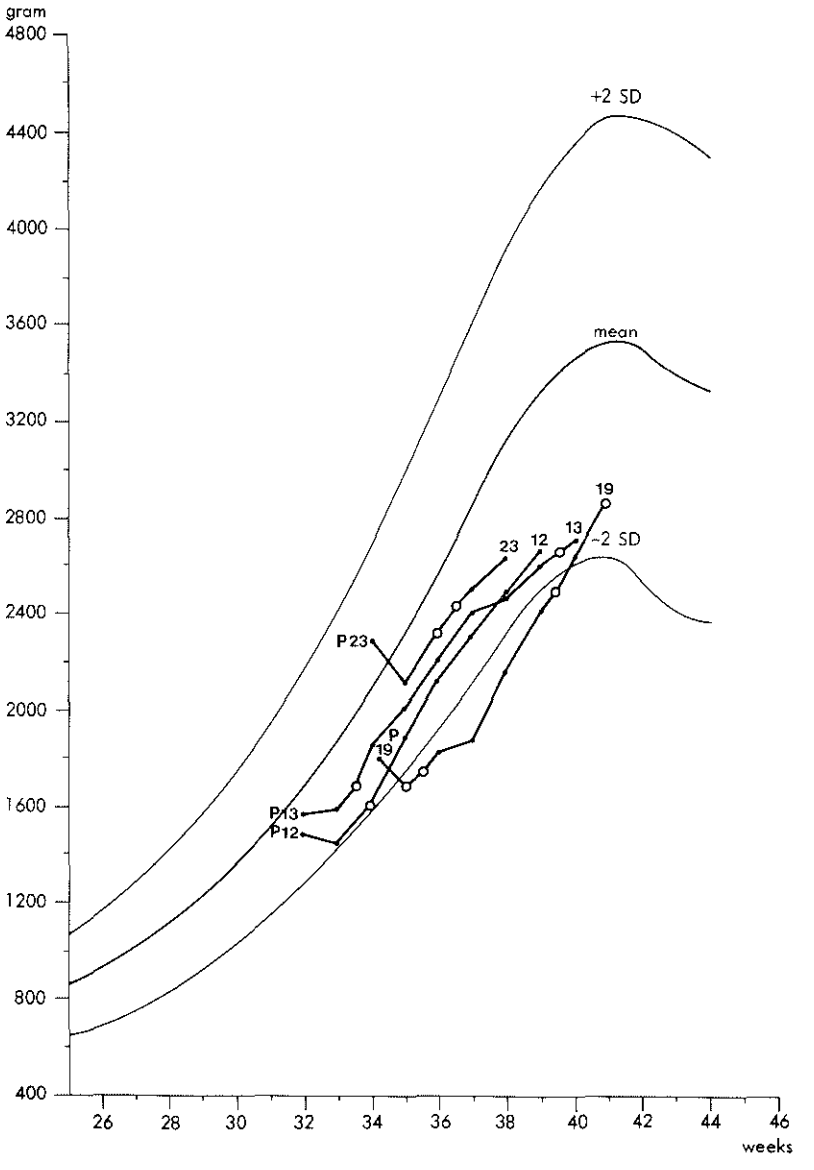


Fig. 18 Growth curves for weight of patients P12, P13, P19 and P23, during their stay in hospital. Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XIII.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
P12	F	32/1500	hyperbilirubinaemia (max. 235 µmol/L)	phototherapy	well, day 43, 2680 g.
P13	F	32/1580	twin transfusion syndrome, donor (Hb 10,1 g%)	blood-transfusion	well, day 52, 2760 g.
			hyperbilirubinaemia (max. 176 µmol/L)	phototherapy	
P19	M	34/1840	AO-antagonism hyperbilirubinaemia (max 179 µmol/L)	phototherapy	well, day 50, 2980 g.
P23	M	34/2300	hyperbilirubinaemia (max. 255 µmol/L)	XCT phototherapy	well, day 25, 2640 g.

Table XIII Clinical data of patients P12, P13, P19 and P23
G.a. = gestational age in weeks
B.wt. = birth weight in grams
XCT = exchange transfusion

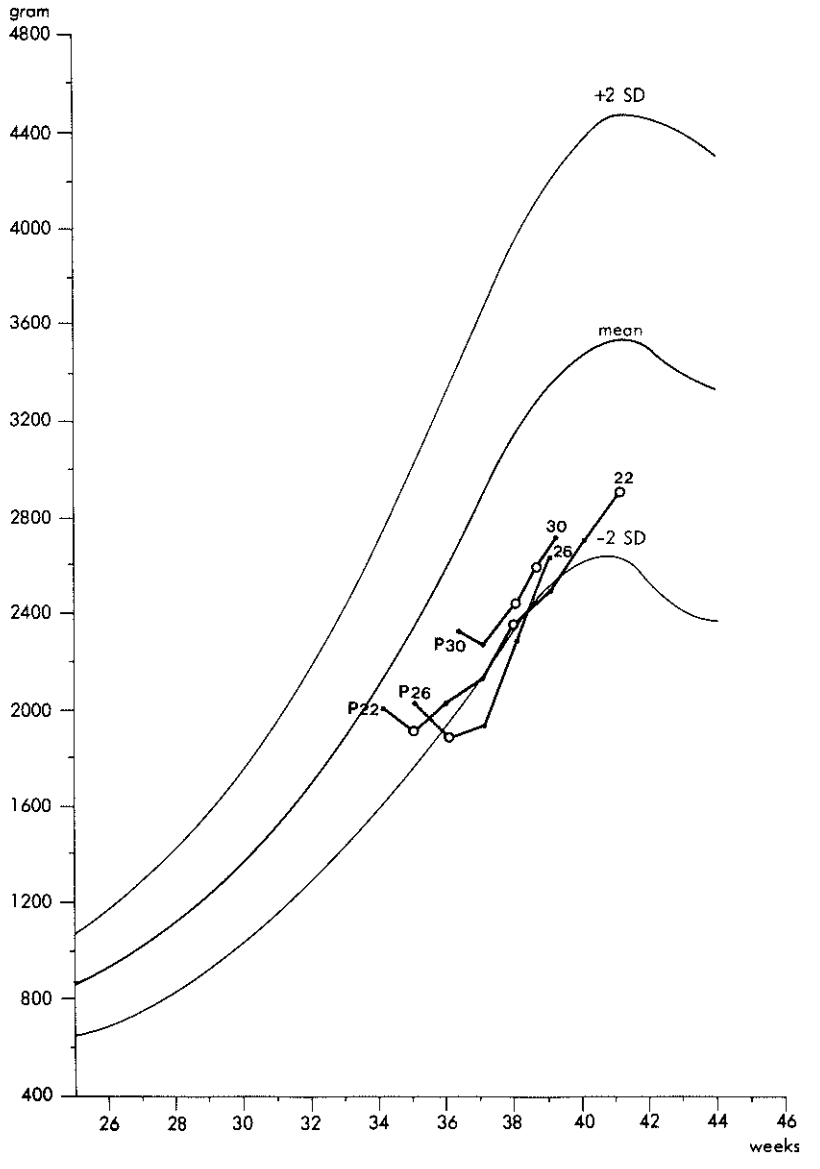


Fig. 19 Growth curves for weight of patients P22, P26 and P30, during their stay in hospital. Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XIV.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
P22	F	34/2010	uncomplicated clinical course		well, day 51, 2960 g.
P26	F	35/2040	hyperbilirubinaemia (max. 250 µmol/L)	phototherapy	well, day 31, 2650 g.
P30	M	36/2380	hyperbilirubinaemia (max. 170 µmol/L)	phototherapy	well, day 25, 2740 g.

Table XIV Clinical data of patients P22, P26 and P30
G.a. = gestational age in weeks
B.wt. = birth weight in grams

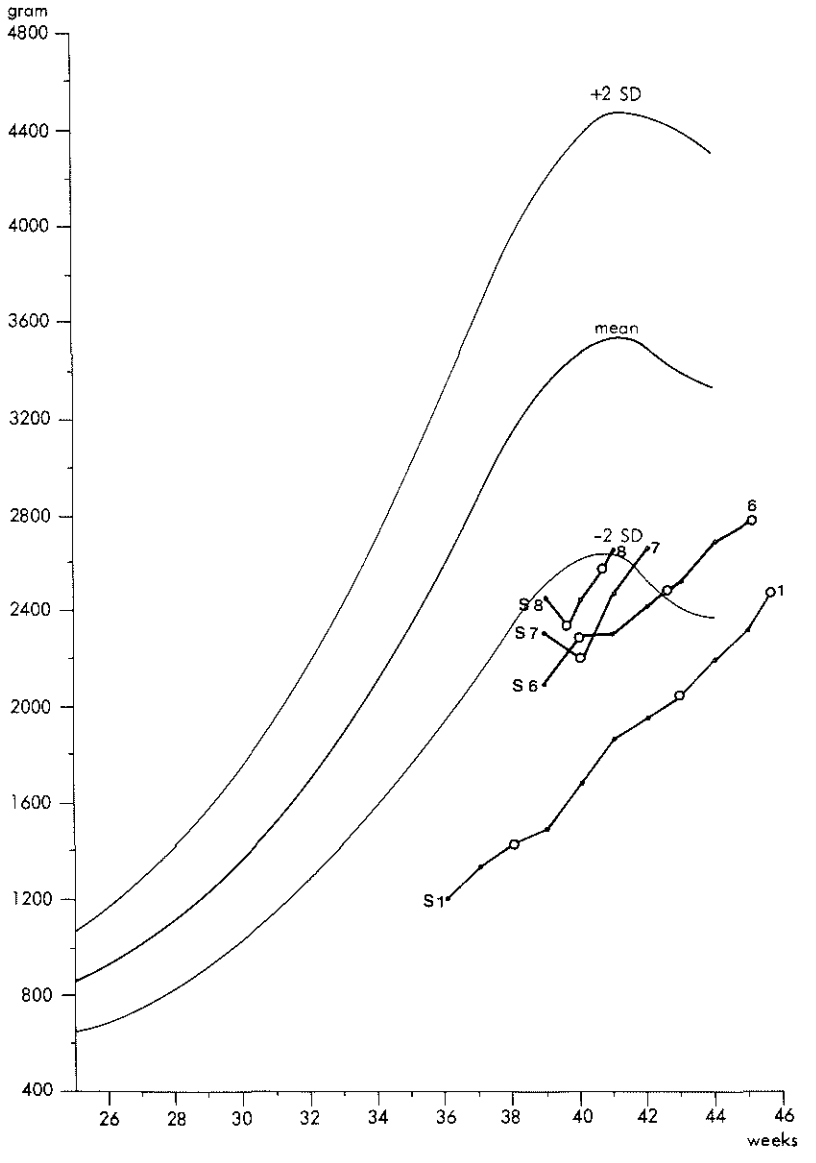


Fig. 20 Growth curves for weight of patients S1, S6, S7 and S8, during their stay in hospital.
Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XV.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
S1	M	36/1220	IGR due to placental insufficiency (signs of toxaemia) transient hypoglycaemia		well, day 85, 2780 g.
S6	F	39/2110	IGR of unknown origin		well, day 44, 2800 g.
S7	F	39/2325	IGR of unknown origin transient hypoglycaemia		well, day 22, 2660 g.
S8	F	39/2460	IGR of unknown origin		well, day 14, 2680 g.

Table XV Clinical data of patients S1, S6, S7 and S8
G.a. = gestational age
B.wt. = birth weight in grams
IGR = intra-uterine growth retardation

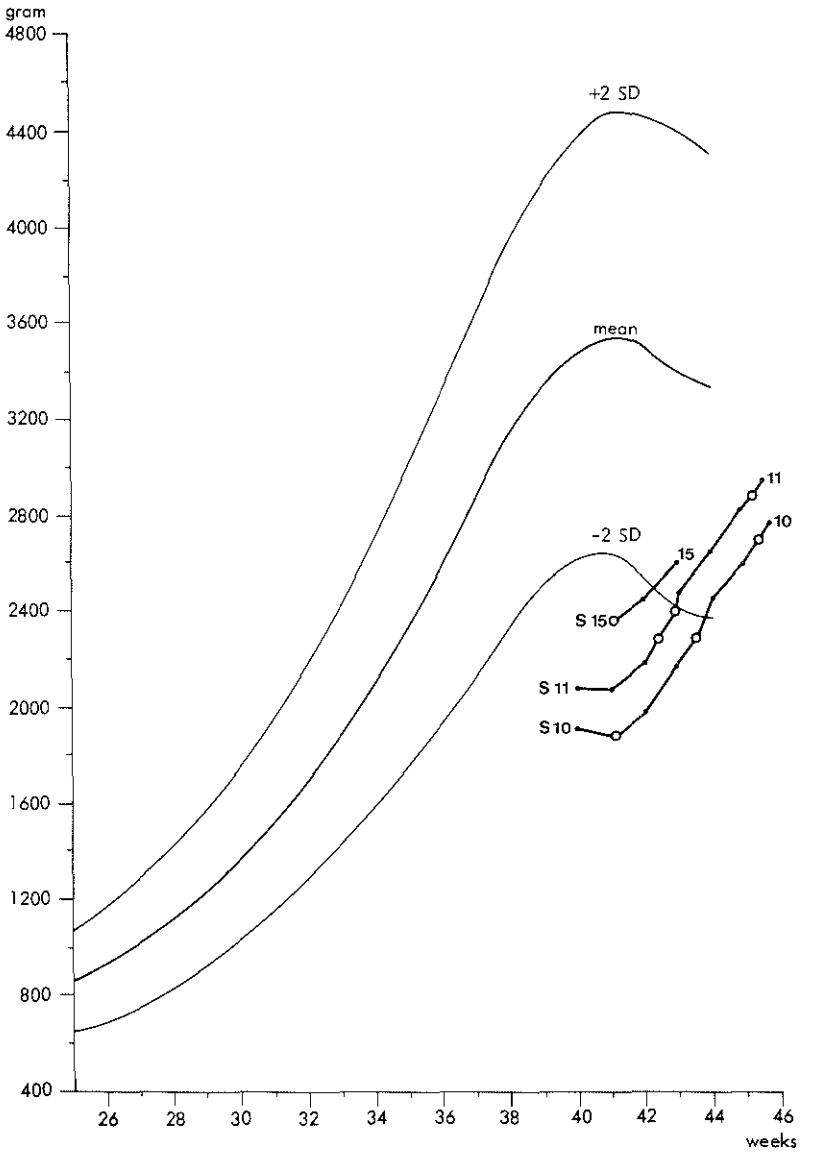


Fig. 21 Growth curves for weight of patients S10, S11 and S15 during their stay in hospital.
Open circles indicate day of measurement of total body fat.
Clinical data of these patients are summarized in table XVI.

Pat.	M/F	G.a./ B.wt.	Clinical problems	Treatment	Condition and age on discharge
S10	F	40/1930	IGR due to placental insufficiency (pre-eclampsia) hyperbilirubinaemia (max. 238 µmol/L)	phototherapy	well, day 43, 2780 g.
S11	M	40/2100	IGR of unknown origin		well, day 37, 2750 g.
S15	F	41/2390	IGR of unknown origin transient hypoglycaemia		well, day 11 2610 g.

Tabel XVI Clinical data of patients S10, S11 and S15
G.a. = gestational age
B.wt. = birth weight in grams
IGR = intra-uterine growth retardation

Chapter V

RESULTS AND DISCUSSION OF THE PRESENT STUDY

V.1. Animal experiments

The results of the animal experiments described in Chapter III.6 are summarized in table XVII (p.126). This table gives a comparison for three guinea-pigs between the total body fat (in grams and in percent by weight) measured by the Xe-absorption method and that measured by carcass analysis. The chemical analysis was done shortly after the last Xe-measurement.

Comparison of the results from both methods forms the basis of the study. The Xe-absorption method can only be used independently if the results in animal experiments are in good agreement with the data from carcass analysis.

The data in table XVII (p. 126) require the following commentary. During the period in which the Xe measurements were done the guinea-pigs had a virtually constant body weight. The percentage variation from the average body weight is only in one instance greater than 2% and for the rest is always less than 1%. The animals are in a steady state as far as their body weight is concerned. The small fluctuations in weight that are seen from day to day must be ascribed to small changes in the extracellular water compartment e.g. the content of the bowel and the urinary bladder.

In guinea-pig A the variation in total body fat measured by the Xe absorption method is greater than the variation in total body weight. However, the result of the second Xe-measurement is in good agreement with that found by carcass analysis.

Good duplicate values for total body fat measured by Xe-absorption are found in guinea-pig B. At the end of the serial measurements there is a clear increase in the body weight. This growth continues between the last Xe-measurement and the carcass analysis. It appears that the percentage fat by weight measured by carcass analysis is virtually identical to that measured previously by Xe absorption.

The increase in weight in this guinea-pig can almost certainly be accounted for by an increase in the fat-free mass (FFM) as well as the total body fat. This is in agreement with the work of Pitts (1962, 1963) on the body composition of the guinea-pig. When the weight of fat tissue increases then the muscles and skeleton also increase in weight as a result of the greater load which must be carried.

Guinea-pig C shows reasonable duplicate values for the total body fat measured by Xe absorption. However, growth is also clearly seen here in the time between the last Xe-measurement and the carcass analysis.

In contrast to the observation in guinea-pig B, it appears that in this case the weight increase can be completely accounted for by an increase in fat. No good explanation can be given for this.

In general it can be said that the results of the fat measurements by the Xe-absorption method in the animal experiments presented are in reasonably good agreement with the results of carcass analysis. However, in a few cases there are differences which are not easily explained. The number of measurements is too small to allow statistical analysis. A number of animal experiments which were performed in the early stages of the study cannot be used in assessment of the technique. The conditions under which measurements were done, were then not optimal and this led to results which did not allow mathematical processing. In the future it will definitely be necessary to do a series of additional animal experiments.

Meanwhile however further support for the reliability of the method is given by the comparison between the results of Xe-measurements in babies and the results, taken from the literature, from carcass analysis. These results are given in V.2.3.

V.2. Investigation in patients

V.2.1. Total body water

Results of the measurements of total body water by the D_2O-dilution method are given in table XVIII (p. 128) for the preterm babies and in table XIX (p. 130) for the S.F.D. babies. In table XX (p. 131) the data on total body water from carcass analysis of foetuses and neonates are given. These are taken from 5 studies (Fehling, 1877; Givens and Macy, 1933; Iob and Swanson, 1934; Widdowson and Spray, 1951; Widdowson and Dickerson, 1964).
The classification in weight groups is explained in table XXI (p. 132).

Table XXII a (p. 133) gives the chemical composition of the male "reference infant" according to Fomon (1966). Table XXII b (p. 133) gives the chemical composition of the "reference fetus", according to Ziegler et al. (1976). The data from tables XVIII (p. 128) and XIX (p. 130) are presented graphically in Fig. 22 and 23 respectively. The total body water in litres is plotted against the body weight in kg. There appears to be very little difference in the amount of body water per kg body weight between preterm and S.F.D. babies. This was also apparently shown in the antipyrine studies of Cassady and Milstead (1971). It seems therefore justifiable to use data from both groups of patients to form a single curve as done in fig. 24.

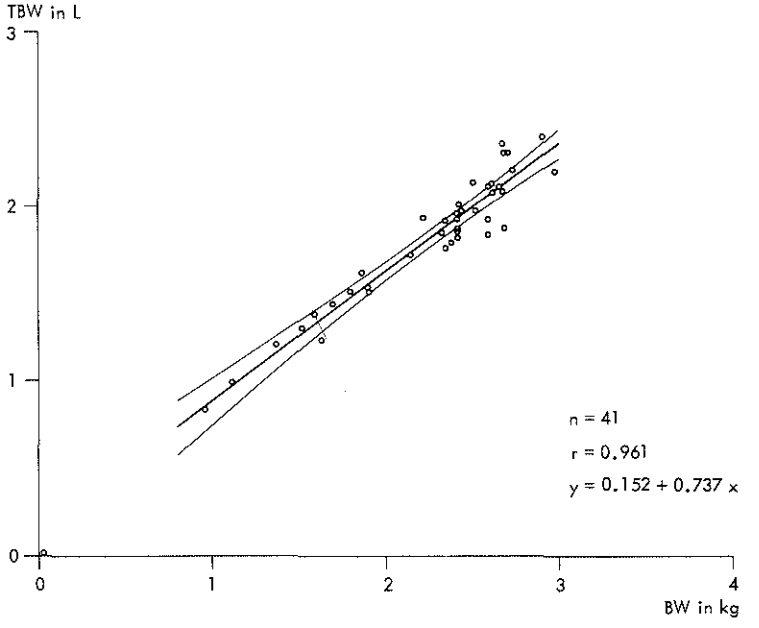


Fig. 22 Total body water (TBW) in litres plotted against body weight (BW) in kg.
Results of 41 measurements in 33 preterm babies are given (∘).
A regression line (———) and 95% confidence limits (———) are drawn.

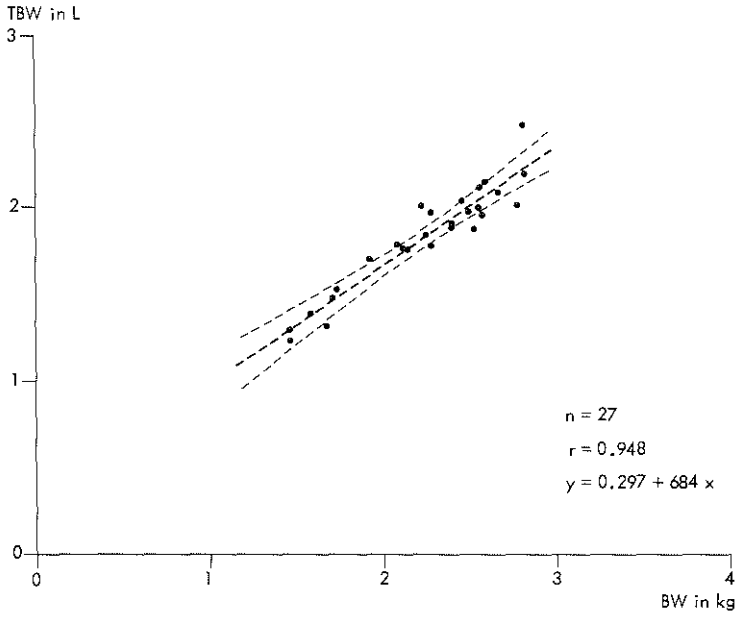


Fig. 23 Total body water (TBW) in litres plotted against body weight (BW) in kg. Results of 27 measurements in 18 S.F.D. babies are given (•). A regression line (-------) and the 95% confidence limits (- - - - - -) are drawn.

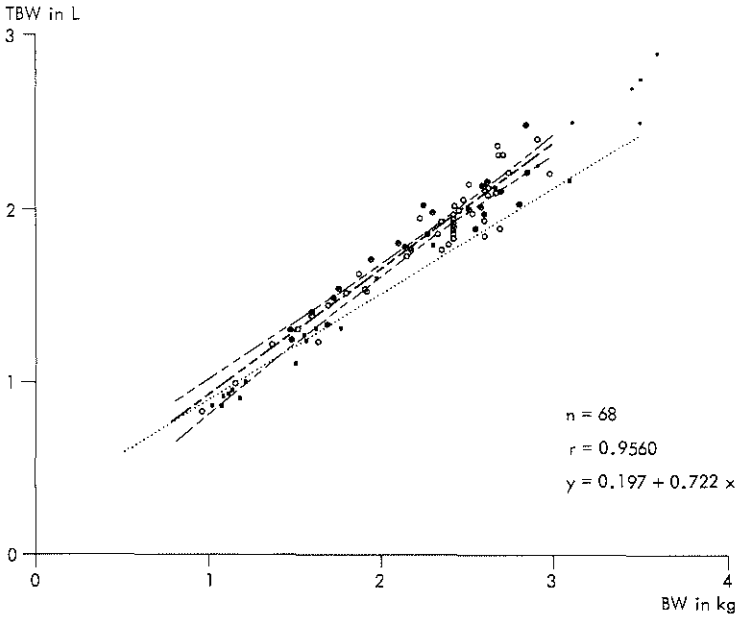


Fig. 24 Total body water (TBW) in litres plotted against body weight (BW) in kg. Results of 68 measurements in 33 preterm babies (○) and 18 S.F.D. babies (•) are given. A regression line and the 95% confidence limits are drawn (--------). Data derived from the literature (table XXIII and Friis-Hansen, 1957) are also given (•), but not included in the calculations for the regression line. The regression line from the antipyrine-space study of Cassady and Milstead (1971) is also given (·····).

Alongside our own figures for the TBW of preterm and S.F.D. babies, the values for TBW obtained from carcass analysis by the authors mentioned in table XX (p.131) are found. The values for TBW are only given for patients who, at the time of the study, were of less than 37 weeks post-conceptional age and of more than 1000 grams body weight.

In this case therefore it only concerns groups IV, V and VI in table XXI (p. 132). The individual figures for TBW, total body fat and total body protein for these patients are given in table XXIII (p. 134).

The results of a number of measurements of TBW by the D_2O-dilution method taken from Friis-Hansen (1957) are plotted in Fig. 24. Finally the regression line for the results of the antipyrine-space study of Cassady and Milstead (1971) is also given. So as would be expected, the regression line of the above named antipyrine-space study lies a little lower than our regression line obtained by the D_2O-dilution method.

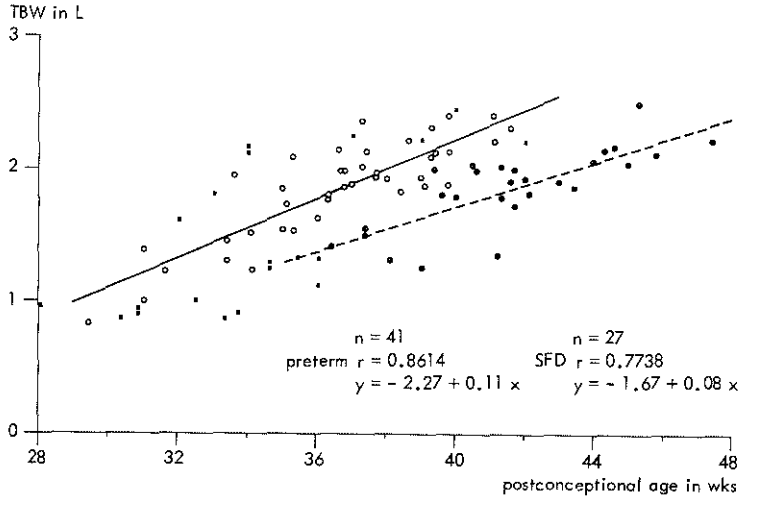


Fig. 25 TWB in litres plotted against post-conceptional age in weeks.
Results of 68 measurements in 33 preterm babies (○) and 18 S.F.D. babies (●) are given. Regression lines are drawn for preterm babies (————) and for S.F.D. babies (-------) separately.
Data derived from the literature (table XXIII) are added but are not included in the calculations for the regression curves (▪).

In Fig. 25 the TBW (in litres) is plotted against the post-conceptional age. In this manner the group of preterm babies is somewhat separated from the group of S.F.D. babies although some overlap remains because the TBW was measured at different post-conceptional and post-natal ages. Although there is a considerable scatter around the calculated regression lines in both groups, a justifiable conclusion would seem to be that in general S.F.D. babies have a lower TBW than preterm babies of a similar post-conceptional age. This would appear a contradiction of the observations of Cassady and Milstead (1971) who found a rather high value for TBW in S.F.D. babies. It should be noted however, that they performed their studies on newly born babies whereas the babies in our study were at least a few days, and sometimes a few weeks, old at the time of measurement of TBW. In newly born babies, who had had growth retardation during the intra-uterine period, Cassady and Milstead (1971) found a high percentage of both extra- and intra-cellular water. They found that the intra-cellular water compartment decreases rapidly after birth (within a few hours) while the extra-cellular water decreases more slowly. The patients whom we studied were all beyond this immediate postnatal period. Various factors, such as initial weight loss and the previously described regime of intravenous and oral fluid administration had already had their influence on the water compartment of the children in our study.

If we consider Fig. 24 then it can be said that there is a well defined relationship between body weight and TBW. It seems justifiable, in future studies on the body composition of low birth weight babies, to use the regression line which we have found for the estimation of the total body water and so to avoid the necessity of D_2O administration and subsequent blood sampling in these babies.

V.2.2. The extra-cellular fluid volume (ECV)

The results of the measurements of the extra-cellular fluid volume, using the NaBr-dilution method, are given in table XXIV (p. 135) for preterm babies and in table XXV (p. 137) for the S.F.D. babies. These results are displayed graphically in Fig. 26 and Fig. 27 where the corrected bromide space (CBS), as a measure of the ECV, is plotted against body weight.

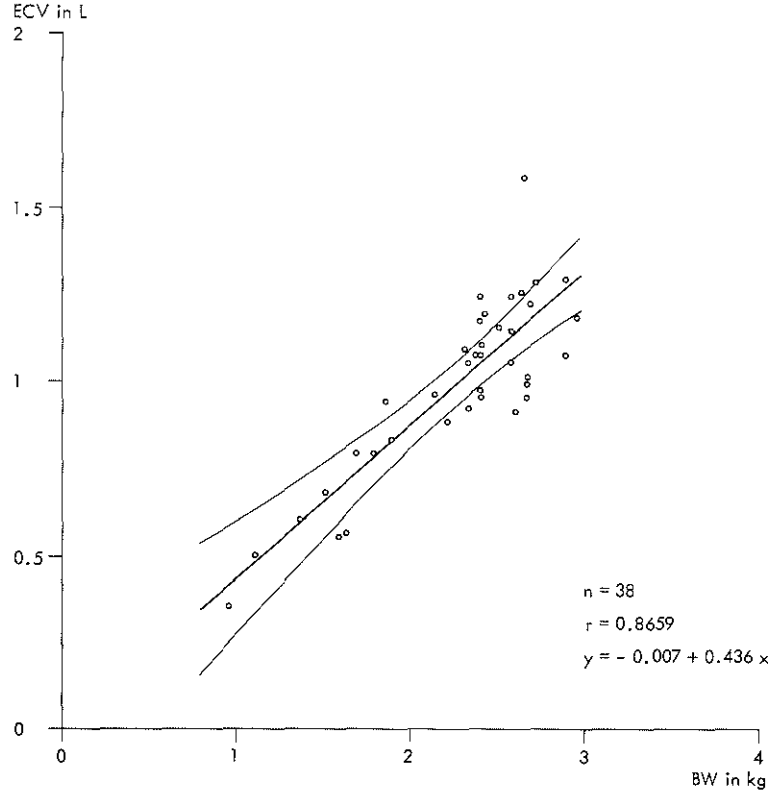


Fig. 26 Corrected bromide space (CBS) in litres as a measure of the extracellular fluid volume (ECV) plotted against body weight (BW) in kg.
Results of 38 measurements in 30 preterm babies (∘) are given. A regression line (————) and 95% confidence limits (————) are drawn.

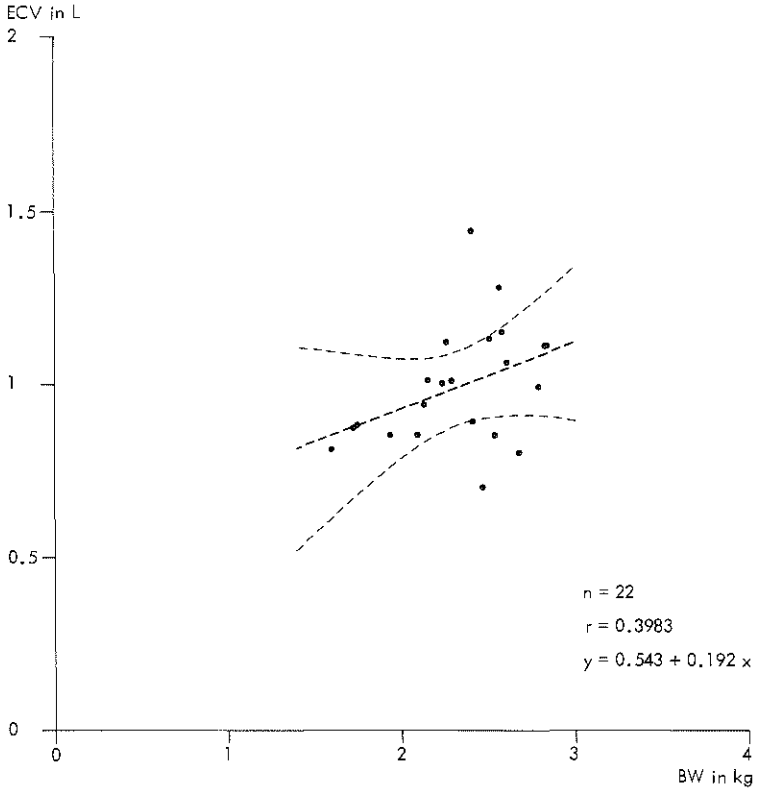


Fig. 27 Corrected bromide space (CBS) in litres as a measure of the extracellular fluid volume (ECV) plotted against body weight (BW) in kg. Results of 22 measurements in 16 S.F.D. babies (•) are given. A regression line (--------) and 95% confidence limits (-------) are drawn.

It is noticeable that, particularly in the S.F.D. babies, the range of values which were found is considerable. The explanation for this can be sought in the fact that this concerns a rather heterogeneous group. It contains both preterm and full-term S.F.D. babies who vary from each other in the timing and duration of the intra-uterine

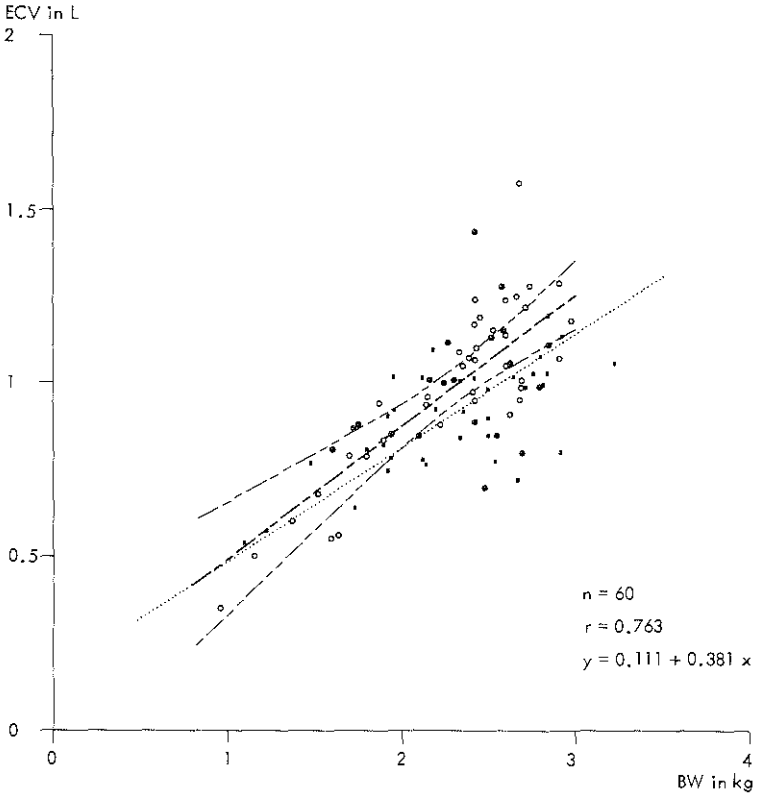


Fig. 28 Corrected bromide space (CBS) in litres as a measure of the extracellular fluid volume (ECV) plotted against body weight (BW) in kg.
Results of 60 measurements in 30 preterm (∘) and 16 S.F.D. (•) babies are combined.
A regression line (——·——) and 95% confidence limits (— — —) are drawn.
Data derived from the literature (Bhakoo and Scopes, 1971) are added (•). The regression line from the study by Cassady (1970) is also given (·····).

growth retardation which they had sustained. Furthermore, the measurements were not performed at the same post-natal age in all babies.

One can assume that both the stage of development when intra-uterine growth retardation begins and its duration will influence the volume of the extra-cellular fluid. Severe and longstanding intra-uterine growth retardation leading to a significant reduction in the total cell mass of the body may lead to a relatively high extracellular volume. There are some indications in this direction in our patients: of the 6 S.F.D. babies with the highest ECV there were 3 (S2, S3 and S4) in whom the delivery was induced at 36 weeks gestation because of intra-uterine growth retardation, while the 3 other babies (S12, S16, S17) were born spontaneously at term and turned out to be seriously growth retarded at birth.
In Fig. 28 the data are plotted against body weight for both preterm and S.F.D. babies. At the same time the extracellular fluid volumes from the study of Bhakoo and Scopes (1971) are plotted. These were also measured by the NaBr-dilution method. The regression line from a study by Cassady (1970) is also given on this graph.

It is notable that the measurements of Bhakoo and Scopes also show a wide range. They give no explanation for this in their paper.

Meanwhile it should be noted that knowledge of the ECV is not necessary for the measurement of total body fat.

V.2.3. The total body fat (TBF)

V.2.3.1. The Xe-absorption method

The results of the measurements of TBF by Xe-absorption are given in table XXVI (p. 138) for preterm babies and in table XXVII (p. 141) for S.F.D. babies. Total body fat is given in percent by weight and in grams.

Where the measurements were made within a period of three days, then the average result is also given.

In Fig. 29a the TBF in grams is plotted against the body weight in grams. The points which are connected by lines indicate serial measurements in the same baby. Since the number of measurements is not the same for each baby, weighed regression lines are calculated and plotted for the group of preterm babies and the group of S.F.D. babies separately [1]. A statistical procedure to test if the regression lines differ significantly from each other is not permissible, since each line is based on related data. Therefore a different procedure has been followed, as is shown in Fig. 29b. In this graph, only the result of the first measurement (or the average value of the first set of measurements) on each baby is plotted. Thus, all patients are equally represented and the regression lines based on these data may be tested statistically. There appears to be a significant difference between the intercepts of the two regression lines. This means that the two groups of patients may be considered as two separate populations. We have assumed that the same is true for the two groups of babies shown in Fig. 29a.

[1] In figures 29a to 35a inclusive no correlation coefficients have been calculated because these give no relevant information over the closeness of fit in weighed regression curves.

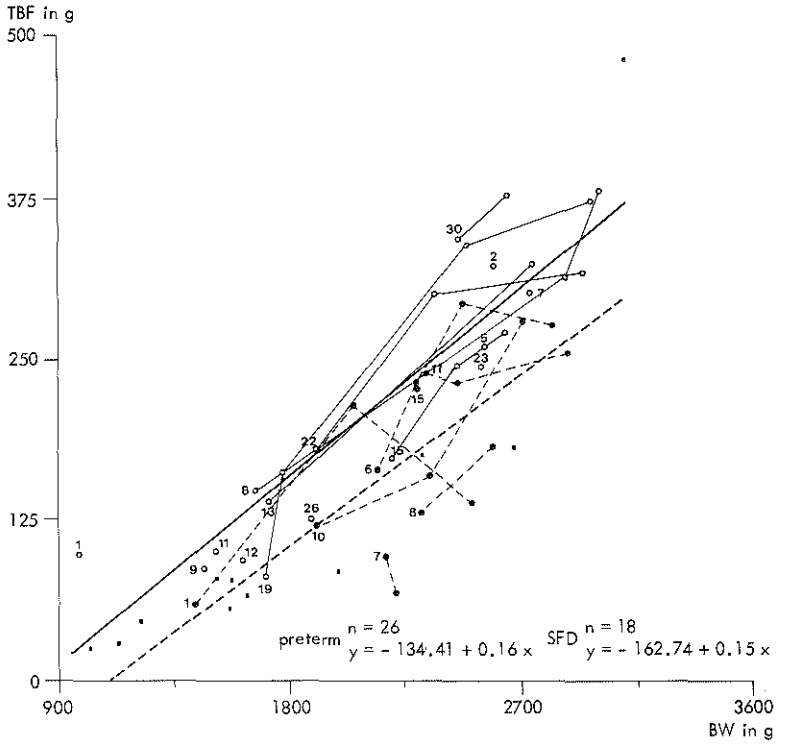


Fig. 29 a. Total body fat (TBF) in grams plotted against body weight (BW) in grams.
Results of 26 measurements in 15 preterm babies (o) and 18 measurements in 7 S.F.D. babies (•) are given. Numbers refer to corresponding numbers given to patients in table XI to XVI. Regression lines are drawn for preterm babies (————) and S.F.D. babies (------) separately. Data derived from the literature (table XXIII) are also given (▪), but not included in the calculations for the regression lines.

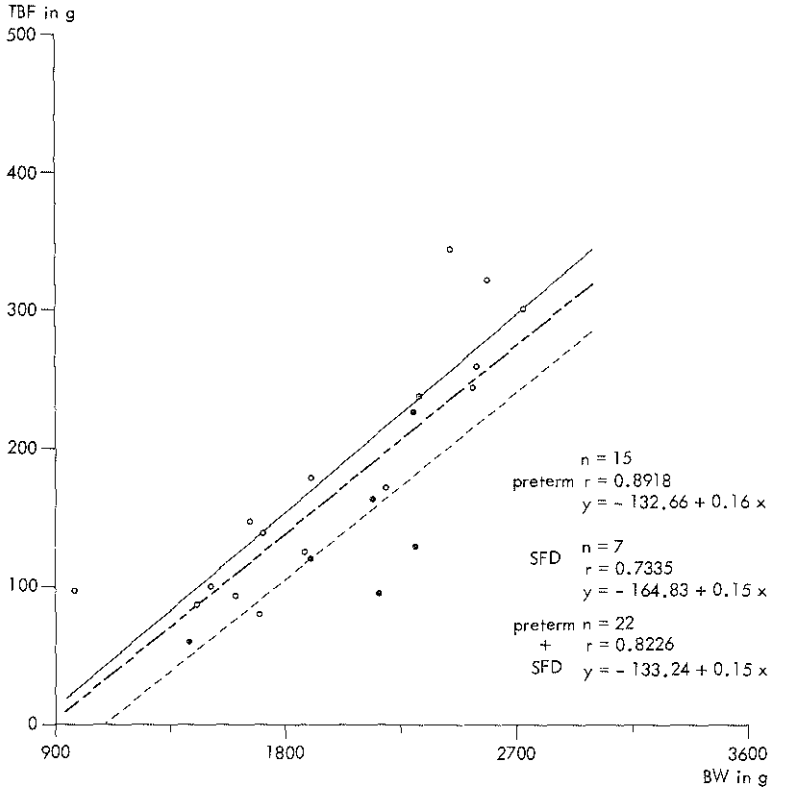


Fig. 29 b. Total body fat (TBF) in grams plotted against body weight (BW) in grams.
The results of the first measurements (or average value of the first set of measurements) in 15 preterm (∘) and 7 S.F.D. babies (•) are given.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------).
The difference between the slopes of the two lines is not significant; the intercepts differ significantly ($0.01<p<0.05$). A regression line for the two groups of babies combined is also given (— — — —).

The same procedure has been followed in figs. 30a and 30b, in which the TBF in percent by weight is plotted against the body weight in grams. The results of all measurements are plotted in fig. 30a whereas in fig. 30b only the result of the first measurement on each baby is given. It appears that the intercepts of the two regression lines in fig. 30b differ significantly. It may be assumed that there exists also a difference between the two groups of babies shown in fig. 30a. In fig. 31a and fig. 31b the TBF is plotted as percent by weight against the postconceptional age in weeks. The same procedure which has been described above has been followed. There is a significant difference between the intercepts of the two regression lines in fig. 31b. It is assumed that a difference also exists between the two groups of babies shown in fig. 31a.

In the figs. 29a, 30a and 31a the data from carcass analysis taken from the literature (table XXIII) are also plotted.

The following comments can be made based on the data presented in figs. 29a and 30a.

Preterm babies have only slightly more TBF than S.F.D. babies of a similar body weight, but the differences are small. This is in agreement with the clinical impression. There is good evidence that most of the fat is found subcutaneously in low birth weight infants (Dauncey et al., 1977). Preterm and S.F.D. babies have little subcutaneous fat. Preterm babies are born at a time when subcutaneous fat has not yet, or hardly, begun to develop. S.F.D. babies have, it is true, a longer gestation behind them, but the development of the subcutaneous fat layer is apparently retarded. From the figs. 29a and 30a it is further apparent that the increase in body weight, especially in preterm babies, goes together with an increase in the TBF. This is clearly shown in the few patients on whom longitudinal studies were done. In the group of S.F.D. babies whom we studied there was sometimes an increase in TBF with increase in weight, but also sometimes a decrease.

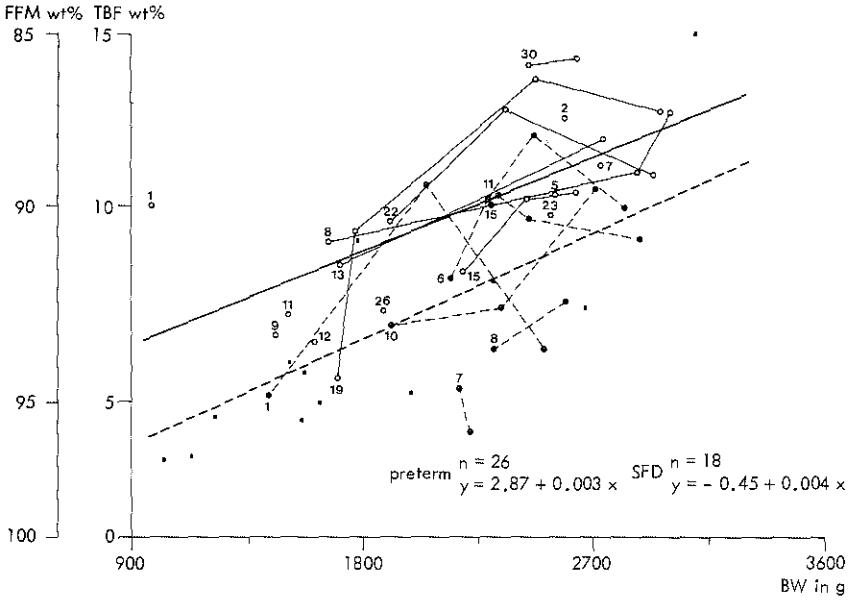


Fig. 30 a. Total body fat (TBF) and fat free mass (FFM) in percent by weight (wt%) plotted against body weight (BW) in grams.
Results of 26 measurements in 15 preterm babies (∘) and 18 measurements in 7 S.F.D. babies (•) are given. Numbers refer to corresponding numbers given to patients in tables XI to XVI.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------) separately.
Data derived from the literature (table XXIII) are also given (▪), but not included in the calculations for the regression lines.

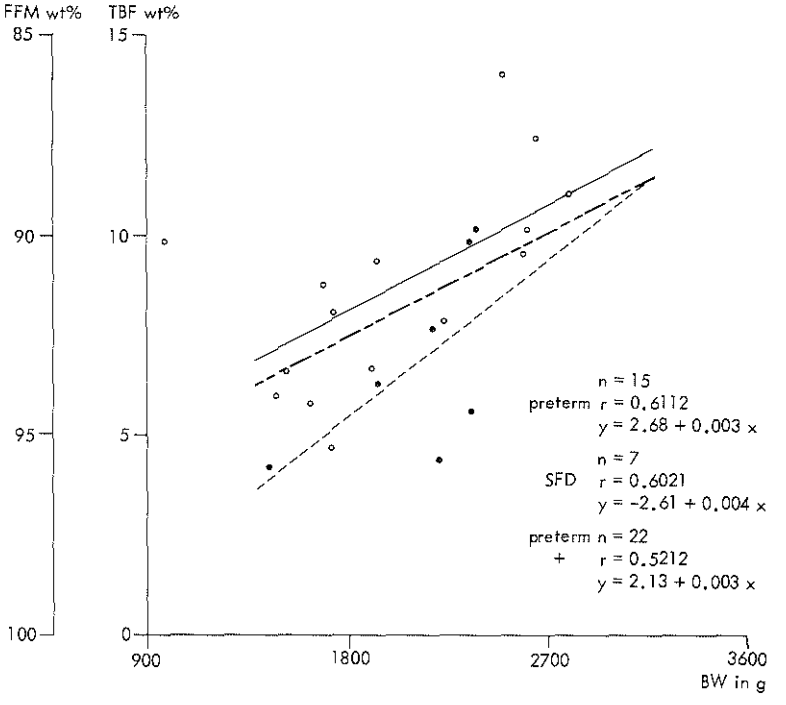


Fig. 30 b. Total body fat (TBF) and fat free mass (FFM) in percent by weight (wt%) plotted against body weight (BW) in grams.
The results of the first measurements (or average value of the first set of measurements) in 15 preterm (∘) and 7 S.F.D. babies (•) are given.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------).
The difference between the slopes of the two lines is not significant; the intercepts differ significantly ($0.01<p<0.05$). A regression line for the two groups of babies combined is also given (—·—·—).

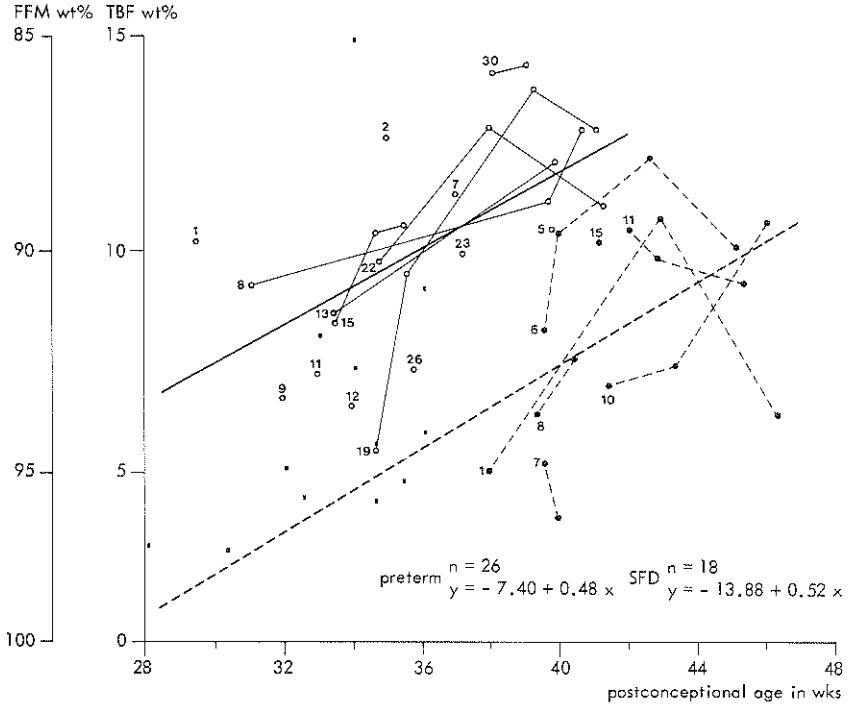


Fig. 31 a. Total body fat (TBF) and fat free mass (FFM) in percent by weight (wt%) plotted against postconceptional age in weeks.
Results of 26 measurements in 15 preterm babies (∘) and 18 measurements in 7 S.F.D. babies (•) are given. Numbers refer to corresponding numbers given to patients in tables XI to XVI.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (- - - - - - - -) separately.
Data derived from the literature (table XXIII) are also given (▪), but not included in the calculations for the regression lines.

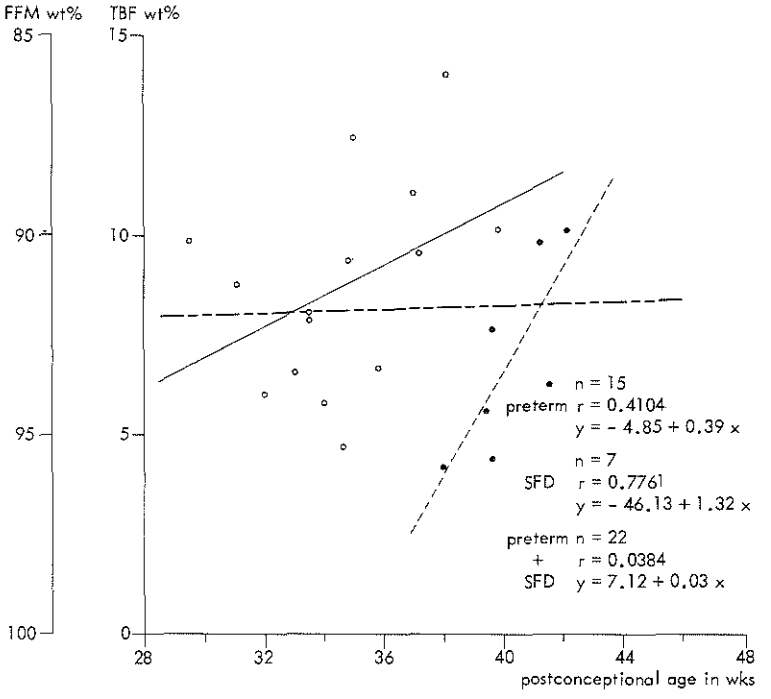


Fig. 31 b. Total body fat (TBF) and fat free mass (FFM) in percent by weight (wt%) plotted against postconceptional age in weeks.
The results of the first measurements (or average value of the first set of measurements) in 15 preterm (∘) and 7 S.F.D. babies (•) are given.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------) separately.
The difference between the slopes of the two lines is not significant; the intercepts differ significantly ($p < 0.01$). A regression line for the two groups of babies combined is also given (—·—·—).

These differences within the group of S.F.D. babies are possibly explained by the fact that they were not a homogeneous group. The development of the fat compartment of S.F.D. babies could well be dependent on the timing and duration of the intra-uterine growth retardation. We shall come back to this point later.

It will be noticed that many of the data from carcass analysis fall in the same area as the data from our S.F.D. babies. Indeed it appears that 11 of the 16 babies, from whom data from carcass analysis are given, fall below the -2 S.D. of the intra-uterine growth curve for weight. There is however one baby whose result lies above the rest. This baby was one of the group reported by Widdowson and Dickerson (1964) with a body weight of 3090 g and a weight of TBF of 479 g at 34 weeks of gestation. This was perhaps a baby of a diabetic mother although no information is given.

On the data presented in Fig. 31a the following comments can be made.

In general preterm babies clearly have more TBF than S.F.D. babies of the same postconceptional age. Preterm babies, under optimal conditions, show a clear increase in TBF during the first few weeks after birth. This is illustrated by the serial measurements on patients P13 and P19. In a period of 6-7 weeks, the body weight of these babies increases by about a half, while the amount of fat (in g) more than doubles. The same pattern is shown in baby P8 over a period of 9 weeks. In contrast, S.F.D. babies in general show only a small increase in TBF during the first weeks after birth. There is however much greater variability within this group. Patients S1, S6 and S8 show an increase in TBF which is comparable to that of the preterm babies. The increase in TBF in baby S10 is initially much smaller. In baby S7, TBF was measured twice in the first week of life; here, the results are much lower than in the other babies. No good explanation, based on either the ob-

stetric history or the clinical data, could be found. The findings in babies S11, born after 40 weeks gestation with a weight of 2100 g and S15, born after 41 weeks gestation with a weight of 2390 g, are somewhat different. In both babies no reason for the intra-uterine growth retardation could be found. Clinically these babies appeared much less wasted than babies S7 and S10. The initial measurements of TBF in babies S11 and S15 were clearly higher than in the other S.F.D. babies.

Percentages of fat by weight of 15-16%, as given in the literature by for instance Widdowson (1974), for full term babies, were never found in those of our babies who were studied at 40 weeks post-conceptional age. We found in preterm babies, of about 40 weeks post-conceptional age, that the percentage of fat by weight was between 11 and 13%. This is in good agreement with the calculated percentage of fat by weight of the "reference fetus" (Ziegler et al., 1976), mentioned in table XXIIb. Our babies were fed, as described in chapter IV, with a "humanized" milk (Almiron M_2, Nutricia) which has an approximately similar percentage of fat to that of breast milk. Under normal conditions in utero, the build up of fat tissue may be faster than occurs after birth. There is insufficient known about this.

The S.F.D. babies whom we studied did not achieve a percentage of fat by weight of 11-13% by a post-conceptional age of 40 weeks. In a few patients, described above, a percentage of fat by weight of about 10% was found. However, most of the S.F.D. babies had not more than 5-8% TBF at this post-conceptional age. Both groups of babies followed growth curves which ran parallel to the normal intra-uterine growth curves as shown in figures 14 and 15. Thus while the gross increase in weight per week is approximately similar in both groups of patients, the growth of the fat compartment in the S.F.D. babies clearly remains retarded.

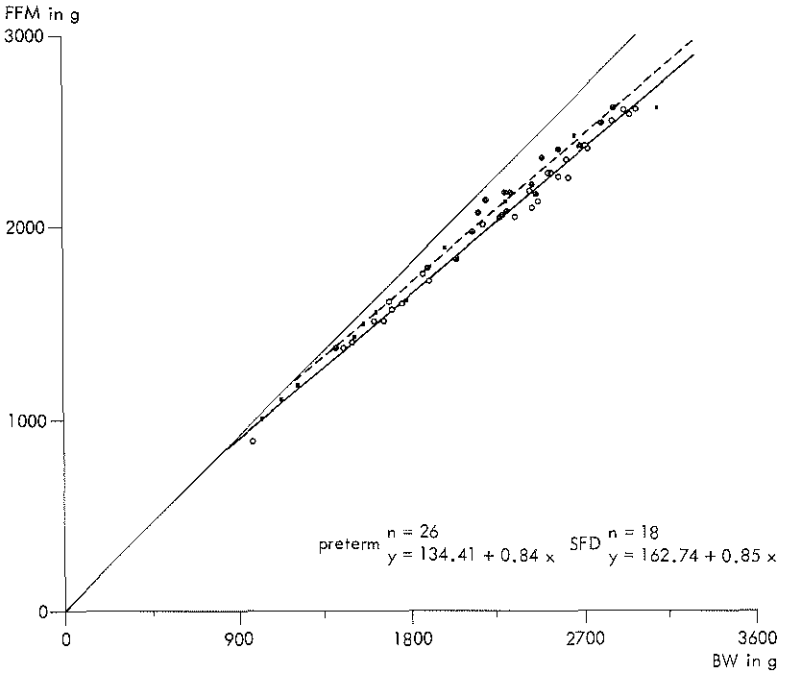


Fig. 32 a. Fat free mass (FFM) in grams plotted against body weight (BW) in grams.
Results of 26 measurements in 15 preterm babies (∘) and 18 measurements in 7 S.F.D. babies (•) are given.
Regression lines are drawn for preterm babies (————) and S.F.D. babies separately.
Data derived from the literature (table XXIII) are also given (·), but not included in the calculations for the regression lines. The line y = x is drawn.

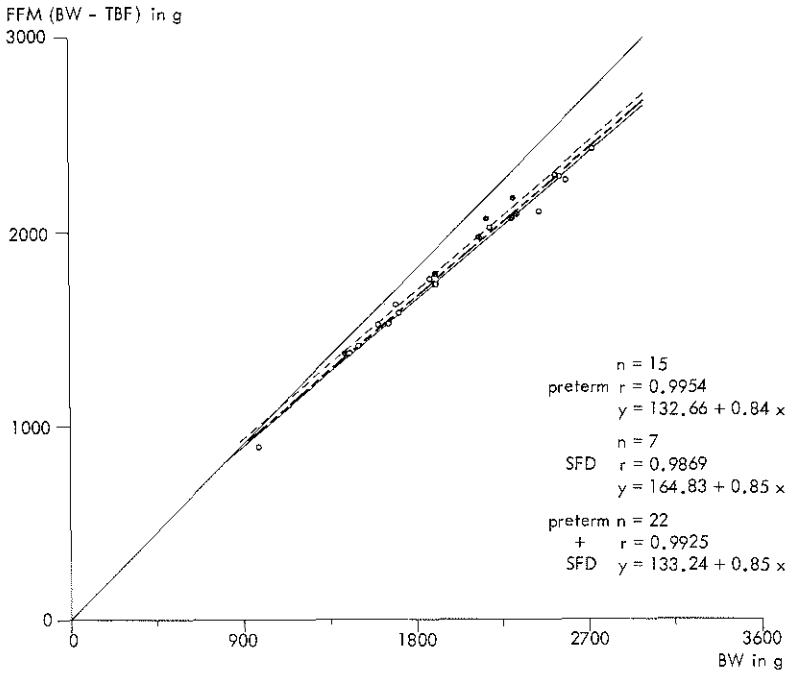


Fig. 32 b. Fat free mass (FFM) is grams plotted against body weight (BW) in grams.
The results of the first measurements (or average of the first set of measurements) in 15 preterm (∘) and 7 S.F.D. babies (•) are given.
Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------). The difference between the slopes of the two lines is not significant; the intercepts differ significantly ($0.01 < p < 0.05$). A regression line for the two groups of babies combined is also given (—·—·—). The line $y = x$ is drawn.

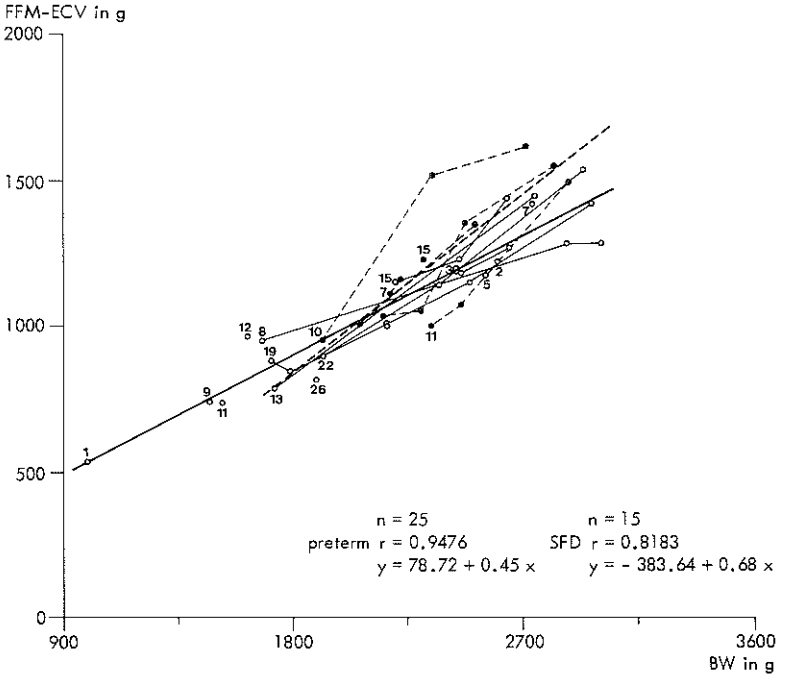


Fig. 33 a. Fat free mass minus extracellular volume (FFM-ECV) in grams plotted against body weight (BW) in grams.

The results of 25 measurements in 14 preterm babies (∘) and 15 measurements in 6 S.F.D. babies (•) are given. Numbers refer to numbers given to patients in tables XI to XVI. Regression lines are drawn for preterm babies (————) and S.F.D. babies (------) separately.

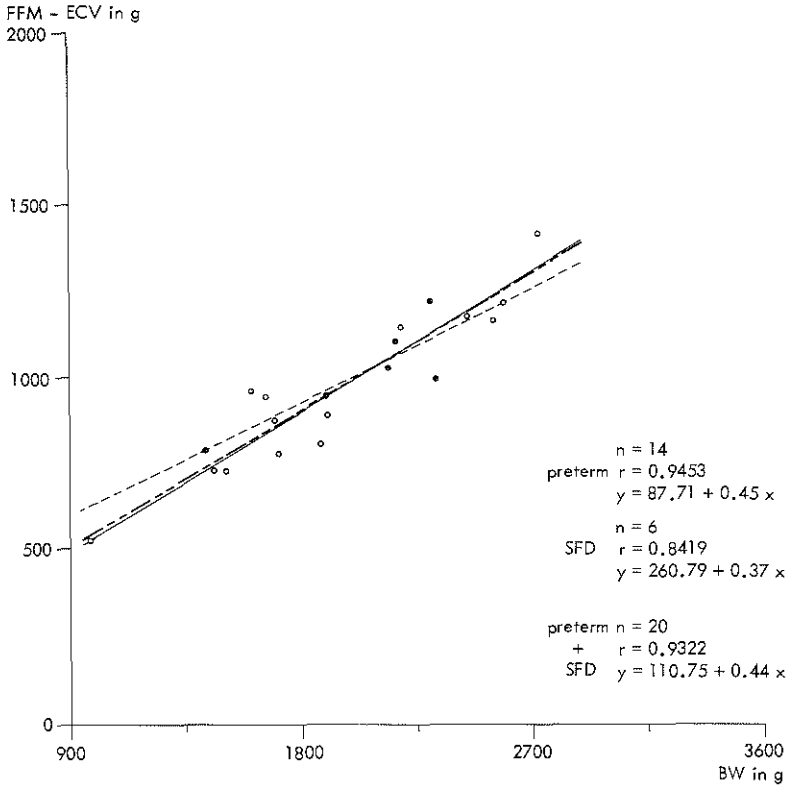


Fig. 33 b. Fat free mass minus extracellular volume (FFM-ECV) in grams plotted against body weight (BW) in grams. The results of the first measurements (or average value of the first set of measurements) in 14 preterm (∘) and 6 S.F.D. babies (•) are given. Regression lines are drawn for preterm babies (————) and S.F.D. babies (--------) separately. Neither the slopes nor the intercepts of the two lines differ significantly. A regression line for the two groups of babies combined is also given.(———)

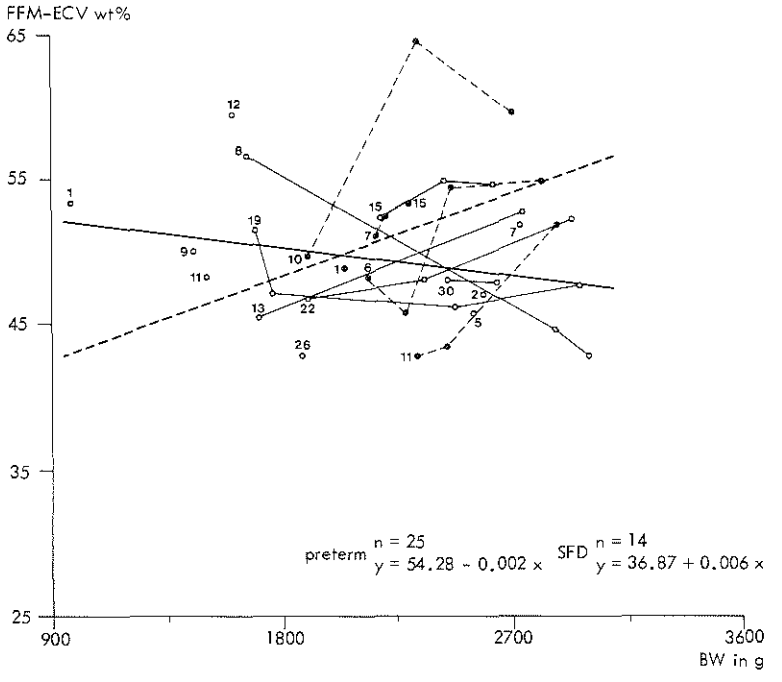


Fig. 34 a. Fat free mass minus extracellular volume (FFM-ECV) in percent by weight (wt%) plotted against body weight (BW) in grams. Results of 25 measurements in 14 preterm babies (∘) and 14 measurements in 6 S.F.D. babies (•) are given. Numbers refer to numbers given to patients in tables XI to XVI. Regression lines are drawn for preterm babies (————) and S.F.D. babies (-------) separately.

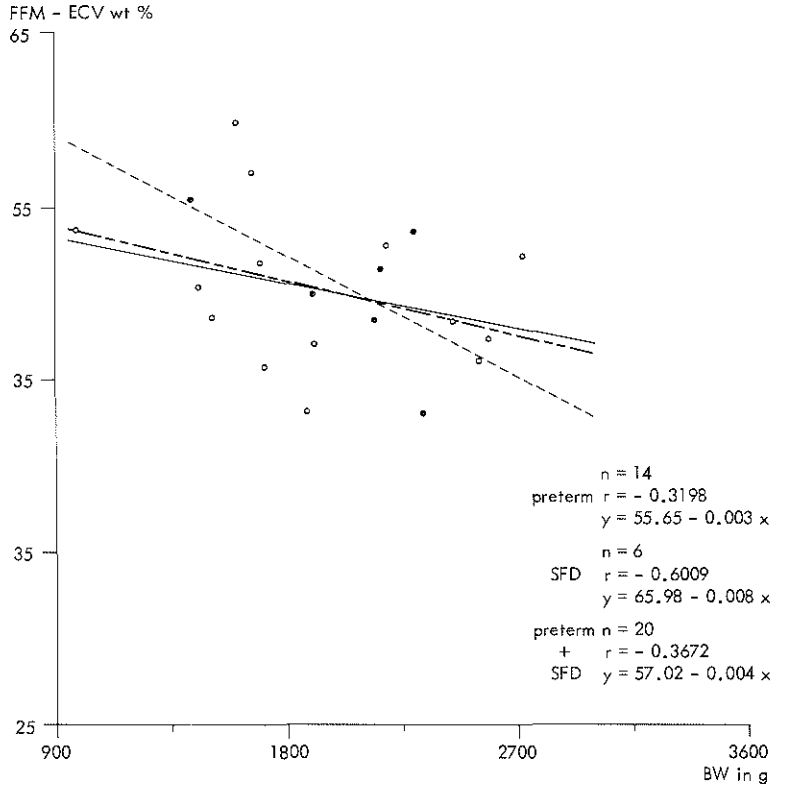


Fig. 34 b. Fat free mass minus extracellular volume (FFM-ECV) in percent by weight (wt%) plotted against body weight (BW) in grams. The results of the first measurements (or average of the first set of measurements) in 14 preterm (∘) and 6 S.F.D. babies (•) are given. Regression lines are drawn for preterm babies (————) and S.F.D. babies (------) separately. Neither the slopes nor the intercepts of the two lines differ significantly. A regression line for the two groups of babies combined is also given (—·—·—).

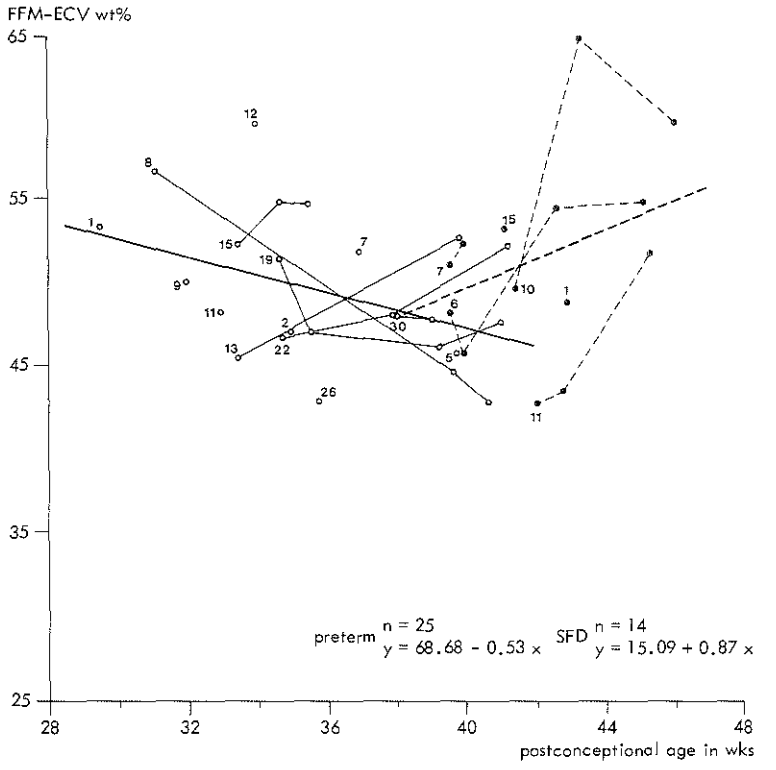


Fig. 35 a. Fat free mass minus extracellular volume (FFM-ECV) in percent by weight (wt%) plotted against postconceptional age in weeks. Results of 25 measurements in 14 preterm babies (∘) and 14 measurements in 6 S.F.D. babies (•) are given. Numbers refer to numbers given to patients in tables XI to XVI. Regression lines are drawn for preterm babies (————) and S.F.D. babies (--------) separately.

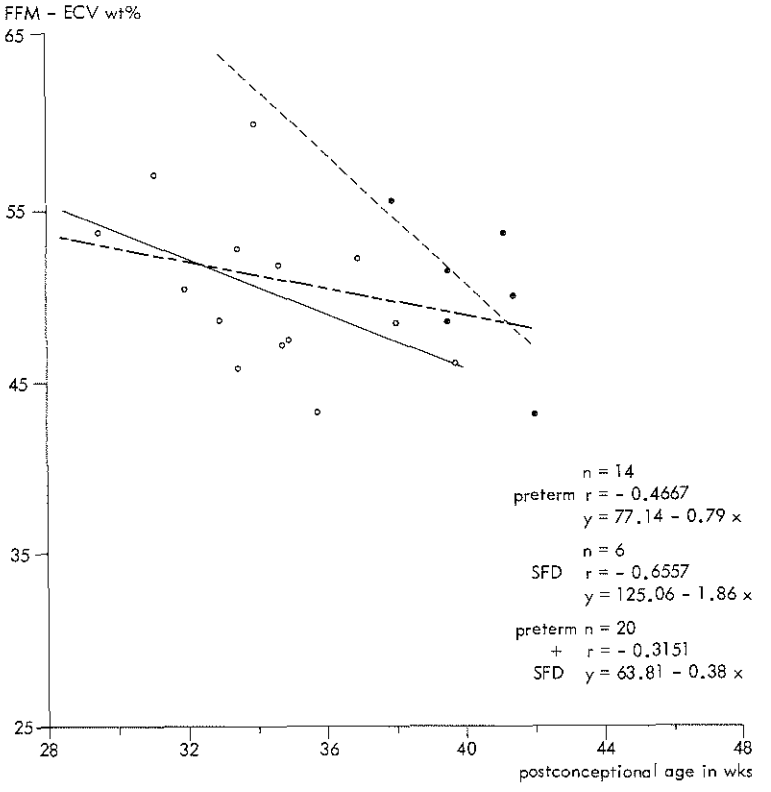


Fig. 35 b. Fat free mass minus extracellular volume (FFM-ECV) in percent by weight (wt%) plotted against postconceptional age in weeks. The results of the first measurements (or average of the first set of measurements) in 14 preterm (∘) and 6 S.F.D. babies (•) are given. Regression lines are drawn for preterm babies (————) and S.F.D. babies (------) separately. Neither the slopes nor the intercepts of the two lines differ significantly. A regression line for the two groups of babies combined is also given (–––––).

In figs. 32a and 32b the fat free mass (FFM, defined as the body weight less the TBF) in grams is plotted against the body weight in grams. There is a slight difference in the amount of FFM between preterm and S.F.D. babies. In fact, the same difference between preterm and S.F.D. babies as is shown in fig. 29a, is presented graphically in a different form in fig. 32a.

After subtraction of the extracellular volume (ECV) from the FFM a body compartment results, which could be called the "fat free cell mass". We calculated this compartment in our patients. In figs. 33a and 33b the "fat free cell mass" in grams is plotted against body weight in grams, and in figs. 34a and 34b the "fat free cell mass" in percent by weight is plotted against body weight in grams. In figs. 35a and 35b the "fat free cell mass" is plotted against the postconceptional age in weeks. There is no significant difference between preterm and S.F.D. babies. It follows, that the difference between these two groups of babies mainly involves the fat compartment. The difference in slope of the regression lines calculated from the data of S.F.D. babies, using either all measurements or only the first measurement, could be explained by the fact that the ECV changes considerably in S.F.D. babies during the first postnatal weeks. When using only the first measurement on each baby, this information is lost. Therefore the longitudinal data give a better picture of what is happening in these babies during the first weeks after birth. Looking at fig. 35a it even seems that in the group of S.F.D. babies a certain amount of catch up growth of the "fat free cell mass" occurs, whereas the fat compartment lags behind.

If it is indeed true that S.F.D. babies are retarded in growth mainly with regard to their fat compartment, then it would be interesting to know whether giving a milk formula with a greater fat content and higher calorific value than the "humanized" milk used, would also lead to catch-up

growth of the fat compartment. The adapted milk formula which is now used (Nenatal, Nutricia) contains more fat and more calories. This milk formula was introduced on our unit after the series of measurements of total body fat by Xe-absorption was already completed. It would seem to be worthwhile, in the future, to study the effect of various feeding regimes on the development of the fat compartment of babies and in particular of the S.F.D. baby.

It could be, however, that, in spite of a greater intake of fat, no catch up growth in the fat compartment would result. In accordance with the theory of Winick and Noble (1966) these babies may have sustained intra-uterine growth retardation of the fat compartment in the phase of growth which is critical for the increase in numbers of fat cells. The babies are then born with a permanently low number of fat cells and continue for the rest of their lives with a relatively small fat compartment. In the light of the obesity problem, to which great importance is attached at present from both medical and social viewpoints, it may be that a certain amount of intra-uterine growth retardation would have an unexpected advantage. The question which can be asked in this respect is not "Do fat babies stay fat?" (Poskitt and Cole, 1977) but rather "Do slim babies stay slim?"

When following up S.F.D. babies it is frequently seen that there is catch-up growth in both length and head circumference but that the weight remains relatively low for length and age. The question of whether it is in fact so that, at least in some S.F.D. babies, the fat compartment is permanently retarded in growth can probably best be answered by longitudinal studies of the TBF using the Xe-absorption method in the neonatal period, followed by the anthropometric method of Dauncey et al. (1977) in infancy and childhood.

V.2.3.2. The anthropometric method of Dauncey et al.

In four patients we were in a position to be able to compare the results for total body fat obtained by the Xe-absorption method with those obtained by the method described by Dauncey et al. (1977). These results are given in table XXVIII.

Patient	g.a./b.wt.	Postconc. age (wks)	TBF (Xe) wt. %	TBF (skin-fold) wt. %
P15	32 / 2100	35.5	10.3	13.9
S7	39 / 2325	40.0	3.1	4.6
S8	39 / 2460	40.5	7.0	8.5
S15	41 / 2390	41.2	9.9	9.1

Table XXVIII Comparison of total body fat (TBF), in percent by weight, measured by the Xe-absorption method and by the anthropometric method of Dauncey et al. (1977).

This concerns only a few observations and the number is too small for statistical analysis. However, it can be said that, in these patients, there is reasonably good agreement between the results of both methods. It is interesting that the percentage of fat of the S.F.D. babies also remains below 10% when measured by the method of Dauncey et al. The only preterm baby (who has the youngest post-conceptional age but the oldest post-natal age) has a percentage of fat which, in agreement with that measured by the Xe-absorption method, is higher than the three S.F.D. babies. It would seem important in future studies on the body composition of babies to use both methods for comparison.

V.3. Conclusions

With the method which has been described for the measurement of total body fat by Xe-absorption, the possibility is opened up of measuring the fat compartment of babies in a non-invasive manner. Longitudinal study of total body fat now becomes possible. In the method which we used, it was still necessary to measure total body water using the D_2O-dilution method, because knowledge of its volume is necessary for the final calculation. If the relationship bwtween body weight and total body water, which we found, is used in future studies, then the D_2O measurement becomes unnecessary and may be left out. Thus we are left with a method for the measurement of total body fat which is non-invasive and which is not taxing for the patient. It is also a method which, because it has no drawbacks for the patient, can be regularly repeated, thus furnishing important information on body composition in relation to growth in the neonatal period.

The questions which were underlying this study can now be answered.

Are there differences in body composition between preterm and S.F.D. babies of similar body weight?

There are small differences in body composition between preterm and S.F.D. babies of a similar body weight. Both groups have about the same amount of total body water. Preterm babies have a little more body fat than S.F.D. babies of similar weight, but the differences are small. On the other hand, when comparing S.F.D. and preterm babies of similar post-conceptional ages, the S.F.D. babies obviously have less fat.

How does the body composition of low birth weight babies change during the course of growth in the first weeks after birth? Preterm babies, with optimal treatment, generally exhibit a clear increase in the fat compartment in the first post-natal weeks. The increase in the fat com-

partment occurs much more slowly in S.F.D. babies under the same conditions. There is however large variability within this group, possibly related to the timing and duration of intra-uterine growth retardation.

Are there differences in body composition between preterm and S.F.D. babies at the time that the "at term" date is reached? From the answer to the second question it follows that, with regard to total body fat, the differences between preterm and S.F.D. babies are evident by this time. The S.F.D. babies lie behind the preterm babies with regard to their TBF, but with regard to the "fat free cell mass" expressed as percent by weight practically no difference between the two groups of babies exists; some S.F.D. babies even have a slightly higher "fat free cell mass". Our preterm babies do not achieve a percentage of TBF of 15-16% as is given in the literature for full term babies. Obviously the treatment regime, even for preterm babies, is not yet such that the extra-uterine milieu can be compared with the conditions in utero.

Further study of these phenomena is necessary using the technique described here. The development of a method for measuring total body fat by Xe-absorption has not been without its difficulties. Significant problems of both a technical and mathematical nature had to be solved before some degree of reliability could be obtained in the results. This has cost a considerable amount of time. The existing apparatus is obviously capable of improvement.
An improvement and refinement in the technique for the measurement of Xenon is the first problem which must be tackled. By changing from gas chromatographic to mass spectrometric measurement of Xenon, it will perhaps be possible to follow the absorption process so accurately that measurement in the desorption phase will become unnecessary.

Further lines of research can then be:

- study of the influence of various feeding regimes on the development of the fat compartment.
- a longitudinal study in babies who have had intra-uterine growth retardation in order to see if the theory formulated by Winick and Noble (1966) concerning growth, applies to fat tissue. Longitudinal study of groups of S.F.D. babies should provide insight into the timing of the various growth phases of the fat tissue.

Both lines of research may be of great practical importance for the future management of low birth weight babies. On the one hand, knowledge of the influence of growth retardation on body composition of the fetus may alter the obstetrical management of the unborn baby with this problem. While, on the other hand, knowledge of the influence of feeding on the body composition of the newborn low birth weight baby could lead to a feeding regime which takes more account, than hitherto, of the differences in body composition between the preterm and S.F.D. baby.

Guinea-pig	Day	Method	B.wt. (g)	TBF (g)	TBF wt. %	FFM (g)	TBW (g)	TBW % of FFM	TBW % of BW
A	1	Xe	870	103 ± 3	11,8 ± 0,3	767 ± 4			
	2	Xe	874	117 ± 4	13,4 ± 0,4	757 ± 4			
			ave 872						
	9	c.a.	883	120 ± 4	13,6 ± 0,4	763 ± 4	565	74	64
B	1	Xe	1083	174 ± 4	16,1 ± 0,3	909 ± 4			
	2	Xe	1083	175 ± 4	16,1 ± 0,3	908 ± 4			
	9	Xe	1083	175 ± 3	16,1 ± 0,3	908 ± 3			
	10	Xe	1082	174 ± 4	16,1 ± 0,4	908 ± 4			
	12	Xe	1081	171 ± 3	15,8 ± 0,3	910 ± 3			
	15	Xe	1109	178 ± 6	16,1 ± 0,5	931 ± 6			
			ave 1087						
	30	c.a.	1135	184 ± 6	16,2 ± 0,6	951 ± 6	726	76	64
C	1	Xe	896	153 ± 5	17,1 ± 0,5	743 ± 5			
	2	Xe	910	147 ± 5	16,1 ± 0,6	763 ± 5			
			ave 903						
	5	c.a.	936	188 ± 4	20,1 ± 0,4	748 ± 4	599	80	64

Table XVII Total body fat (TBF) (in grams) of 3 guinea-pigs measured by Xe-absorption method and by carcass analysis. Total body water (TBW) measured by exsiccation during carcass analysis. Fat free mass (FFM) obtained by subtraction of the weight of fat from the body weight.
Xe = Xe-absorption measurement
c.a. = carcass analysis

TBF from c.a. is given as the mean value ± 1 S.D. (Guinea-pig A: 9 measurements; guinea-pig B: 7 measurements and guinea-pig C: 5 measurements).
TBF from Xe-measurements is given as the mean value ± 95% confidence limits of the desorption curve.

Pat.	G.a. (wks)	B.wt. (g)	At time of measurement: Postc. age (wks)	Wt. (g)	TBW: % by wt.	TBW: L
P1	28	970	29.4	960	85.7	0.83
P2	28	1360	35.0	2600	70.6	1.84
P3	29	1080	41.6	2710	85.3	2.31
P4	30	1070	36.3	2350	74.9	1.76
P5	30	1260	31.0	1155	86.3	0.99
P5			39.8	2600	81.7	2.12
P6	30	1450	36.3	2390	74.9	1.79
P7	30	1630	36.5	2690	69.8	1.88
P8	30	1690	31.0	1595	86.3	1.38
P8			39.8	2910	82.5	2.40
P9	31	1490	31.6	1370	88.5	1.21
P10	31	1755	36.8	1530	78.2	1.98
P11	32	1480	33.4	1520	85.5	1.30
P12	32	1500	34.1	1635	75.3	1.23
P13	32	1580	33.4	1700	84.6	1.44
P13			39.3	2680	78.1	2.09
P14	32	1610	38.6	2740	80.7	2.21
P15	32	2100	33.6	2225	87.1	1.94
P15			35.3	2620	79.3	2.08
P16	32	1850	36.3	1910	80.0	1.52
P17	33	2090	35.1	2150	80.2	1.72
P18	34	1810	37.7	2420	81.0	1.96
P19	34	1840	34.1	1800	83.7	1.51
P19			39.1	2420	76.8	1.86
P19			41.1	2980	73.9	2.20
P20	34	1880	37.7	2420	79.7	1.93
P21	34	1990	36.8	2330	79.4	1.85
P22	34	2010	35.0	1905	80.1	1.53
P22			38.0	2350	81.6	1.92
P22			41.1	2910	82.5	2.40
P23	34	2300	37.4	2620	81.2	2.13
P24	34	2300	36.5	2510	85.3	2.14

Pat.	G.a. (wks)	B.wt. (g)	At time of measurement: Postc. age (wks)	Wt. (g)	TBW: % by wt.	TBW: L
P25	34	2500	36.5	2510	85.3	2.14
P26	35	2040	36.0	1870	86.8	1.62
P27	35	2200	39.0	2600	74.3	1.93
P28	36	2100	38.4	2420	75.3	1.82
P29	36	2210	39.8	2420	77.4	1.87
P30	36	2380	39.3	2690	86.0	2.31
P31	36	2500	36.7	2450	80.8	1.98
P32	37	2380	39.4	2660	79.5	2.11
P33	37	2590	37.3	2430	82.9	2.01

Table XVIII 41 measurements of total body water (TBW) in 33 preterm babies in percent by weight and litres.

Pat.	G.a. (wks)	B.wt.	At time of measurement		TBW	
			Postc. age (wks)	Wt. (g)	% by Wt.	L
S1	36	1220	38.1	1475	88.1	1.30
S1			42.1	2100	85.5	1.80
S2	36	1590	37.4	1725	86.5	1.49
S3	36	1720	36.4	1600	87.4	1.40
S3			39.6	2300	77.8	1.79
S4	36	1760	37.4	1750	88.0	1.54
S5	39	1480	39.0	1480	83.8	1.24
S5			41.2	1690	78.9	1.33
S6	39	2110	40.0	2140	83.1	1.78
S6			43.0	2550	74.1	1.89
S6			45.0	2800	72.4	2.03
S7	39	2325	40.5	2245	89.9	2.02
S8	39	2460	39.4	2300	86.0	1.98
S8			40.6	2600	76.0	1.97
S9	40	1670	47.4	2850	77.6	2.21
S10	40	1930	41.7	1940	88.2	1.71
S10			44.0	2480	82.8	2.05
S10			45.8	2690	77.9	2.10
S11	40	2100	41.3	2160	82.1	1.77
S11			45.3	2840	87.8	2.49
S12	40	2160	44.6	2620	82.5	2.16
S13	40	2300	41.7	2520	79.0	1.99
S14	40	2600	41.3	2580	78.1	2.01
S15	41	2390	41.6	2420	78.4	1.90
S16	41	2550	42.0	2420	79.5	1.92
S17	42	2010	43.4	2270	84.1	1.85
S18	42	2220	44.3	2590	82.1	2.13

Table XIX 27 measurements of total body water (TBW) in 18 S.F.D. babies; in percent by weight and in litres.

		Fehling (1877) n = 21	Givens and Macy (1933) n = 25	Iob and Swanson (1934) n = 17	Widdowson and Spray (1951) and Widdowson and Dickerson (1964) n = 24
Total body water	I	92.0(89.8-97.5)	88.7(84.0-92.3)	93.0(88.7-95.4)	89.7(87.0-92.4)
	II	89.1(88.9-89.3)	86.1(79.0-88.6)	87.5(87.3-87.7)	88.5(87.9-89.3)
	III	83.9(82.6-86.4)	-	84.7(83.1-85.5)	87.9(87.5-88.7)
	IV	77.8(73.9-84.8)	81.8(77.2-84.9)	82.2(79.6-85.5)	81.3
	V	-	-	75.5	78.8(77.8-79.7)
	VI	74.1	-	-	69.2(58.5-72.6)
Total body fat	I	0.5(0.3-0.6)	-	0.6(0.5-0.7)	0.5(0.5-0.6)
	II	0.6(0.5-0.7)	-	0.7(0.7-0.8)	0.5(0.5-0.6)
	III	2.3(1.1-3.5)	-	1.6(1.2-2.2)	1.2(0.9-1.5)
	IV	5.4(2.4-8.7)	-	3.6(2.2-4.8)	4.2
	V	-	-	6.7	7.1(6.7-7.5)
	VI	9.1	-	-	16.1(11.0-28.3)
Total body protein	I	5.9(4.9-7.1)	-	5.0(3.6-7.4)	7.5(5.1-11.0)
	II	7.2(6.7-7.7)	-	8.0(7.8-8.3)	8.5(8.1-9.3)
	III	10.2(7.8-11.8)	-	9.7(9.3-10.6)	8.4(7.7-9.1)
	IV	13.2(9.1-17.8)	-	10.1(9.0-10.9)	11.8
	V	-	-	12.3	11.4(11.0-11.8)
	VI	11.8	-	-	11.9(10.6-13.8)

Table XX Total body water, fat and protein of foetuses and neonates measured by carcass analysis.
Results of 5 studies subdivided into 6 groups by weight (see table XXI).
Averages and ranges are given. All results in percent by weight.

Weight group	Fehling (1877) n = 21	Givens and Macy (1933) n = 25	Iob and Swanson (1934) n = 17	Widdowson and Spray (1951) and Widdowson and Dickerson (1964) n = 24
I 0- 249 g	8	16	5	7
II 250- 499 g	3	5	3	5
III 599- 999 g	6	0	3	3
IV 1000-1999 g	3	4	5	1
V 2000-2999 g	0	0	1	2
VI 3000 g and higher	1	0	0	6

Table XXI Carcass analysis from foetuses and neonates. Results of 5 studies. Weight group classification and the number analysed per weight group.

Body weight	3500 g
Total body water	75,1% of body weight
Total body fat	11,0% of body weight
Total body protein	11,4% of body weight

Table XXIIa Chemical composition of male "reference infant" at birth, according to Fomon (1966).

G.a. (wks)	B.wt. (g)	Per 100 g Body Weight		
		Water (g)	Protein (g)	Lipid (g)
24	690	88.6	8.8	0.1
25	770	87.8	9.0	0.7
26	880	86.8	9.2	1.5
27	1010	85.7	9.4	2.4
28	1160	84.6	9.6	3.3
29	1318	83.6	9.9	4.1
30	1480	82.6	10.1	4.9
31	1650	81.7	10.3	5.6
32	1830	80.7	10.6	6.3
33	2020	79.8	10.8	6.9
34	2230	79.0	11.0	7.5
35	2450	78.1	11.2	8.1
36	2690	77.3	11.4	8.7
37	2940	76.4	11.6	9.3
38	3160	75.6	11.8	9.9
39	3330	74.8	11.9	10.5
40	3450	74.0	12.0	11.2

Table XXIIb Chemical composition of the "reference fetus", obtained by calculation from data on carcass analyses of human foetuses. (Ziegler et al., 1976)

Postc. age (wks)	B.wt. (g)	TBW (g)	TBF (g)	TBP (g)
Fehling (1877) (n = 3)				
28	1117	946	26	102
36	1496	1105	76	266
36	1761	1305	153	222
Givens and Macy (1933) (n = 4)				
30.8	1107	929	-	-
30.8	1071	910	-	-
33.3	1060	861	-	-
33.7	1170	903	-	-
Iob and Swanson (1934) (n = 5)				
30.3	1010	863	22	91
32.5	1205	994	42	116
34.6	1555	1238	75	166
34.6	1545	1270	53	159
35.4	1615	1308	63	176
Widdowson and Spray (1951) Widdowson and Dickerson (1964) (n = 4)				
32	1966	1598	83	229
33	2295	1786	172	271
34	2652	2114	178	292
34	3090	2160	479	363

Table XXIII Figures for total body water (TBW), fat (TBF) and protein (TBP) measured by carcass analysis by a number of workers.

Pat.	G.a. (wks)	B.wt. (g)	At time of measurement			
			Postc. age (wks)	Wt. (g)	CBS % by Wt.	L
P1	28	970	29.4	960	36.6	0.35
P2	28	1360	35.0	2600	40.3	1.05
P3	29	1080	41.6	2710	44.9	1.22
P4	30	1070	36.3	2350	44.6	1.05
P5	30	1260	31.0	1155	43.7	0.50
P5			39.8	2600	43.9	1.14
P6	30	1450	36.3	2390	44.6	1.07
P7	30	1630	36.5	2690	36.9	0.99
P8	30	1690	31.0	1595	34.4	0.55
P8			39.8	2910	44.3	1.29
P9	31	1490	31.6	1370	43.8	0.60
P10	31	1755	36.8	2530	45.6	1.15
P11	32	1480	33.4	1520	45.0	0.68
P12	32	1500	34.1	1635	34.5	0.56
P13	32	1580	33.4	1700	46.3	0.79
P13			39.3	2680	35.3	0.95
P14	32	1610	38.6	2740	46.4	1.28
P15	32	2100	33.6	2225	39.6	0.88
P15			35.1	2620	34.9	0.91
P17	33	2090	35.1	2150	44.5	0.96
P18	34	1810	37.7	2410	44.2	1.07
P19	34	1840	34.1	1800	43.7	0.79
P19			39.1	2420	40.0	0.97
P19			41.1	2980	39.5	1.18
P20	34	1880	37.7	2420	48.5	1.17
P21	34	1990	36.8	2330	46.9	1.09
P22	34	2010	35.0	1905	43.7	0.83
P22			38.0	2350	39.0	0.92
P22			41.1	2910	36.8	1.07
P25	34	2500	37.3	2680	59.0	1.58
P26	35	2040	36.0	1870	50.3	0.94
P27	35	2200	39.0	2600	47.8	1.24

Pat.	G.a. (wks)	B.wt. (g)	At time of measurement		CBS	
			Postc. age (wks)	Wt. (g)	% by Wt.	L
P28	36	2100	38.4	2420	39.4	0.95
P29	36	2210	39.8	2420	51.2	1.24
P30	36	2380	39.3	2690	37.7	1.01
P31	36	2500	36.7	2450	48.5	1.19
P32	37	2380	39.4	2660	47.1	1.25
P33	37	2590	37.3	2430	45.4	1.10

Table XXIV 38 measurements of corrected bromide space (CBS) in 30 preterm babies.
(No measurement was done in patients P16, P23 and P24).

Pat.	G.a. (wks)	B.wt. (g)	At time of measurement		CBS	
			Postc. age (wks)	Wt. (g)	% by Wt.	L
S1	36	1220	42.1	2100	40.5	0.85
S2	36	1590	37.4	1725	50.2	0.87
S3	36	1720	36.4	1600	50.9	0.81
S3			39.6	2300	44.1	1.01
S4	36	1760	37.4	1750	50.1	0.88
S6	39	2110	40.0	2140	44.0	0.94
S6			43.0	2550	33.4	0.85
S6			45.0	2800	35.2	0.99
S7	39	2325	40.5	2245	44.4	1.00
S9	40	1670	47.4	2850	39.0	1.11
S10	40	1930	41.7	1940	43.9	0.85
S10			44.0	2480	28.3	0.70
S10			45.8	2690	29.8	0.80
S11	40	2100	41.3	2160	46.9	1.01
S11			45.3	2840	39.2	1.11
S12	40	2160	44.6	2620	40.4	1.06
S13	40	2300	41.7	2520	44.8	1.13
S14	40	2600	41.3	2580	49.6	1.28
S15	41	2390	41.6	2420	36.7	0.89
S16	41	2550	42.0	2420	59.3	1.44
S17	42	2010	43.4	2270	49.4	1.12
S18	42	2220	44.3	2590	44.5	1.15

Table XXV 22 measurements of corrected bromide space (CBS), as a measure of extracellular fluid volume (ECV) in 16 S.F.D. babies. (No measurement was done in patients S5 and S8).

Table XXVI

Pat.	G.a./B.wt. (wks) (g)	Postnatal age (days)	At time of measurement: Wt. (g)	Postc. age (wks)	TBF (g)	TBF % by weight
P1	28/ 970	9	960	29.3	91±7	9.5±0.8
		11	1000	29.6	103±6	10.3±0.5
		ave.	980	29.5	97±4	9.9±0.5
P2	28/1360	48	2560	34.9	277±18	10.8±0.7
		50	2620	35.1	370±28	14.1±1.1
		ave.	2590	35.0	323±17	12.5±0.7
P5	30/1260	67	2510	39.6	281±17	11.2±0.7
		70	2590	40.0	239±10	9.2±0.4
		ave.	2550	39.8	260±10	10.2±0.4
P7	30/1630	48	2710	36.9	291±20	10.7±0.7
		50	2750	37.1	313±23	11.4±0.8
		ave.	2730	37.0	302±15	11.1±0.5
P8	30/1690	8	1665	31.1	147±14	8.8±0.9
		68	2870	39.7	314±14	10.9±0.5
		75	3000	40.7	382±15	12.7±0.5
P9	31/1490	6	1530	31.9	84±8	5.5±0.5
		8	1395	32.1	90±8	6.5±0.5
		ave.	1460	32.0	87±6	6.0±0.4
P11	32/1480	7	1510	33.0	100±10	6.6±0.6
P12	32/1500	13	1610	33.9	84±11	5.2±0.7
		14	1610	34.0	102±16	6.3±1.0
		ave.	1610	34.0	93±10	5.8±0.6

Pat.	G.a./B.wt. (wks) (g)	Postnatal age (days)	At time of measurement Wt. (g)	Postc. age (wks)	TBF (g)	TBF % by weight
P13	32/1580	9	1710	33.3	139±5	8.1±0.3
		11	1720	33.6	139±13	8.1±0.8
		ave.	1715	33.5	139±7	8.1±0.4
		55	2740	39.9	325±14	11.9±0.6
P15	32/2100	9	2160	33.3	173±9	8.0±0.4
		12	2220	33.7	170±8	7.7±0.3
		ave.	2190	33.5	172±6	7.9±0.3
		19	2440	34.7	245±17	10.1±0.6
		24	2620	35.4	261±9	10.0±0.3
		25	2640	35.6	280±8	10.6±0.3
		ave.	2630	35.5	271±6	10.3±0.2
P19	34/1840	5	1700	34.7	80±8	4.7±0.5
		11	1770	35.6	161±12	9.1±0.7
		36	2420	39.1	315±13	13.0±0.6
		38	2540	39.4	364±12	14.3±0.5
		ave.	2480	39.3	340±9	13.7±0.4
		49	2950	41.0	380±11	12.9±0.3
		50	2980	41.1	368±12	12.4±0.4
		ave.	2965	41.1	374±8	12.7±0.3
P22	34/2010	5	1890	34.7	166±11	8.8±0.5
		6	1920	34.9	191±12	10.0±0.6
		ave.	1905	34.8	179±8	9.4±0.4
		27	2340	37.9	295±9	12.6±0.4
		29	2380	38.1	306±11	12.9±0.4
		ave.	2360	38.0	301±7	12.8±0.3
		51	2910	41.3	317±14	10.9±0.5
		52	2960	41.4	319±10	10.8±0.3
		ave.	2935	41.3	318±9	10.8±0.3

Pat.	G.a./B.wt. (wks) (g)	Postnatal age (days)	At time of measurement Wt. (g)	Postc. age (wks)	TBF (g)	TBF % by weight
P23	34/2300	21	2470	37.0	282±10	11.4±0.4
		22	2510	37.1	238±11	9.5±0.4
		24	2580	37.4	284±14	11.0±0.5
		24	2580	37.4	172±11	6.6±0.5
		ave.	2535	37.2	244±6	9.6±0.2
P26	35/2040	5	1890	35.7	121±11	6.4±0.6
		6	1870	35.9	128±10	6.9±0.5
		ave.	1880	35.8	125±7	6.7±0.4
P30	36/2380	15	2450	38.1	344±8	14.1±0.3
		22	2640	39.1	378±8	14.3±0.3

Table XXVI Total body fat (TBF), measured by the Xe-absorption method, in 15 preterm babies. Results in grams and percent by weight.
G.a. = gestational age (in weeks)
B.wt. = birth weight (in grams)
ave. = average value of two preceding values.

The weight and the percent by weight are given as the mean ± the 95% confidence limits the extrapolated Xe-desorption curve.

Table XXVII

Pat.	G.a./B.wt. (wks) (g)	Postnatal age (days)	At time of measurement Wt. (g)	Postc. age (wks)	TBF (g)	TBF % by weight
S1	36/1220	13	1440	37.9	57±7	4.0±0.5
		14	1425	38.0	63±7	4.4±0.5
		ave.	1432	38.0	60±5	4.2±0.4
		49	2050	43.0	215±8	10.5±0.4
		72	2500	46.3	149±11	5.9±0.5
		73	2510	46.4	129±13	5.2±0.4
		ave.	2505	46.4	139±9	5.6±0.3
S6	39/2110	4	2140	39.6	164±11	7.7±0.5
		7	2290	40.0	232±11	10.1±0.5
		25	2510	42.6	268±11	10.7±0.4
		26	2430	42.7	320±14	13.2±0.5
		ave.	2470	42.7	294±9	12.0±0.3
		43	2820	45.1	313±12	11.1±0.4
		44	2820	45.3	240±12	8.5±0.4
		ave.	2820	45.2	277±9	9.8±0.3
S7	39/2325	4	2170	39.6	96±8	4.4±0.4
		7	2210	40.0	68±11	3.1±0.5
S8	39/2460	2	2320	39.3	167±15	7.2±0.6
		3	2300	39.4	93±9	4.0±0.4
		ave.	2310	39.4	130±9	5.6±0.4
		10	2580	40.4	139±15	5.4±0.6
		11	2600	40.6	223±17	8.6±0.6
		ave.	2590	40.5	181±11	7.0±0.4

Pat.	G.a./B.wt. (wks) (g)	Postnatal age (days)	At time of measurement: Wt. (g)	Postc. age (wks)	TBF (g)	TBF % by weight
S10	40/1930	9	1890	41.3	131±4	6.9±0.3
		10	1920	41.4	108±7	5.6±0.4
		ave.	1905	41.5	120±4	6.3±0.3
		25	2340	43.4	159±8	6.8±0.3
		42	2690	46.0	261±11	9.7±0.4
		43	2720	46.1	298±7	11.0±0.2
		ave.	2705	46.1	280±7	10.4±0.2
S11	40/2100	15	2330	42.1	239±11	10.2±0.5
		20	2450	42.9	232±12	9.5±0.4
		37	2840	45.3	278±13	9.8±0.4
		38	2920	45.4	234±7	8.0±0.2
		ave.	2880	45.4	256±8	8.9±0.2
S15	41/2390	1	2300	41.1	245±11	10.7±0.4
		2	2300	41.3	208±15	9.0±0.7
		ave.	2300	41.2	227±9	9.9±0.4

Table XXVII Total body fat (TBF), measured by the Xe-absorption method, in 7 S.F.D. babies. Results in grams and percent by weight.
G.a. = gestational age (in weeks)
B.wt. = birth weight (in grams)
ave. = average value of two preceding values.

The weight and the percent by weight are given as the mean ± the 95% confidence limits of the extrapolated Xe-desorption curve.

Summary

This thesis describes a non-invasive, "in vivo" method for the measurement of total body fat in newborn babies. The method was tested in animal experiments and the results obtained were checked by carcass analysis. Thereafter the method was used on a number of low birth weight babies.

The questions which we hoped to answer in this study are introduced and formulated in chapter I.
Under normal conditions the human foetus grows very quickly in the last trimester of pregnancy (150-200 grams per week). This results in a birth weight at term of about 3500 g. (the P50 of the Kloosterman intra-uterine growth curve). It appears from the chemical analysis of foetuses of various ages given in the literature that there are clear changes in the relationship between the body compartments during this rapid growth. The total body water, expressed in percent by weight, decreases from about 86 to about 70% between the 26th and 40th week of gestation. During the same period, the total body protein increases from about 9 to about 12 percent by weight and the total body fat increases from about 1 to 12-16 percent by weight.

Low birth weight is defined, more or less arbitrarily, as a birth weight of 2500 g. or less. Low birth weight babies, on whom this study concentrates, can be divided into two groups:

- preterm babies born after a gestation of 28 to 37 weeks inclusive.
- small for dates (S.F.D.) babies in whom the intra-uterine growth is so retarded that their body weight is on or under the 2.3 percentile line of the intra-uterine growth

curve of Kloosterman (1969).

Preterm babies can be differentiated from S.F.D. babies clinically. Using the present fluid and feeding regimes it is generally possible to obtain a growth velocity in these children which compares with the intra-uterine growth curve. It is however not certain that the body composition of these babies when they have reached an "at term" post-conceptional age is similar to that of a normal baby born at term. In order to be able to establish whether this is so, it is important to know the body composition at various times during treatment. This results in the following questions which form the basis for the research presented in this thesis:

- are there differences in the body composition between preterm and S.F.D. babies of similar body weight?
- how does the body composition of these babies change during growth in the first few weeks after birth?
- are there differences in body composition between preterm and S.F.D. babies when they have reached a post-conceptional age of 40 weeks?

A brief summary of the methods used in the literature for the measurement of body composition is given in chapter II. Some of the "in vivo" methods described are less suitable for young babies either because they give practical problems (e.g. densitometry and creatinine excretion) or because they are taxing for the patient (e.g. some dilution methods) or because the disadvantage of radiation is inherent (e.g. the radiographic methods and the neutron activation method). Experience with K^{40} measurement in babies is still very limited and requires a considerable investment.

The experimental methods which we used are described in chapter III.

The measurement of total body fat was done by the gas-

absorption method using Xenon in low concentrations. The baby being investigated is put for a given time into a closed incubator containing a Xenon-air mixture. The gas is taken up in the body via the lungs. A detailed description is given of the apparatus (figs. 2 and 3) and the procedure. Accurate measurement of the amount of Xenon absorbed is not easily performed because the amount involved is so small. However, by following Xenon desorption by repeated gas-chromatographic analysis, during a consecutive desorption phase, it is possible to obtain a Xenon washout curve. After the application of various corrections a Xenon desorption curve is obtained which must be extrapolated. To do this a method was used, which has not previously been described, in which the values for Xenon and time are expressed as reciprocals. After 40 minutes desorption this gives a straight line.
In the final calculation of the amount of Xenon absorbed, the fact that complete saturation of the organism is not achieved during the limited absorption time must be taken into account. A mathematically definable relationship between the duration of absorption and the amount of Xenon absorbed (the "asymmetry factor") could be shown in the animal studies but was not found in the studies on patients. For practical reasons therefore, the asymmetry factor from the animal experiments was used in the calculations in patients. The final formula for the calculation of the fat volume is explained .

There follows a description of the measurement of total body water by the deuterium oxide (D_2O) dilution method and of the extracellular volume by the Bromide dilution method. The total amount of body protein could not be measured in the patients, so figures were taken from the literature on carcass analysis.
The total body fat was calculated in a few patients using the anthropometric method described by Dauncey et al. (1977).
A number of guinea-pig experiments were done using the

Xenon-absorption method followed by carcass analysis.

A description of the patients in whom total body fat was measured (figs. 14 to 21, tables XI to XVI) is given in chapter IV. A review of the various aspects of their treatment is also given.

The results of the study are given and discussed in chapter V.
Reasonable agreement was found between the results of the Xenon absorption method and those of carcass analysis in the animal experiments. However, the numbers were too small to allow statistical analysis.
Total body water (TBW) was measured in 33 preterm babies and in 18 S.F.D. babies (tables XVIII and XIX, figs. 22, 23 and 24). There appears to be little difference in the TBW between the two groups of patients. The results are in agreement with those from carcass analysis given in the literature.
The extracellular volume (ECV) was measured in 30 preterm and 16 S.F.D. babies (tables XXIV and XXV, figs. 26 and 27). The large scatter in measurement results, especially in the S.F.D. babies, can probably be ascribed to the heterogeneity of this group.
Total body fat (TBF) was measured in 15 preterm and 7 S.F.D. babies (tables XXVI and XXVII, figs. 29 to 35). Preterm babies have only slightly more TBF than S.F.D. babies of a similar weight. However, preterm babies show a more obvious increase in the TBF in the first few post-natal weeks than S.F.D. babies even though the increase in body weight is virtually identical in each group (figs. 14 and 15). Preterm babies attain a fat content of 12-13% by weight by the time that they reach an "at term" post-conceptional age. This agrees with figures given in the literature. S.F.D. babies, however, only achieve 5-8% fat by weight at this age. The difference in body composition

between preterm and S.F.D. babies would seem therefore to lie mainly in the amount of TBF, while the fat free mass is virtually identical in both groups.
The amount of TBF measured by Xenon absorption was compared with the results of the anthropometric method of Dauncey et al. (1977) in a few patients. However, the number of measurements by the latter technique was limited (table XXVIII).
Further study of total body fat in young babies will take place using both the Xenon absorption method and the anthropometric method of Dauncey. Hereby longitudinal studies on the influence of various feeding regimes on the development of the fat compartment and also on the influence of early versus late intra-uterine growth retardation in S.F.D. babies will be undertaken.

Samenvatting

Dit proefschrift bevat de beschrijving van een niet invasieve in-vivo methode ter bepaling van het totale lichaamsvet bij pasgeborenen. Deze methode werd in het dierexperiment beproefd en de verkregen resultaten werden aan de gegevens van de karkasanalyse getoetst. Voorts werden bij een aantal kinderen met laag geboortegewicht metingen uitgevoerd.

In hoofdstuk I worden de vragen, die aan dit onderzoek ten grondslag liggen, ingeleid en geformuleerd.
Onder normale omstandigheden groeit de menselijke foetus in het laatste trimester van de zwangerschap zeer snel, namelijk 150-200 gram per week, hetgeen op de à terme leeftijd resulteert in een geboortegewicht van ongeveer 3500 gr (P50 van de intra-uteriene groeicurve volgens Kloosterman). Uit de literatuur betreffende chemische analyse van foetussen van verschillende leeftijden blijkt, dat tijdens deze snelle foetale groei beduidende verschuivingen optreden in de verhouding tussen verschillende lichaamscompartimenten. Zo neemt in de periode van de 26e tot de 40e zwangerschapsweek het totale lichaamswater af van ongeveer 86 tot ongeveer 70 gewichtsprocent; in dezelfde periode neemt het totale lichaamseiwit toe van ongeveer 9 tot ongeveer 12 gewichtsprocent en het totale lichaamsvet van ongeveer 1 tot 12-16 gewichtsprocent.

Men spreekt, min of meer arbitrair, van een laag geboortegewicht wanneer het geboortegewicht 2500 gr of minder bedraagt. Kinderen met een laag geboortegewicht, waarop deze studie zich in het bijzonder richt, zijn in twee groepen onder te verdelen:

- preterm geborenen, waaronder men verstaat kinderen geboren na een zwangerschap van 28 t/m 37 weken;
- "small-for-dates" (S.F.D.) kinderen, waaronder men verstaat kinderen, bij wie de intra-uteriene groei zodanig is vertraagd, dat bij de geboorte het lichaamsgewicht zich op of onder de 2.3 percentiellijn van de intra-uteriene groeicurve voor gewicht (Kloosterman, 1969) bevindt.

Preterm en S.F.D. geborenen zijn op grond van het klinisch aspect goed van elkaar te onderscheiden. Hoewel het over het algemeen met het huidige vocht- en voedingsregime goed gelukt bij deze kinderen een met de intra-uteriene groeicurve overeenkomende groeisnelheid te verkrijgen, is het niet zeker of zo tevens op de à terme datum een lichaamssamenstelling wordt bereikt, die overeenkomt met die van een normale à terme geborene. Teneinde dit te kunnen beoordelen, is kennis van de lichaamssamenstelling op verschillende tijdstippen tijdens behandeling van belang. Dit resulteert in de volgende vragen, die de basis vormen van het in dit proefschrift beschreven onderzoek:

- zijn er verschillen in lichaamssamenstelling tussen preterm en S.F.D. geborenen van vergelijkbaar lichaamsgewicht?
- hoe wijzigt zich de lichaamssamenstelling van deze kinderen gedurende de groei in de eerste weken na de geboorte?
- zijn er verschillen in lichaamssamenstelling tussen preterm en S.F.D.-geborenen wanneer zij de postconceptionele leeftijd van 40 weken hebben bereikt?

In hoofdstuk II wordt een beknopt overzicht gegeven van de literatuur betreffende methoden van onderzoek naar de lichaamssamenstelling. Van de "in-vivo" methoden die worden besproken, zijn sommigen voor de jonge zuigeling minder geschikt omdat zij praktische problemen opleveren (zoals de densitometrie en de creatinine excretie), belastend zijn voor de patiënt (sommige dilutiemethoden) of omdat zij een stralingsrisico inhouden (zoals de röntgenographie en de

neutron activatie methode). De ervaring met K^{40}-meting bij jonge zuigelingen is nog zeer beperkt, en vereist een beduidende investering.

De door ons gebruikte methoden van onderzoek worden in hoofdstuk III beschreven.

Voor de bepaling van het totale lichaamsvet werd gebruik gemaakt van de gas-absorptie methode met behulp van Xenon in lage concentratie. De te onderzoeken baby wordt in een gesloten incubator gebracht, waarin zich een Xenon-lucht mengsel bevindt en gedurende bepaalde tijd wordt via de luchtwegen het gas in het lichaam opgenomen. Een gedetailleerde beschrijving van de gebruikte apparatuur (fig. 2 en 3) en de gang van zaken bij de meting wordt gegeven. Daar de geabsorbeerde hoeveelheid Xenon slechts zeer gering is, is nauwkeurige meting van de geabsorbeerde hoeveelheid gas niet goed mogelijk. Daarom is een tweede, aansluitende desorptie-fase nodig, tijdens welke door middel van frequente gaschromatographische metingen een Xenon-uitwascurve kan worden verkregen. Na toepassing van verschillende correcties volgt extrapolatie van de desorptie curve. Hierbij wordt een nog niet eerder beschreven methode gevolgd, volgens welke de waarden van Xenon en tijd worden omgezet in de reciproke waarden, waarna, voor een desorptietijd van meer dan 40 min., een rechte lijn resulteert. Bij de uiteindelijke berekening van de geabsorbeerde hoeveelheid Xenon moet voorts rekening worden gehouden met het feit, dat tijdens een eindige absorptieduur geen volledige verzadiging van het organisme wordt verkregen. Een mathematisch goed definieerbare relatie tussen absorptieduur en geabsorbeerde hoeveelheid Xenon ("asymmetrie-factor"), werd in het dierexperiment wel, bij de patiënten niet gevonden. Om praktische redenen werd de bij het dierexperiment gevonden asymmetrie-factor ook bij de patiëntenmetingen gebruikt. De uiteindelijke formule voor de berekening van het vetvolume wordt toegelicht.

Vervolgens wordt een beschrijving gegeven van de meting van het totale lichaamswater door middel van de dilutie-methode met behulp van deuterium-oxyde (D_2O) en van de meting van het extracellulaire volume door middel van de broom-dilutie methode. De totale hoeveelheid lichaamseiwit kon bij de patiënten niet worden bepaald doch werd afgeleid uit de literatuurgegevens van karkasanalyse.
Bij enkele patiënten werd de hoeveelheid lichaamsvet berekend volgens de anthropometrische methode van Dauncey et al. (1977).
Een aantal dierexperimenten met caviae werd verricht volgens de Xenon-absorptiemethode, gevolgd door karkasanalyse.

In hoofdstuk IV wordt een beschrijving gegeven van de patiënten, bij welke het totale lichaamsvet werd gemeten (fig. 14 t/m 21, tabel XI t/m XVI). Tevens wordt een overzicht gegeven van de verschillende aspecten van behandeling.

In hoofdstuk V worden de resultaten van het onderzoek medegedeeld en besproken.
In de dierexperimenten werd een redelijke overeenstemming gevonden tussen Xe-absorptie-methode en karkasanalyse. Het aantal dierproeven was echter te gering voor statistische analyse.
Totaal lichaamswater (TBW) werd gemeten bij 33 preterm geborenen en 18 S.F.D. geborenen (tabel XVIII en XIX, fig. 22, 23 en 24). Er blijkt weinig verschil in TBW te bestaan tussen de beide groepen patiënten. De uitkomsten stemmen goed overeen met de literatuur-gegevens van de karkasanalyse.
Het extracellulaire volume (ECV) werd gemeten bij 30 preterm geborenen en 16 S.F.D. geborenen (tabel XXIV en XXV, fig. 26 en 27). De grote spreiding in de meetresultaten, vooral bij de S.F.D. geborenen is waarschijnlijk toe te schrijven aan de heterogeniteit van deze groep.

Totaal lichaamsvet (TBF) werd gemeten bij 15 preterm geborenen en 7 S.F.D. geborenen (tabel XXVI en XXVII, fig. 29 t/m 35). Preterm geborenen bezitten slechts weinig meer TBF dan S.F.D. geborenen van vergelijkbaar gewicht. Preterm geborenen tonen gedurende de eerste postnatale weken een duidelijker toename van het TBF dan S.F.D.-geborenen, terwijl toch de toename van het totaalgewicht in beide groepen vrijwel gelijk is (fig. 14 en 15). Preterm geborenen bereiken op de à terme leeftijd vetpercentages van 12-13 gew. %, overeenkomstig de literatuur, terwijl S.F.D. geborenen slechts 5-8% bereiken. Het verschil tussen preterm geborenen en S.F.D.-geborenen lijkt dus voornamelijk te liggen in de hoeveelheid TBF, terwijl de vetvrije massa bij beide groepen vrijwel gelijk is.
Bij enkele patiënten werd het totale lichaamsvet bepaald door middel van Xe-absorptie vergeleken met de resultaten van de anthropometrische methode volgens Dauncey et al. (1977). Het aantal metingen volgens deze techniek is echter beperkt (tabel XXVIII).
Verdere studie van het totale lichaamsvet bij jonge zuigelingen, zowel met de Xenon-absorptie-methode als met de anthropometrische methode volgens Dauncey, zal plaatsvinden, waarbij enerzijds de invloed van verschillende voedingsregimes op de ontwikkeling van het vetcompartiment zal worden bestudeerd, terwijl anderzijds door middel van longitudinaal onderzoek van S.F.D. geborenen de invloed van vroege versus late intra-uteriene groeiretardatie zal worden bestudeerd.

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Appendix

Calculator programs

All calculations were performed on an Hewlett Packard Calculator type 9810 A, equipped with a statistics ROM and a plotter-control ROM.

Complete print-outs and operating instructions of the program for the following calculations are given:

- Correction for sampling (Ch. III.1.4.1)
- Correction for clearing phase (Ch. III.1.4.2)
- Extrapolation of the desorption curve (Ch. III.1.6.1)
- Calculation of total body fat (Ch. III.1.6.3)

Correction for sampling

Instructions:

1. Press: clear, run, end, FMT, goto
2. Insert program card
3. Enter: factor δ
4. Press: x $\rightarrow$ 002
5. Enter: any factor by which the Xe values should be divided if they are too large to be handled conveniently in their original form.
   If no reduction is required enter: 1.
6. Press: x $\rightarrow$ 004
7. Press: clear, end, continue
8. Enter: t_1 in y
   Xe_1 in x
9. Press: continue
   print-out: in z : t_1
   in y : Xe_1 uncorrected
   in x : Xe_1 corrected

Repeat steps 8 and 9 for all sets of data (t_i, Xe_i).

Print-out:

```
0000--CLR---20    0016-- X ---36    0032--PNT---45
0001--XTO---23    0017-- 2 ---02    0033--PNT---45
0002-- 1 ---01    0018--XTO---23    0034--XFR---67
0003--XTO---23    0019-- 1 ---01    0035-- - ---34
0004-- 3 ---03    0020--CLR---20    0036-- 1 ---01
0005--STP---41    0021--STP---41    0037--XFR---67
0006--XEY---30    0022--XEY---30    0038-- X ---36
0007--PNT---45    0023--PNT---45    0039-- 2 ---02
0008--XEY---30    0024--XEY---30    0040--XTO---23
0009--PNT---45    0025--PNT---45    0041-- + ---33
0010--XFR---67    0026--XFR---67    0042-- 1 ---01
0011--DIV---35    0027--DIV---35    0043--GTO---44
0012-- 4 ---04    0028-- 4 ---04    0044-- 2 ---02
0013--PNT---45    0029--XFR---67    0045-- 0 ---00
0014--PNT---45    0030-- + ---33    0046--END---46
0015--XFR---67    0031-- 1 ---01
```

Correction for clearing phase
(no plotted control block needed)

Instructions:

1. Press: clear, run, end, FMT, goto
2. Insert program card
3. Press: clear, end, continue
4. Enter: x max. (usually: 30)
5. Press: continue
6. Enter: x min. (usually: -2)
7. Press: continue
8. Enter: y max.
9. Press: continue
10. Enter: y min.
11. Press: continue
12. Enter: scale factor
13. Press: continue X- and Y-axis are drawn
14. Enter: Xe_1 in y
    t_i in x
15. Press: continue
    Repeat steps 14 and 15 until all data
    (Xe_i, t_i) have been entered
16. Press: set flag, continue
    print-out: b_o, b_1, b_2 and r
17. Enter: interval between absorption and desorption
    in minutes (usually: 2)
18. Press: continue
    print-out: correction value
    curve is drawn.

Correction for clearing phase

Print-out:

```
0000--CLR---20
0001--FMT---42
0002--FMT---42
0003--  E  ---60
0004--  N  ---73
0005--XTO---23
0006--  E  ---60
0007--  a  ---13
0008--CNT---47
0009--  YE---24
0010--CNT---47
0011--  M  ---70
0012--  A  ---62
0013--  YE---24
0014--  .  ---21
0015--CLR---20
0016--CLR---20
0017--FMT---42
0018--STP---41
0019--PNT---45
0020--PNT---45
0021--XTO---23
0022--  5  ---05
0023--  0  ---00
0024--CLX---37
0025--FMT---42
0026--FMT---42
0027--  E  ---60
0028--  N  ---73
0029--XTO---23
0030--  E  ---60
0031--  a  ---13
0032--CNT---47
0033--  YE---24
0034--CNT---47
0035--  M  ---70
0036--  I  ---65
0037--  N  ---73
0038--  .  ---21
0039--CLR---20
0040--CLR---20
0041--FMT---42
0042--STP---41
0043--PNT---45
0044--PNT---45
0045--XTO---23
0046--  5  ---05
0047--  1  ---01
0048--CLX---37
0049--FMT---42
0050--FMT---42
0051--  E  ---60
0052--  N  ---73
0053--XTO---23
0054--  E  ---60
0055--  a  ---13
0056--CNT---47
0057--XFR---67
0058--CNT---47
0059--  M  ---70
0060--  A  ---62
0061--  YE---24
0062--  .  ---21
0063--CLR---20
0064--CLR---20
0065--FMT---42
0066--STP---41
0067--PNT---45
0068--PNT---45
0069--XTO---23
0070--  5  ---05
0071--  4  ---04
0072--CLX---37
0073--FMT---42
0074--FMT---42
0075--  E  ---60
0076--  N  ---73
0077--XTO---23
0078--  E  ---60
0079--  a  ---13
0080--CNT---47
0081--XFR---67
0082--CNT---47
0083--  M  ---70
0084--  I  ---65
0085--  N  ---73
0086--  .  ---21
0087--CLR---20
0088--CLR---20
0089--FMT---42
0090--STP---41
0091--PNT---45
0092--PNT---45
0093--XTO---23
0094--  5  ---05
0095--  5  ---05
0096--XFR---67
0097--  5  ---05
0098--  0  ---00
0099--XFR---67
0100--  -  ---34
0101--  5  ---05
0102--  1  ---01
0103--XTO---23
0104--  5  ---05
0105--  2  ---02
0106--CLX---37
0107--FMT---42
0108--FMT---42
0109--  E  ---60
0110--  N  ---73
0111--XTO---23
0112--  E  ---60
0113--  a  ---13
0114--CNT---47
0115--YTO---40
0116--  C  ---61
0117--  A  ---62
0118--  L  ---72
0119--  E  ---60
0120--CLR---20
0121--  F  ---16
0122--  A  ---62
0123--  C  ---61
0124--XTO---23
0125--  Ü  ---71
0126--  a  ---13
0127--CLR---20
0128--CLR---20
0129--FMT---42
0130--STP---41
0131--PNT---45
0132--PNT---45
0133--  UP---27
0134--  9  ---11
0135--  9  ---11
0136--  9  ---11
0137--  9  ---11
0138--XEY---30
0139--DIV---35
0140--YTO---40
0141--  5  ---05
0142--  3  ---03
0143--  YE---24
```

```
0144--DIV---35
0145--  5 ---05
0146--  2 ---02
0147--YTO---40
0148--  5 ---05
0149--  2 ---02
0150--  DN--25
0151--XFR---67
0152--  5 ---05
0153--  4 ---04
0154--XFR---67
0155--  - ---34
0156--  5 ---05
0157--  5 ---05
0158--XFR---67
0159--DIV---35
0160--  5 ---05
0161--  3 ---03
0162--1/X---17
0163--XTO---23
0164--  5 ---05
0165--  6 ---06
0166--CLX---37
0167--FMT---42
0168--  UP--27
0169--XFR---67
0170--  5 ---05
0171--  3 ---03
0172--FMT---42
0173--  DN--25
0174--RUP---22
0175--RUP---22
0176--FMT---42
0177--  UP--27
0178--  DN--25
0179--FMT---42
0180--  DN--25
0181--  UP--27
0182--  UP--27
0183--FMT---42
0184--  UP--27
0185--CLR---20
0186--  K ---55
0187--  3 ---03
0188--  A ---62
0189--  0 ---71
0190--FMT---42
0191--FMT---42
0192--  E ---60
0193--  N ---73
0194--XTO---23
0195--  E ---60
0196--  a ---13
0197--CNT---47
0198--  YE--24
0199--  E ---60
0200--CNT---47
0201--  A ---62
0202--  N ---73
0203--  D ---63
0204--CNT---47
0205--XTO---23
0206--  . ---21
0207--FMT---42
0208--STP---41
0209--IFG---43
0210--  0 ---00
0211--  3 ---03
0212--  0 ---00
0213--  5 ---05
0214--XTO---23
0215--  5 ---05
0216--  0 ---10
0217--YTO---40
0218--  5 ---05
0219--  9 ---11
0220--XFR---67
0221--  - ---34
0222--  5 ---05
0223--  1 ---01
0224--XFR---67
0225--  X ---36
0226--  5 ---05
0227--  2 ---02
0228--  YE--24
0229--  - ---34
0230--  5 ---05
0231--  5 ---05
0232--  YE--24
0233--  X ---36
0234--  5 ---05
0235--  6 ---06
0236--RUP---22
0237--  2 ---02
0238--  0 ---00
0239--XTO---23
0240--  5 ---05
0241--  7 ---07
0242--  - ---34
0243--  DN--25
0244--FMT---42
0245--  UP--27
0246--FMT---42
0247--  DN--25
0248--XFR---67
0249--  + ---33
0250--  5 ---05
0251--  7 ---07
0252--XFR---67
0253--  + ---33
0254--  5 ---05
0255--  7 ---07
0256--FMT---42
0257--  DN--25
0258--XFR---67
0259--  - ---34
0260--  5 ---05
0261--  7 ---07
0262--FMT---42
0263--  DN--25
0264--  YE--24
0265--  - ---34
0266--  5 ---05
0267--  7 ---07
0268--FMT---42
0269--  DN--25
0270--  YE--24
0271--  + ---33
0272--  5 ---05
0273--  7 ---07
0274--  YE--24
0275--  + ---33
0276--  5 ---05
0277--  7 ---07
0278--FMT---42
0279--  DN--25
0280--FMT---42
0281--  UP--27
0282--CLX---37
0283--  UP--27
0284--  UP--27
0285--XFR---67
0286--  5 ---05
0287--  9 ---11
```

Correction for clearing phase

Print-out: (cont.)

```
0288--  UP---27
0289--XFR---67
0290--  5  --05
0291--  8  --10
0292--  UP---27
0293--XSQ---12
0294--XEY---30
0295--  0  --71
0296--CLX---37
0297--  UP---27
0298--  UP---27
0299--  1  --01
0300--GTO---44
0301--  0  --00
0302--  2  --02
0303--  0  --00
0304--  8  --10
0305--  E  --60
0306--FMT---42
0307--FMT---42
0308--  A  --62
0309--  0  --00
0310--FMT---42
0311--RUP---22
0312--PNT---45
0313--FMT---42
0314--FMT---42
0315--  A  --62
0316--  1  --01
0317--FMT---42
0318--  DN---25
0319--PNT---45
0320--FMT---42
0321--FMT---42
0322--  A  --62
0323--  2  --02
0324--FMT---42
0325--  DN---25
0326--PNT---45
0327--PNT---45
0328--FMT---42
0329--FMT---42
0330--  C  --61
0331--  O  --71
0332--  a  --13
0333--  a  --13
0334--  ,  --21
0335--CNT---47
0336--  C  --61
0337--  O  --71
0338--  E  --60
0339--  F  --16
0340--  F  --16
0341--  .  --21
0342--FMT---42
0343--  B  --66
0344--  r  --76
0345--PNT---45
0346--PNT---45
0347--FMT---42
0348--FMT---42
0349--  E  --60
0350--  N  --73
0351--XTO---23
0352--  E  --60
0353--  a  --13
0354--CNT---47
0355--XTO---23
0356--  I  --65
0357--  M  --70
0358--  E  --60
0359--CLR---20
0360--  I  --65
0361--  N  --73
0362--XTO---23
0363--  E  --60
0364--  a  --13
0365--INT---64
0366--  A  --62
0367--  L  --72
0368--CNT---47
0369--  B  --66
0370--  E  --60
0371--XTO---23
0372--IND---31
0373--  E  --60
0374--  E  --60
0375--  N  --73
0376--CNT---47
0377--  A  --62
0378--  B  --66
0379--YTO---40
0380--  .  --21
0381--CNT---47
0382--  A  --62
0383--  N  --73
0384--  D  --63
0385--CNT---47
0386--  D  --63
0387--  E  --60
0388--YTO---40
0389--  .  --21
0390--FNT---42
0391--CLX---37
0392--  UP---27
0393--  UP---27
0394--STP---41
0395--PNT---45
0396--PNT---45
0397--XSQ---12
0398--  r  --76
0399--CHS---32
0400--XTO---23
0401--  9  --11
0402--  0  --00
0403--  E  --60
0404--XFR---67
0405--  X  --36
0406--  9  --11
0407--  0  --00
0408--  YE---24
0409--  X  --36
0410--  9  --11
0411--  0  --00
0412--  YE---24
0413--  X  --36
0414--  9  --11
0415--  0  --00
0416--  +  --33
0417--  DN---25
0418--  +  --33
0419--  DN---25
0420--PNT---45
0421--PNT---45
0422--  E  --60
0423--XTO---23
0424--  1  --01
0425--YTO---40
0426--  2  --02
0427--RUP---22
0428--XTO---23
0429--  3  --03
0430--CLR---20
0431--FNT---42
```

Correction for clearing phase

Print-out: (cont.)

```
0432-- UP---27
0433--XFR---67
0434-- 5 ---05
0435-- 1 ---01
0436--XTO---23
0437-- 7 ---07
0438-- 5 ---05
0439--XFR---67
0440-- 3 ---03
0441--XTO---23
0442-- 8 ---10
0443-- 0 ---00
0444--XFR---67
0445-- 7 ---07
0446-- 5 ---05
0447--XFR---67
0448-- X ---36
0449-- 1 ---01
0450--XTO---23
0451-- + ---33
0452-- 8 ---10
0453-- 0 ---00
0454--XFR---67
0455-- 7 ---07
0456-- 5 ---05
0457--XSQ---12
0458--XFR---67
0459-- X ---36
0460-- 2 ---02
0461--XTO---23
0462-- + ---33
0463-- 8 ---10
0464-- 0 ---00
0465--CLX---37
0466-- UP---27
0467-- YE---24
0468-- + ---33
0469-- 8 ---10
0470-- 0 ---00
0471-- YE---24
0472-- - ---34
0473-- 5 ---05
0474-- 5 ---05
0475-- YE---24
0476-- X ---36
0477-- 5 ---05
0478-- 6 ---06
0479--XFR---67
0480-- 7 ---07
0481-- 5 ---05
0482--XFR---67
0483-- - ---34
0484-- 5 ---05
0485-- 1 ---01
0486--XFR---67
0487-- X ---36
0488-- 5 ---05
0489-- 2 ---02
0490--FMT---42
0491-- DN---25
0492--XFR---67
0493-- 7 ---07
0494-- 5 ---05
0495-- UP---27
0496--XFR---67
0497-- 5 ---05
0498-- 0 ---00
0499--X>Y---53
0500-- 0 ---00
0501-- 5 ---05
0502-- 1 ---01
0503-- 0 ---00
0504--CLX---37
0505-- UP---27
0506--FMT---42
0507-- UP---27
0508--GTO---44
0509-- 0 ---00
0510-- . ---21
0511-- 1 ---01
0512--XTO---23
0513-- + ---33
0514-- 7 ---07
0515-- 5 ---05
0516--CLX---37
0517-- UP---27
0518-- UP---27
0519--XTO---23
0520-- 8 ---10
0521-- 0 ---00
0522--GTO---44
0523-- 0 ---00
0524-- 4 ---04
0525-- 3 ---03
0526-- 9 ---11
0527--END---46
```

Extrapolation of desorption curve

1. Press: clear, run, end, FMT, goto
2. Insert program card
3. Insert punched tape (data: Xe, t) into tape reader
4. Press: end, continue

   When tape has run through:
5. Press: set flag, continue

   print-out: total S.S.
6. Press: regresssion
7. Press: $y^{(a)} \rightarrow 030$

   $x^{(b)} \rightarrow 031$
5. Insert tape
6. Press: goto 0080, continue

   When tape has run through:
7. Press: goto 0135, continue

   $(a-a_o)$ and $(b-b_o)$ of the Taylor-series appear
8. Press: continue:

   new a and b appear

   Repeat steps 5-8 : 3 or 4 times
9. Insert tape
10. Press: goto 0250

    When tape has run through
11. Press: continue
12. Enter: total S.S.
13. Press: continue

    print-out: r

Extrapolation of desorption curve

Print-out:

```
0000--CLR---20
0001--  K  ---55
0002--  3  ---03
0003--  A  ---62
0004--  0  ---71
0005--  K  ---55
0006--  2  ---02
0007--FMT---42
0008--CNT---47
0009--IFG---43
0010--  0  ---00
0011--  0  ---00
0012--  4  ---04
0013--  5  ---05
0014--  UP---27
0015--FMT---42
0016--CNT---47
0017--  UP---27
0018--  3  ---03
0019--  8  ---10
0020--X<Y---52
0021--  0  ---00
0022--  0  ---00
0023--  2  ---02
0024--  8  ---10
0025--CLR---20
0026--GTO---44
0027--  7  ---07
0028--  DN---25
0029--YTO---40
0030--  +  ---33
0031--  6  ---06
0032--RUP---22
0033--RUP---22
0034--XSQ---12
0035--XTO---23
0036--  +  ---33
0037--  7  ---07
0038--DIV---35
0039--  DN---25
0040--XEY---30
0041--1/X---17
0042--  0  ---71
0043--GTO---44
0044--  7  ---07
0045--FMT---42
0046--FMT---42
0047--XTO---23
0048--  0  ---71
0049--XTO---23
0050--  A  ---62
0051--  L  ---72
0052--CNT---47
0053--YTO---40
0054--YTO---40
0055--CLR---20
0056--CLR---20
0057--CLR---20
0058--FMT---42
0059--XFR---67
0060--  7  ---07
0061--  UP---27
0062--XFR---67
0063--  6  ---06
0064--XSQ---12
0065--XFR---67
0066--DIV---35
0067--  0  ---00
0068--  -  ---34
0069--  DN---25
0070--PNT---45
0071--PNT---45
0072--STP---41

0080--CLR---20
0081--  K  ---55
0082--  3  ---03
0083--  A  ---62
0084--  0  ---71
0085--FMT---42
0086--CNT---47
0087--  UP---27
0088--FMT---42
0089--CNT---47
0090--  UP---27
0091--  3  ---03
0092--  8  ---10
0093--X<Y---52
0094--  0  ---00
0095--  1  ---01
0096--  0  ---00
0097--  1  ---01
0098--GTO---44
0099--  8  ---10
0100--  5  ---05
0101--  DN---25
0102--  UP---27
0103--1/X---17
0104--XFR---67
0105--  X  ---36
0106--  3  ---03
0107--  1  ---01
0108--XFR---67
0109--  +  ---33
0110--  3  ---03
0111--  0  ---00
0112--1/X---17
0113--XTO---23
0114--  α  ---13
0115--CHS---32
0116--RUP---22
0117--  +  ---33
0118--  α  ---13
0119--XSQ---12
0120--RUP---22
0121--CHS---32
0122--1/X---17
0123--  X  ---36
0124--  α  ---13
0125--XSQ---12
0126--CHS---32
0127--  0  ---71
0128--CLR---20
0129--GTO---44
0130--  8  ---10
0131--  5  ---05
0132--CNT---47
0133--STP---41
0134--  0  ---00
0135--CLR---20
0136--XFR---67
0137--  2  ---02
0138--XTO---23
0139--  2  ---02
0140--  0  ---00
0141--CNT---47
0142--XFR---67
0143--  4  ---04
```

Extrapolation of desorption curve

Print-out: (cont.)

```
0144--XTO---23
0145--  2  ---02
0146--  1  ---01
0147--XFR---67
0148--  5  ---05
0149--XTO---23
0150--  2  ---02
0151--  2  ---02
0152--XFR---67
0153--  7  ---07
0154--XTO---23
0155--  2  ---02
0156--  3  ---03
0157--XFR---67
0158--  9  ---10
0159--XTO---23
0160--  2  ---02
0161--  4  ---04
0162--CLR---20
0163--  K  ---55
0164--  2  ---02
0165--  A  ---62
0166--  0  ---71
0167--XFR---67
0168--  2  ---02
0169--  3  ---03
0170--XFR---67
0171--DIV---35
0172--  2  ---02
0173--  0  ---00
0174--  UP--27
0175--XFR---67
0176--  2  ---02
0177--  1  ---01
0178--XFR---67
0179--DIV---35
0180--  2  ---02
0181--  0  ---00
0182--  0  ---71
0183--CLR---20
0184--XFR---67
0185--  2  ---02
0186--  4  ---04
0187--XFR---67
0188--DIV---35
0189--  2  ---02
0190--  1  ---01
0191--  UP--27
0192--XFR---67
0193--  2  ---02
0194--  2  ---02
0195--XFR---67
0196--DIV---35
0197--  2  ---02
0198--  1  ---01
0199--  0  ---71
0200--  E  ---60
0201--STP---41
0202--  YE--24
0203--  +  ---33
0204--  3  ---03
0205--  0  ---00
0206--XFR---67
0207--  +  ---33
0208--  3  ---03
0209--  1  ---01
0210--YTO---40
0211--  3  ---03
0212--  0  ---00
0213--XTO---23
0214--  3  ---03
0215--  1  ---01
0216--STP---41
0217--  0  ---00
0218--  0  ---00
0219--  0  ---00
0220--  0  ---00
0221--  0  ---00
0222--  0  ---00
0223--  0  ---00
0224--  0  ---00
0225--  0  ---00
0226--  0  ---00
0227--  0  ---00
0228--  0  ---00
0229--  0  ---00
0230--  0  ---00
0231--  0  ---00
0232--  0  ---00
0233--  0  ---00
0234--  0  ---00
0235--  0  ---00
0236--  0  ---00
0237--  0  ---00
0238--  0  ---00
0239--  0  ---00
0240--  0  ---00
0241--  0  ---00
0242--  0  ---00
0243--  0  ---00
0244--  0  ---00
0245--  0  ---00
0246--  0  ---00
0247--  0  ---00
0248--  0  ---00
0249--  0  ---00
0250--CLR---20
0251--FMT---42
0252--CNT---47
0253--IFG---43
0254--  0  ---00
0255--  2  ---02
0256--  9  ---11
0257--  7  ---07
0258--  UP--27
0259--FMT---42
0260--CNT---47
0261--  UP--27
0262--  3  ---03
0263--  8  ---10
0264--X<Y---52
0265--  0  ---00
0266--  2  ---02
0267--  7  ---07
0268--  6  ---06
0269--CLX---37
0270--  UP--27
0271--  UP--27
0272--GTO---44
0273--  2  ---02
0274--  5  ---05
0275--  1  ---01
0276--  DN--25
0277--1/X---17
0278--XFR---67
0279--  X  ---36
0280--  3  ---03
0281--  1  ---01
0282--XFR---67
0283--  +  ---33
0284--  3  ---03
0285--  0  ---00
0286--1/X---17
0287--  -  ---34
```

Extrapolation of desorption curve

Print-out: (cont.)

```
0288-- DN---25
0289--XSQ---12
0290--XTO---23
0291-- + ---33
0292-- a ---13
0293--GTO---44
0294-- 2 ---02
0295-- 5 ---05
0296-- 1 ---01
0297--FMT---42
0298--FMT---42
0299-- E ---60
0300-- N ---73
0301--XTO---23
0302-- E ---60
0303-- a ---13
0304--CNT---47
0305--YTO---40
0306--YTO---40
0307--CNT---47
0308--XTO---23
0309-- 0 ---71
0310--XTO---23
0311-- . ---21
0312--CLR---20
0313--CLR---20
0314--FMT---42
0315--CLX---37
0316-- UP---27
0317-- UP---27
0318--STP---41
0319-- UP---27
0320-- a ---13
0321--DIV---35
0322-- 1 ---01
0323--XEY---30
0324--1/X---17
0325-- - ---34
0326-- DN---25
0327--FMT---42
0328--FMT---42
0329-- a ---13
0330--CLR---20
0331--CLR---20
0332--FMT---42
0333--FMT---42
0334-- 1 ---01
0335-- 8 ---10
0336-- 4 ---04
0337-- r ---76
0338--PNT---45
0339--PNT---45
0340--CLR---20
0341--STP---41
0342--END---46
```

Calculation of total body fat

1. Press: clear, run, end, FTM, goto
2. Insert program card.
   The quadratic interpolation for the calculation of the Bunsen absorption coefficients at t_i is included in this program. The constants C_2, C_1 and C_o for the calculation of α_f have to be entered; the constants for the calculation of α_p and α_w are present in the program.
3. Press: goto 0187 program
4. Enter: C_2 : human : 9945, EE, CHS, 8
   guinea-pig: 2103, EE, CHS, 7
5. Press: run, goto, 0198, program
6. Enter: C_1 : human : 2673, CHS, EE, CHS, 5
   guinea-pig: 3523, CHS, EE, CHS, 5
7. Press: run, goto, 0207, program
8. Enter: C_o : human : 2.475
   guinea-pig: 2.587
9. Press: run, clear, end, continue
10. Enter: t_i
11. Press: continue (t_i → reg. o)
12. Enter: t_s
13. Press: continue (t_s → reg. 1)
14. Enter: O_I
15. Press: continue (O_I → reg. 2)
16. Enter: O_{II}
17. Press: continue (O_{II} → reg. 3)
18. Enter: spurious absorption (S.A.)
    O_I^{sp}/O_{II}^{sp} as percentage x 10^{-2}
19. Press: continue (S.A. → reg. 4)
20. Enter: V_w
21. Press: continue (V_w → reg. 5)
22. Enter: V_p
23. Press: continue (V_p → reg. 6)

24. Enter: V_b
25. Press: continue (V_b → reg. 7)
26. Enter: V_s
27. Press: continue (V_s → reg. 8)
28. Enter: asymmetry factor
29. Press: continue (A.F. → reg. 9)
    Mistakes may be corrected here
30. Press: continue
    print-out: fat volume in ml.

Calculation of total body fat

Print-out:

```
0000--CLR---20
0001--FMT---42
0002--FMT---42
0003--XTO---23
0004--CNT---47
0005--  I  ---65
0006--  N  ---73
0007--XTO---23
0008--  .  ---21
0009--FMT---42
0010--STP---41
0011--XTO---23
0012--  0  ---00
0013--PNT---45
0014--PNT---45
0015--FMT---42
0016--FMT---42
0017--XTO---23
0018--CNT---47
0019--YTO---40
0020--XFR---67
0021--YTO---40
0022--XTO---23
0023--  .  ---21
0024--FMT---42
0025--CLR---20
0026--STP---41
0027--XTO---23
0028--  1  ---01
0029--PNT---45
0030--PNT---45
0031--FMT---42
0032--FMT---42
0033--  0  ---71
0034--  -  ---34
0035--  1  ---01
0036--FMT---42
0037--CLR---20
0038--STP---41
0039--XTO---23
0040--  2  ---02
0041--PNT---45
0042--PNT---45
0043--FMT---42
0044--FMT---42
0045--  0  ---71
0046--  -  ---34
0047--  2  ---02
0048--FMT---42
0049--CLR---20
0050--STP---41
0051--XTO---23
0052--  3  ---03
0053--PNT---45
0054--PNT---45
0055--FMT---42
0056--FMT---42
0057--YTO---40
0058--  .  ---21
0059--  A  ---62
0060--  .  ---21
0061--FMT---42
0062--FMT---42
0063--  1  ---01
0064--  8  ---10
0065--  5  ---05
0066--CLR---20
0067--STP---41
0068--XTO---23
0069--  4  ---04
0070--PNT---45
0071--PNT---45
0072--FMT---42
0073--FMT---42
0074--INT---64
0075--  .  ---21
0076--IND---31
0077--  .  ---21
0078--FMT---42
0079--FMT---42
0080--  1  ---01
0081--  8  ---10
0082--  1  ---01
0083--CLR---20
0084--STP---41
0085--XTO---23
0086--  5  ---05
0087--PNT---45
0088--PNT---45
0089--FMT---42
0090--FMT---42
0091--INT---64
0092--CNT---47
0093--  ¶  ---56
0094--  o  ---13
0095--  O  ---P1
0096--XTO---23
0097--FMT---42
0098--CLR---20
0099--STP---41
0100--XTO---23
0101--  6  ---06
0102--PNT---45
0103--PNT---45
0104--FMT---42
0105--FMT---42
0106--INT---64
0107--CNT---47
0108--  B  ---66
0109--  A  ---62
0110--  B  ---66
0111--XFR---67
0112--FMT---42
0113--CLR---20
0114--STP---41
0115--XTO---23
0116--  7  ---07
0117--PNT---45
0118--PNT---45
0119--FMT---42
0120--FMT---42
0121--INT---64
0122--CNT---47
0123--YTO---40
0124--XFR---67
0125--YTO---40
0126--XTO---23
0127--  .  ---21
0128--FMT---42
0129--CLR---20
0130--STP---41
0131--XTO---23
0132--  8  ---10
0133--PNT---45
0134--PNT---45
0135--FMT---42
0136--FMT---42
0137--  A  ---62
0138--YTO---40
0139--XFR---67
0140--  M  ---70
0141--  M  ---70
0142--  .  ---21
0143--  F  ---16
```

Calculation of total body fat

Print-out: (cont.)

```
0144--  A  ---62
0145--  C  ---61
0146--XTO----23
0147--  0  ---71
0148--  a  ---13
0149--FMT----42
0150--FMT----42
0151--  1  ---01
0152--  8  ---10
0153--  2  ---02
0154--CLR----20
0155--STP----41
0156--XTO----23
0157--  9  ---11
0158--PNT----45
0159--PNT----45
0160--FMT----42
0161--  1  ---01
0162--  8  ---10
0163--  1  ---01
0164--CLR----20
0165--FMT----42
0166--FMT----42
0167--  M  ---70
0168--  I  ---65
0169--YTO----40
0170--XTO----23
0171--  A  ---62
0172--  K  ---55
0173--  E  ---60
0174--YTO----40
0175--CNT----47
0176--CNT----47
0177--IFG----43
0178--CLR----20
0179--CLR----20
0180--CLR----20
0181--FMT----42
0182--STP----41
0183--XFR----67
0184--  0  ---00
0185--  UP---27
0186--  X  ---36
0187--CNT----47
0188--CNT----47
0189--CNT----47
0190--CNT----47
0191--CNT----47
0192--CNT----47
0193--CNT----47
0194--  X  ---36
0195--XFR----67
0196--  0  ---00
0197--  UP---27
0198--CNT----47
0199--CNT----47
0200--CNT----47
0201--CNT----47
0202--CNT----47
0203--CNT----47
0204--CNT----47
0205--CNT----47
0206--  X  ---36
0207--CNT----47
0208--CNT----47
0209--CNT----47
0210--CNT----47
0211--CNT----47
0212--  +  ---33
0213--  DN---25
0214--  +  ---33
0215--YTO----40
0216--  1  ---01
0217--  0  ---00
0218--CLR----20
0219--XFR----67
0220--  0  ---00
0221--  UP---27
0222--  X  ---36
0223--  1  ---01
0224--  7  ---07
0225--  0  ---00
0226--  7  ---07
0227--EEX----26
0228--CHS----32
0229--  7  ---07
0230--  X  ---36
0231--XFR----67
0232--  0  ---00
0233--  UP---27
0234--  1  ---01
0235--  7  ---07
0236--  8  ---10
0237--  1  ---01
0238--CHS----32
0239--EEX----26
0240--CHS----32
0241--  5  ---05
0242--  X  ---36
0243--  .  ---21
0244--  5  ---05
0245--  5  ---05
0246--  6  ---06
0247--  7  ---07
0248--  +  ---33
0249--  DN---25
0250--  +  ---33
0251--YTO----40
0252--  1  ---01
0253--  1  ---01
0254--XFR----67
0255--  0  ---00
0256--  UP---27
0257--  X  ---36
0258--  2  ---02
0259--  0  ---00
0260--  9  ---11
0261--EEX----26
0262--CHS----32
0263--  7  ---07
0264--  X  ---36
0265--XFR----67
0266--  0  ---00
0267--  UP---27
0268--  3  ---03
0269--  0  ---00
0270--  2  ---02
0271--  3  ---03
0272--CHS----32
0273--EEX----26
0274--CHS----32
0275--  6  ---06
0276--  X  ---36
0277--  .  ---21
0278--  1  ---01
0279--  5  ---05
0280--  2  ---02
0281--  1  ---01
0282--  +  ---33
0283--  DN---25
0284--  +  ---33
0285--YTO----40
0286--  1  ---01
0287--  2  ---02
```

Calculation of total body fat

Print-out: (cont.)

```
0288--XFR---67
0289-- 5 ---05
0290-- X ---36
0291--XFR---67
0292-- 1 ---01
0293-- 1 ---01
0294-- UP---27
0295--XFR---67
0296-- 6 ---06
0297-- X ---36
0298-- DN---25
0299-- + ---33
0300--YTO---40
0301-- 1 ---01
0302-- 3 ---03
0303--XFR---67
0304-- 8 ---10
0305-- UP---27
0306-- UP---27
0307--XFR---67
0308-- 7 ---07
0309-- - ---34
0310--YTO---40
0311-- 1 ---01
0312-- 4 ---04
0313-- DN---25
0314--DIV---35
0315--XFR---67
0316-- 4 ---04
0317-- X ---36
0318-- UP---27
0319-- 1 ---01
0320-- - ---34
0321-- DN---25
0322--CHS---32
0323--DIV---35
0324--YTO---40
0325-- 1 ---01
0326-- 5 ---05
0327--XFR---67
0328-- 3 ---03
0329--XFR---67
0330--DIV---35
0331-- 2 ---02
0332--XFR---67
0333-- X ---36
0334-- 9 ---11
0335-- UP---27
0336-- UP---27
0337-- 1 ---01
0338-- - ---34
0339-- DN---25
0340--CHS---32
0341--DIV---35
0342-- YE---24
0343-- - ---34
0344-- 1 ---01
0345-- 5 ---05
0346-- YE---24
0347-- X ---36
0348-- 1 ---01
0349-- 4 ---04
0350--CNT---47
0351-- 2 ---02
0352-- 7 ---07
0353-- 3 ---03
0354-- . ---21
0355-- 1 ---01
0356-- 5 ---05
0357-- X ---36
0358--XFR---67
0359-- + ---33
0360-- 1 ---01
0361--DIV---35
0362-- YE---24
0363-- - ---34
0364-- 1 ---01
0365-- 3 ---03
0366-- YE---24
0367--DIV---35
0368-- 1 ---01
0369-- 0 ---00
0370--CLX---37
0371-- UP---27
0372--RUP---22
0373--FMT---42
0374--FMT---42
0375-- F ---16
0376-- A ---62
0377--XTO---23
0378--CNT---47
0379--INT---64
0380-- 0 ---71
0381-- L ---72
0382--1/X---17
0383-- M ---70
0384-- E ---60
0385--FMT---42
0386--PNT---45
0387--PNT---45
0388--PNT---45
0389--PNT---45
0390--GTO---44
0391-- 0 ---00
0392--CNT---47
0393--END---46
```

Postscriptum

Slechts zelden is het tot stand komen van een wetenschappelijke publicatie toe te schrijven aan de verdiensten van één persoon. Het in dit proefschrift beschreven onderzoek maakt hierop geen uitzondering. Velen hebben op directe of indirecte wijze hieraan bijgedragen, waarvoor ik op deze plaats gaarne mijn dank uitspreek.

Het oorspronkelijk idee om de techniek van de bepaling van het totale lichaamsvet door middel van gas-absorptie toepasbaar te maken bij pasgeborenen is afkomstig van Prof. Dr. H.K.A. Visser. Op zijn initiatief werd deze studie opgezet. Ik ben hem zeer erkentelijk voor zijn stimulerende begeleiding en vooral ook voor zijn geduld, wanneer desondanks de voortgang van het onderzoek soms traag was.

Zonder de onverwoestbare inventiviteit van Dr. H.J. Degenhart zouden vele problemen van technische, methodische en mathematische aard onopgelost gebleven zijn. Ik ben je, Herman, bijzonder dankbaar voor je nimmer aflatende hulp. De vet-bepaling is in niet geringe mate ook jouw geesteskind!

Prof. Dr. M.W. van Hof en Prof. Dr. J.J. van der Werff ten Bosch dank ik voor de waardevolle kritiek, die zij als co-referenten op het gepresenteerde werk leverden.

Veel dank is verschuldigd aan de medewerkers van de Centrale Research Werkplaats van de Medische Faculteit, Erasmus Universiteit (hoofd: Ir. H.A. Bak), voor het ontwerpen en de constructie van de gebruikte apparatuur. In het bijzonder dank ik Ir. W.P.J. Holland voor zijn belangrijke inbreng, met name bij het oplossen van problemen van theoretisch-mathematische aard; hierbij heeft je onverstoorbaarheid mij immer geïmponeerd, Wim. Naast hem waren

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Gerdie Abeln verrichtte in de beginfase van het onderzoek (1972-73) nauwgezet de bepalingen van D_2O en broom.

De heer Groeneveld verzorgde de proefdieren.

Eric Duiverman verrichtte, aanvankelijk als keuzepraktikant en later als student-assistent, een beduidend aantal Xenonmetingen, vaak gedurende lange nachtelijke uren. Dat hij desondanks toch kinderarts wil worden, toont dat hij uit het goede hout is gesneden.

Marian Duiverman-Oudijk nam het typerwerk voor de nederlandse versie voor haar rekening. De engelse tekst werd getypt door Ans Stenger-Oosting, daarin bijgestaan door Marianne de Bruijne-Scharrenberg en Ineke Hermans-Schulp.

Ellen Nelemans-van den Broek verzorgde de literatuurlijst.

De figuren werden vervaardigd door Noud Kempers, Loek Baars, Cora van Nieuwkerk en Carla Schweinsberg van de Audio-visuele Dienst van het Sophia Kinderziekenhuis. De wijze, waarop één en ander in korte tijd werd gereedgemaakt, heb ik zeer gewaardeerd.

De engelse vertaling werd door Richard Pearse verzorgd. Met genoegen releveer ik de langdurige linguïstische discussies, die het inzicht in elkanders dialect aanmerkelijk verdiepten.

Trix von Ruhe-Zurcher was een ware paranimf, door mij moed in te fluisteren, wanneer dat nodig was. Ook was zij behulpzaam bij het correctiewerk.

Mijn collegae in het Sophia Kinderziekenhuis, in het bijzonder Richard Pearse, Ralph Spritzer, Pieter Sauer en Rudy Boersma dank ik voor de bereidwilligheid, waarmede

zij in de afgelopen maanden vele malen klinische en poliklinische werkzaamheden van mij overnamen.

Tenslotte dank ik Ali, Janet, Jaap, Han en Marjel voor de lichtelijk relativerende belangstelling, waarmede zij de groei van dit boekje hebben gevolgd.

Curriculum Vitae

De schrijver van dit proefschrift werd in 1930 te Koog aan de Zaan geboren. In 1949 behaalde hij aan het Zaanlands Lyceum het diploma Gymnasium-β. Hierna studeerde hij geneeskunde aan de Gemeentelijke Universiteit van Amsterdam, waar in 1960 het artsdiploma werd behaald.
Vervolgens was hij van 1961 tot 1963 als gouvernementsarts werkzaam in het voormalig Nederlands Nieuw-Guinea (thans: Irian Barat, Indonesia). Van 1963 tot eind 1965 verbleef hij in Algerije, waar hij, in het kader van een multidisciplinair ontwikkelingsproject uitgaande van de Wereldraad van Kerken, leiding gaf aan een stuk overwegend extramurale medische zorg. Tijdens deze werkperiode ontstond speciale belangstelling voor de pediatrie.
Terug in Nederland specialiseerde hij zich in deze richting in het Sophia Kinderziekenhuis te Rotterdam (hoofd van de afdeling Kindergeneeskunde: Prof. Dr. H.K.A. Visser). Op 1 februari 1970 werd hij als kinderarts in het specialistenregister ingeschreven. Nadien verdiepte hij zich in het bijzonder in de ziekten van de pasgeborene.
Van februari 1970 tot september 1975 was hij als wetenschappelijk hoofdmedewerker in deelaanstelling verbonden aan de afdeling Kindergeneeskunde van de Faculteit der Geneeskunde, Erasmus Universiteit te Rotterdam. In dezelfde periode was hij als kinderarts-consulent verbonden aan de Rijkskweekschool voor Vroedvrouwen te Rotterdam. Sinds september 1975 is hij, in volledig dienstverband bij het Academisch Ziekenhuis Rotterdam, werkzaam op de afdeling Pasgeborenen Pathologie van de afdeling Kindergeneeskunde, Sophia Kinderziekenhuis te Rotterdam. Tevens is hij kinderarts-consulent voor de afdeling Verloskunde en Gynaecologie van het Academisch Ziekenhuis Rotterdam, Dijkzigt.