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A metabolic unit for studies on human nutrition

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SUMMARY. In clinical nutrition we are used to dealing with the clinical management of patients, but of equal or greater importance is the study of how nutrition affects the development of diseases or modifies its manifiestations. This field is complex and links physiological studies of nutrition to the epidemiological analyses which form the basis of thinking in public health in Mexico today. Thus a number of studies have investigated the nutritional risk factors leading to the development of diseases such as heart disease and cancer (1-10). This epidemiological research requires the difficult task of accurately assessing the food consumption of individuals: with poor methodologies the chances of erroneous results are very high. This has implications for both group and individual comparison. Physiological studies on the effects of highly controlled changes in food intake on risk factors then allows the epidemilogy to be interpreted in metabolic terms. In this paper we illustrate some of the benefits of metabolic studies and some of the requirements for this succesfull conduct.

RESUMEN. Una unidad metabólica para estudios de nutrición en humanos. En nutrición clínica estamos acostumbrados al manejo clínico de pacientes pero de igual importancia es el estudio de como la nutrición afecta el desarrollo de enfermedades y como modifica sus manifestaciones. Este campo es complejo y vincula los estudios fisiológicos de nutrición con los análisis epidemiológicos. Esto conforma las bases del pensamiento en salud pública en el México de hoy. Así, varios estudios han investigado los factores nutricionales de riesgo que conllevan al desarrollo de enfermedades tales como la cardiovascular y el cáncer. La investigación epidemiológica requiere de la difícil labor de valorar con precisión el consumo de alimentos en individuos: las probabilidades de obtener resultados erróneos si se utilizan metodologías inapropiadas es muy alto. Esto tiene implicaciones tanto para la comparación de grupos de personas así como de individuos. Los estudios fisiológicos en donde se observan los efectos en los factores de riesgo con dietas modificadas y altamente controladas, permiten la interpretación de la epidemiología en términos metabólicos. En este artículo ilustramos algunos de los beneficios que se obtiene con estudios metabólicos de nutrición así como algunos de los requisitos necesarios para lograr el éxito de los mismos.

CLINICAL VERSUS HUMAN NUTRICION STUDIES

Metabolic Units have traditionally been used, as the name implies, for metabolic studies of patients at a hospital. These types of metabolic units are very expensive to run since sick patients require highly qualified nursing care day and night as well as expensive equipment. Studies on human nutrition, however, do not have to be necessarily undertaken with sick patients and so they can be much cheaper. Less expensive metabolic units can be run by only a qualified nutritionist and a cook. Such Metabolic Units function as a type of «hotel suite» where rooms and meals are provided free of charge to those «healthy» persons who are interested in participating in studies of human nutrition (11-14). The nutritionist needs to be trained however to understand the significance of meticulous attention to detail at every stage of the study, i.e. the preparation of foods, and when collecting urine, faeces and sweat. The importance of having reliable subjetcs is also vital, since even the drinking of a single glass of water or eating small food items not included in the metabolic dieta often ruins the study. Usually unrecognized however is the value of undertaking meticulous studies on only a small number of individuals. Only eight subjects, are needed to attain statistical as well as biological significant changes provided very tight control of the experiment is achieved. When we are looking for the impact of diets, studying the effect of dietary change on the same individual is much better than studying two groups on different diets, as happens in some forms of epidemiology e.g.a case control study. In a metabolic study everybody is

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under controlled conditions so that when one factor is changed i.e. the introduction of a substance such as lithium, a very small effect can be picked up (e.g., the increased excretion by sweat) (11) (Table 1). It is then possible to establish clear evidence of individual variations in the dietary responsiveness. Human nutrition studies can also be used to develop marker techniques, as in the case of the lithium-marker technique for transferring to an epidemiological setting. This technique was used to assess the different sources of salt in the diet (15-17).

In a «hotel suite» form of metabolic unit it is possible to run several types of nutritional studies:

TABLE 1
THE LITHIUM LOSSES IN SWEAT IN FIVE VOLUNTEERS WHO RECEIVED LITHIUM LABELLED COOKING AND TABLE SALT IN TWO PERIODS DURING A METABOLIC STUDY (11).

Subject	NG	RT	JP (μMol)	JW	RG	
Periods (days)						Group Mean ± SD
2	3.2	1.7	1.9	1.0	0.5	1.7±1.0
3*	6.9	18.3	2.4	6.3	2.9	7.6±6.2
4	2.3	1.6	2.7	3.5	1.5	2.3±0.8
5**	8.0	11.6	15.3	7.1	4.2	9.0±4.7
6	1.6	1.8	2.5	1.5	1.5	1.8±0.4

* Ad libitum lithium labelled table salt
** Controlled lithium labelled cooking salt

a. Residential detailed balance studies.

Detailed balance studies e.g. on trace elements require people to live in the Unit and eat their meals so that the collection of urine, faeces and sometime sweat can be supervised. Some studies are so dependent on total compliance e.g. studies on overweight and obese people that it is best to have these people confined to the hotel facility unless they go out for controlled exercise under scrutiny. This is the most rigorous control and is a good way to start learning how to use these units because it teaches staff the importance of absolute control over subjects behaviour, and ensuring their complete compliance.

In energy balance studies more effort is often required from the subjetcs as well as from the investigator since volunteers may need to live in a calorimeter chamber within the metabolic unit for long periods of time and this can cause apprehension or anxiety. This is minimised by bringing the subject to live in the Unit several days before he goes in the calorimeter. Such a study on the factors involved in controlling energy balance was described by Dallosso and James (18,19). They used indirect calorimeters which were built within a 6-bedroomed Metabolic Unit. This enabled strict control of the subject's diet for periods of weeks and also allowed the subjects to make complete faecal and urine collections.

The detailed control of metabolic studies can lead, however, to surprising problems. Thus a colleague decided it was unreasonable to ban a volunteer from having his grilfriend to stay for an occasional night during a very long study. Unfortunately fasting blood samples taken the next morning showed marked increases in plasma glycerol and free fatty acids, presumably in response to the excess lipolysis induced by increased sympathetic activity. This problem therefore increased the difficulty of recruiting subjets for prolonged studies on lipid metabolism.

b. Partially Residentail studies.

Other studies can readily be conducted with subjects living a normal working and social life. They can go to work and to carefully assessed social events. They then regard the Unit as a temporary home. An example of such a study is that undertaken by Bingham et al. (2) where adult men were used to evaluate 4-amino-benzoic acid (PABA) as a marker for the completness of urine collections and a no-absorbable marker for faecal collections (21) to establish the relationship between urinary, faecal and extrarenal losses of nitrogen under conditions of varying N intake. These healthy volunteers had to eat their normal diet for 28 days. During this time all urine and faecal specimens were collected and skin losses of nitrogen were measured directly on two occasions in each subjetc. To avoid changes in dietary habits and lifestyle on moving from home to the Metabolic Unit, and possible alterations in nitrogen balance, the subjects were given rigidly controlled diets based on their normal pattern of food.

c. Free living studies.

Controlled diet studied. A third type of study involves free living volunteers. Volunteers are supplied with all their food in cool picnic bags which is either collected by them from the Metabolic Unit or is delivered to their homes by the investigator. This type of study is easier for the subject and can be more readily undertake when only blood samples or other simple tests are needed. An example of this is a dietary oil study where different kinds of oil are fed to free living volunteers and only a blood sample is needed to provide the necessary information (22).

Ethical committee and adequate sampling.

The ethical aspects of nutritional research involving human subjects are extremely important. The value, the procedures involved, the duration, and potential hazards of the projects should be provided in detail by the researcher to an Ethical Committee whose job it is to evaluate any potential hazards for the volunteers. A routine general medical examination is also required before volunteers are accepted because it is surprising how often relevant disorders are picked up in those who volunteer. It is no good doing studies on the dietary aspects of thrombosis in individuals who regularly use aspririn. Other drug use can cause problems, such as the use of contraceptive pills; additionally smoking has a profound effect on energy balance, vitamin E and C metabolism as well as inducing a range of metabolic abnormalities. Young men also often consume surprisingly high amounts of alcohol which then affects the hepatic metabolism of many nutrients, hormones and drugs. A sophisticated assessment of the volunteer is therefore essential.

Running a metabolic unit.

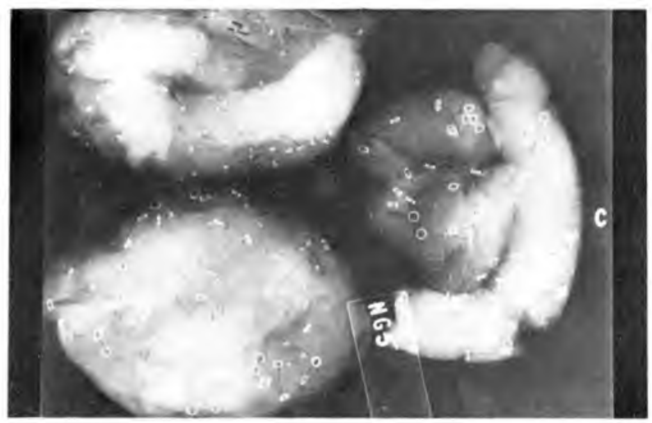
Metabolic studies involve a great deal of care since they require type careful preparation of a specially designed constant diet and often complete collections of urine and faeces and perhaps sweat, with the proper demarcation of the faecal collections, in relation to the dietary periods to which the collections correspond (23). Each collection and form of analysis needs strict quality control measures at every step if high standards are to be attained.

These metabolic units (11,12) therefore usually consist of a metabolic kitchen, a volunteers' dining room, toilets, showers, a homogenising room for sample processing, an x-ray area and often, calorimeters. The kitchen is equipped with all those utensils needed for the preparation and cooking of meals; scales, an homogeniser, short-term storage refrigerators and large cold (-4°C) and freezer (-20°C) rooms for long-term food storage. This last need is vital so that bulk purchases of food can be used to minimise the nutrient variability in the tightly controlled diets. Entry to the kitchen should be limited to the researcher, nutritionist and assistants so that volunteers and others do not interfere with the preparation of the meals.

It is useful to have the dining room next to the kitchen furnished with the usual table and chairs. Shelves are needed for cutlery, plates, glasses, cups, salt cellars etc., and five small fridges are useful for keeping the daily diets of those participating. Each fridge can be labelled with the name of the volunteer and the containers with food for the individual clearly marked with and individual color code and with the date on which it is to be consumed. Food can be provided either hot from the kitchen or for later heating by the subjects in a microware oven. Deionised water for drinking is kept in plastic jars with lids and can be heated in a kettle kept especially for this purpose. Although these arrangements seem very restrictive it is important to have a room which is attractively furnished to provide a domestic rather than an experimental atmosphere. For residential and partially residential studies, the bedrooms also should be as comfortable as possible with the volunteers receiving all the laundry facilities i.e. bed linen, towels and a dressing-gown which they would expect in a good hotel. For the management of sweat collections it is useful to have an area with a washing and drying machine for dealing with the clothes used for collecting sweat losses. The water running through the ashing machine has to be deionised.

Toilets and showers are provided for the use of the volunteers and a especially designed collecting frame is kept on the toilet seat for the collection of faeces. An area opposite to the toilets is usually kept with sterile bottles and plastic bags for the colection of urine samples. Space is also needed for homogenisation and aliquoting the urine and faecal specimens. It is useful to ventilate the room by means of an extractor fan. Scales, homogeniser, deionise water and toilet for the discarding of unneeded specimens are also useful. An area with X-ray equipment is also needed so that the number and type of faecal markers in each stool specimen can be counted on the X-ray plate (Fig. 1).

FIGURA 1
Inert faecal markers in a stool specimen on the X-ray plate



A living room is also a valuable for the volunteers so that they can relax while reading or watching television. This facility improves the atmosphere and makes participants feel at home. Our experience suggest that if this room is not provided then volunteers tend to use their own bedrooms during the day and become detached and dissatisfied with the whole procedure. This then reduces their commitment to the study.

Volunteers.

Volunteers are usually recruited by advertising in Colleges, Libraries, the Medical School and a University Center. When these routes fail, newspaper adverstising is used as a last resort. A list of former volunteers can also be used, since many of then are willing to take part in new experiments if they have been treated well in earlier studies. Subjects are contacted by post or telephone. The fact that they will be provided with accomodation, food and a small financial reward at the end of the study makes this an attractive proposition, especially for students or unemployed individuals. Reliable subjects are essential for success. One of the principal determinants of a succesful study is whether or not the researcher develops a close and pleasant relationship with the participants. The volunteers' anxieties and problems need to be resolved early and with a good relationship it is also possible to detect unintentional errors that arise during the experiment. Total supervision on a minute by minute basis is out of the question so there is a need to encourage the volunteers to perform well and to state quickly that a mistake has been made, e.g. incomplete collections, or a failure to eat the diets; only then can allowances be made or variations in the protocol be developed. A subject who admits to taking a drug or other food is usually inmmediatelly disqualified from the study and sent home.

Problems in the Design of Experimental Diet.

Contrary to popular believe the most difficult aspect of the work is in the design of the diets. A poorly designed diet leads to confusing interpretations of the data and the waste of the study. The planning of metabolic diets also requires a great deal of care because of the many sources of error that can arise in the selection, preparation and consumption of a nutritionally balanced diet (23,24) which takes into account the various interactions of minerals and vitamins.

Many factors need to be considered. Diets, must be sufficiently attractive and, if possible, similar to the volunteers's normal diet unless a prolongued preliminary feeding period is planned. A standard policy has developed over the last 20 years of providing a 3 day rotating menu to allow volunteers to cope with 6-12 week studies. A tighter dietary design with a 2 days rotating menu may be required in studies dealing with mineral balances to reduce the variation but volunteers become bored quickly with this regime. Occasionally one ends up with liquid formulae or similar diets. These used to be a standard method on studies on lipid metabolism but are now recognized to be flawed without an appropriate intake of non-starch polysaccharides (fibre) to retain as near normal as possible the colonic metabolism of food residues, endogenous intestinal secretions and steroidal recycling.

Day to day variations in the composition of the food used is a problem as emphasised by Gephart and Dubois as long ago as 1915 (25). This problem has been described by Reifenstein, Albright and Wells in 1945 (26) who noted that the mineral content of the same foodstuffs fluctuates from time to time depending on such factors as the soil, climate and the method of preservation of the food. This aspect is particularly important as the length of the experiment increases. The variation in the nutritional content of foodstuffs from day to day is a random error and cannot be avoided. Random errors, for example, for sodium will increase when natural foods such as milk and cream or processed foods are incorporated in the menus. However the fluctuation in, for example, the sodium content of raw vegetables is not a problem because it would not account for a big variation in the diets given their naturally low Na content. Thus the sodium content of vegetables will represent only a very small proportion of the total intake. This may not be true however if one is studying vitamin C metabolism

Isaksson an Sjogren (27) reported that the greatest problem in controlling dietary intake is caused by day to day variation in the composition of the food used. They analysed a number of nutrients in standard diets and found that the variation was particulary great in the case of sodium. The diets used by Isaksson for the statistical calculations excluded most processed foods and only occasionally contained items such as boiled ham, canned mushrooms and canned green beans which they considered as one probable cause for a relatively «great» variation in the sodium content of diets. Greater variations occurs with the use of many processed foods. Isaksson and Sjogren did not include table or cooking salt in their analyses but found that the variation in the sodium content of their foods amounted to 6.3%.

Bassett and Van Alstine (28) determined the minerals ingested by human subjects during a long balance experiment with a series of twenty «identical» diets. They obtained a coefficient of variation (CV) of 2.3% for sodium in 10 low calcium diets and of 3.2 % in ten high calcium diets.

Practical steps to limit variability in intake.

Fluctuations in dietary sodium can be reduced if some of the products are purchased in advance in amounts large enough to last the whole trial. Manufactured foods may, for example, be obtained from the same commercial batch (29). Selecting as many similar foods as possible for the different rotating menus will also help to reduce the variation in the composition of the so-called «constant diet». The selected foods must also be easy to handle because so many wieghings are involved (Table 2)

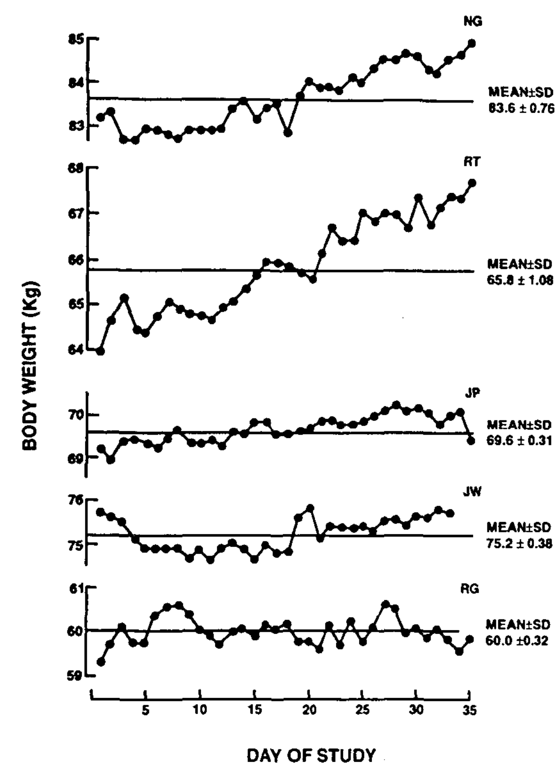
The simpler the diet, the less error is introduced; simple diets may, however, be unpalatable and this can jeopardise a long-term balance study because subjects may refuse food or eat additional unscheduled food; good cooking and attractive menus are therefore important and the diet must fulfil the energy requirements of the individuals so that hunger is not an additional factor leading to innapropriate extra foods being eaten. A strict weight control should also be achieved. In Fig 2 the changes in body weight during a metabolic study are shown. Subject RT had an increase of 6% in body weight. However the energy requirements for this study were calculated using the 1973 WHO energy requirements (3) which does not take account of differences between and within individuals in their basal metabolic rates (BMR) and differences due to daily variation in BMR. In the UK, metabolic units were developed by James in Cambridge and Aberdeen where a new approach was introduced to calculate energy intake based on the individually measured basal metabolic rate (BMR) of volunteers (31). This value of BMR multiplied by a factor (PAL) e.g. 1.4-1.7 which depends particularly on their physical activity for the study allows one to overcome a major complication of weight gain or weight loss. Weight change during a nutritional study can alter the whole interpretation of the study so is best avoided. For this particular study dealing with the recovery of mineral components the weight gain did not affect the interpretation of the data..

TABLE 2
COMPARISON OF THE FOOD AND AMOUNTS GIVEN IN TWO ROTATING MENUS (11)

Basal Diet Day 1 Food	(g)	Basal Diet Day 2 Food	(g)
Runner Beans	100	Carrots	80
Tomato	60	Tomato	100
Chicken Soup (canned & strained)	180	Mushroom Soup (canned & strained)	175
Roast Pork	80	Roast Beef	100
Roast turkey	100	Roast Lamb	80
Tinned Apricots (drained of juice)	100	Tinned Peaches (drained of juice)	100

Identical for both diets	(g)
Egg	50
Milk	50
Cornflakes	28
Anchor Butter	45
Wholemeal bread	200
Milk	350
Sugar	20
Marmalade	30
Coffee	6
Lettuce	30
Cucumber	30
Mayonnaise	15
Fresh Apple	100
Jam Tart	90
Gravy	60
Boiled Potato	200
Double cream	20

FIGURE 2
Changes in body weight during a metabolic study (11)



Minimum losses of food in dishes.

In metabolic balance studies, systematic errors in what has been termed «invisible returns», i.e. unmeasured amounts of nutrients, give rise to large cumulative errors. Isaksson and Sjogren (32) found that the «invisible returns» of sodium on trays, pots and pans of 25 patients receiving normal diets amounted to about 2.7 mmol with a range from 0.2 to 6.3 mmol Na. With trained staff returns were of the order of 0.5 mmol sodium, ranging from 0.1 to 0.8 mmol Na in 4 subjects (33). Other studies have found losses of 4.2±1.2 mmol Na in four individuals and 2.5±0.9 in subjects under metabolic ward conditions depending on whether they cleaned the pots and pans with or without a flexible spatula (32). Since these invisible returns are usually assumed to have been eaten and with total urine and faecal collections being hard to achieve there is a bias towards obtaining a positive balance for the nutrient which in fact is misleading. This is why a meticulous approach is essential and where faecal and urine markers for complete recovery are important This point is particularly relevant in N-balance studies. Unmeasured N in dishes, toilet paper and miscellaneous losses can yield erroenous results.

Testing the diet.

Once the dietitian and principal investigator have worked out the diet to suit the protocol a pilot study is advisable to ensure that the diet is palatable, edible and practical. Available ingredients should be used (it is no good choosing unavailable foods, for example, seasonal foods such as mangos for use in December). Once the diet is tasted by a panel and analyzed, it should be presented to potential volunteers so that they can make their comments. If there are foods which they dislike then changes can be made if then are slight. Particular food fads should however be identified before the diets are planned and all the volunteers should receive the same diet. It is not sensible to cater for individual needs because this changes the composition of each individual's diet and negates the point of feeding a group of volunteers a standard diet. Once all these factors have been dealt with the diet can be prepared in bulk.

Urine collections.

All the polypropylene items including both those to be given to the volunteers and those to be used in the sampling procedure have to be cleaned thouroughly, especially when minerals are to be studied. An example of cleaning procedure for the analysis of lithium and sodium has been described elsewhere (11). Once the material had been cleaned jugs and funnels are kept inside plastic bags to prevent contamination before use. The rest of the material is carefully stored and cylinders for measuring the urinary volumen covered with plastic film». The subjects will be asked every day if any problem had arisen during the last 24 hours so that an inmediate correction can be made. The 24 hour urine bottles will be collected from the subject, usually soon after completion on each day. Careful techniques reduces sample contamination and errors (11).

Obtaining a full twenty-four hour urine collection although apparently easy to fulfil, is in practice very difficult. Most volunteers although commited to being very careful, are not used to the procedures needed for urine collection, not to carrying the materials needed. A special bag is given to the volunteers containing urine bottles with a preservative. A polypropylene funnel and a jug is often needed to help people, especially women, to collect their urine completely. A notebook is also useful to remind them of the details of the study and additional plastic bags or tissues are included to keep the bottles clean. Unintended errors arise: e.g. a failure to empty the bladder completely, the loss of a urine sample to the toilet due to distraction, loss of a sample by accidental splilling of urine, or the loss or removal of bottles by others at home. Some volunteers are also very shy when people notice that they are carrying around bags for collecting urine. The loss of a urine sample to the toilet because of forgetfulness can be prevented by giving volunteers a safety pin which they attach to their underpants as a reminder to collect their urine. Unforseen factors are surprisingly common e.g. a volunteer who left his apparatus in the car while shopping only to find the bag stolen by a thief who broke the window and took the bag.

Care must be taken to give precise instructions to the volunteers both by verbal explanation and by means of a written instruction guide. They are asked to write the precise time when they start and finish the collection and any other features of the day relating to the collection. They will also be asked to write if any sudden excess exercise had to be undertaken: profuse sweating alters catabolite excretion in urine. Cleaning instructions for the apparatus, i.e. funnels and jugs, have to be provided to limit contamination and volunteers have to be warned that preservative solution, which may be concentrated acid, is contained in the bottles. Each volunteer has to be monitored carefully so that difficulties are identified early and so that they can be encouraged to continue to the end of the experiment. Completness of the collections has to be assessed both by a urinary marker (see below) and by the subject's assessment once they have been encouraged to be as frank as possible about any urinary losses.

Completness of urine collections.

Creatinine has been traditionally used to estimate lean body or muscle mass. It is assumed that body pool sizes, rates of creatinine excretion and conversion of creatinine to creatine are reasonably constant from day to day in individuals, altough there are studies proving the opposite. Based on these assumptions the completness of 24h urine collections have been assessed by measuring the constancy of 24h urinary creatinine. This constancy however has been questioned (34,35). Individual daily variation in continuous collections have been found to range from 1 to 36% (33). It is well know that loss of creatine from the creatinine pool depends upon the acid base status and ingested meat certainly leads to an appreciable increase in creatinine output (36-38); since urinary creatinine output is dependent on dietary intake and the range for normal 24h excretion is large it is difficult to use it to detect anything other than gross errors in urine collections. Creatinine excretion is also influenced by level of excersice, menstrual cycle, hydration level and disease.

In choosing an alternative to creatinine an exogenous biological marker, para-aminobenzoic acid (PABA), that can be given by mouth with meals has been developed under rigorous controlled conditions in a metabolic unit and has been proved a safe marker of the completness of 24h urine collections (39). The principles for choosing this substance were that it was a harmless substance with no toxic or pharmacological effects and that it is completely absorbed from the gastrointestinal tract and excreted in the urine either in its original form or as metabolites which can be measured in urine. When eight volunteers living in a calorimeter where complete urine collections could be guaranteed were given varying doses of PABA the urine excretion of PABA and its metabolites and oral dose were directly related (r=1.000). The marker was also tested in 33 reliable free living individuals who ate their normal diet and took 80 mg PABA with meals (240 mg/day). Mean urinary recovery over the 24h period was 223±9 mg or 93±4% of the administered marker. The range in individual recovery from minimum to maximum was 15% compared with 75% for creatinine excretion per kg fat free mass. Detailed explanations of the studies are given elsewhere (20).

Faecal Collections.

The first thing the investigator must be aware of is the difficulties involved in the collection of faecal samples. This will impose an additional strain on the volunteer especially if the study is partially residential or free living where collections of faeces have to be made away from the Unit; the subjects then need to carry a specially designed collecting frame with a plastic bag to place on the toilet seat to help them collect their faeces. Faecal samples may need to be carried around sealed in an air tight box or in a cooled container.

The same unforseen factors apply to faecal collections. A colleague after an enormous effort in collecting faecal samples from individuals had them stolen from the boot of his car: they could not be recovered even after an announcement in the newspapers of a reward for their return.

Metabolic balance studies require accurate information on the output of faecal constituents and this accuracy can only be achieved if the faecal collections are complete. Account of faeces lost in toilet paper need to be considered.

A number of inert markers have been used to check on faecal collections but we must remember to choose one that comes to the criteria for an «ideal» intestinal marker: it should be inert, nontoxic, completely unabsorbed, non-metabolised, easily measured, have no appreciable bulk and mix well with intestinal contents (21). The marker needs to allow faecal material from one period or several periods to be clearly identified while maintaining a continuous flow of marker. Barium sulphate, copper thiocyanate, polyethylene glycol, chromium sesquioxide, are well established markers and widely used but are tedious to measure. Other markers such as carmine and similar dyes have been traditionally used for the demarcation of faeces. These are, however, not acceptable any more since the use of single doses of marker leads to inaccuracies. Carmine does not mix well with faeces and cannot distinguish partial collections of faecal material from a complete collection, not can it differenciate between periods. The tail-off of the carmine marker is also a problem and no correction factor for faeces belonging to other periods or to irregular bowel movement is possible. Thus carmine allows only a very inaccurate or crude measurement of the components under study and does not give any information on other physiological variables such as transit time.

The need for accurate measures of elemental faecal excretion to understand better the physiological behaviour of the intestine and the factors involved in the development of colon cancer led scientists to develop more accurate methods for detecting very small changes in the behaviour of the gut. Radio-opaque barium impregnated plastic pellets (Portex Ltd) of different shapes (cylindrical, cube etc.) have been developed and have a series of advantages as outlined by Branch and Cummings (21): they are easier to measure with an X-ray machine, the handling of specimens is reduced to a minimum, marker determination by radiography does not destroy the specimens and allows individual stool by stool analyses to be done with the result that a mean transit time (a measure of passage of food through the gut) for the marker can be calculated (40) throughout the study. Several different types of marker can be made. This enables different pellets to be given in different weeks or in separate parts of a dietary study. Faecal material from each part of the study can therefore be clearly identified while maintaining a continuous flow of marker to differentiate dietary periods. Samples containing 96% or more of one type of marker are used to make pools for each dietary period. An example of marker recovery is shown in 5 volunteers involved in a study of sodium and lithium balance where different type of pellets were given in the different periods of the study (Table 3) (41).

TABLE 3
AVERAGE FAECAL OUTPUT AND MARKER RECOVERIES

Subject	Mean ± SD*	R5	Cubes	R1	R2	%
NG	201±66	95	98	100	58**	88
RT	148±59	98	99	100	98	99
JP	106±50	98	95	94	99	97
JW	162±65	96	99	100	96	98
RG	178±73	100	100	99	98	99

* Mean ± SD of the whole metabolic study (11)
** This subject stopped faecal collections on the last day of the diet so subsequent excreted markers were not collected.

The correction of variability in output of faecal collections is essential for some types of study. Studies on calcium metabolism need to correct for variability in output because small changes in calcium balance are sought (20).

A Marker Correction Factor (MCF) for faeces belonging to other periods can be made based on the ratio between the number of markers ingested over the period and the number of markers excreted over the same time (as counted by X-ray). The mean daily faecal weight is multiplied by the MCF to get a «marker-corrected mean daily faecal weight. The stability of colonic function in subjects is aided by having a high NSP intake which smooths any irregularities intransit time. If people are on low fibre diets then their faecal output may become very variable and corrections are needed.

Collection of sweat.

Sweat collection is a very laborious task (11,20). The technique involves the use of special clothes with a closely fitting two-piece set of underwear, tennis shoes, cotton socks and towels. Special containers are used for the cleaning and extraction of elements from the clothes and for washing the skin. These include plastic bath tubs with lids, 10 litre plastic bottles, plastic buckets, polypropylene measuring cylinders and plastic gloves.

The cleaning of the clothes is of paramount importance since some of the elements under study e.g. sodium and lithium may differ widely both in their concentration when occuring naturally and when used experimentally. Either contamination or poor extraction of the elements from the clothes will lead to misinterpretation and erroneous values for elemental balance.

All the clothes and materials for the collection and extraction of sweat need to be thoroughly cleaned with dilute nitric acid and deionised water. This cleaning procedure is repeated for each collection. All the plastic material are washed for a few minutes with concentrated nitric acid. Further extractions of the elements are then performed with diluted nitric acid. The washings have to be tested in the spectrophotometer to make sure that the extractions have been complete. Both the preparation of clothing and instructions to volunteers for the collection of sweat can be based on the methods used by Dahl, Stall and Cotzias (42) for quantitating skin losses of electrolytes. For example on the day of the collection subjetc can be provided with a 10 litre container of «warm» deionised water with a plastic tube attached to the tap. Subjects are directed to wash their whole skin surface with soap and water, shampoo their hair and thoroughly rinse it. Afterwards they rinse their bodies with deionised water. No lotions, deodorants, soap or other toilet preparations that could contaminate the body can be applied from then on. Then they dry themselves with special towels and immediately put on the underwear, socks, tennis shoes and their regular clothing and went about their daily routine. Only the shoes are removed at night and special precautions can also be taken with the bed clothes (20).

At the end of the 24 hours, the clothes are removed and deposited in plastic bags. Again the volunteers shower with deionised water which is now collected into an acid washed bath. After thorough mixing, washing samples are taken for element determinations. The extration of sweat from clothes is conducted in the same manner as the system used for cleaning. Checks made to the washings ensure that complete extraction was achieved. The detailed description of the nature of the task has been explained elsewhere (11).

CONCLUSIONS

This overview has been set out to illustrate the simple point that human nutrition studies which assess the impact of diet on metabolism can be performed relatively inexpensively but demand a rigour and understanding of meticulous procedures which most doctors and nutritionists rarely recognize. The design of diets with checking on the collection of duplicate diets, urine, faeces and sweat is extremely demanding but if conducted properly it is possible to show small but statistically significant changes relating to relevant changes in the diet using groups of only 8 volunteers. These highly controlled studies have been neglected because it has not been appreciated that inaccurate dietary design with poor collection procedures in early metabolic studies introduced error which limited the ability of investigators to assess the role of diet do the pathophysiology of disease.

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