

## Animal models for the study of protein–energy malnutrition

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Essentially there are two reasons why scientists interested in protein–energy malnutrition might wish to carry out studies on animal models. The first is to elucidate fundamental biochemical and physiological responses either to primary protein malnutrition, or to primary energy undernutrition. The second reason is to produce a close replica of the two clinical syndromes, kwashiorkor and marasmus. This type of model is essential when it is necessary to continue with one's investigations in a country like the United Kingdom, where the frank human conditions are rare. Alternative circumstances when these animal replicas of the human syndrome might be needed are for studies in which a hypothesis derived from an epidemiological survey has to be tested. It is always wise to bear in mind that epidemiology rarely establishes a theory beyond doubt, it is more likely only to generate a hypothesis, particularly so when one is trying to study pathogenesis at a molecular level. There are sufficient current controversies relating to the reason why kwashiorkor sometimes emerges as the ultimate clinical syndrome rather than marasmus, to make such considerations important.

At first sight it might seem I am splitting hairs by suggesting there are fundamentally two different types of approach, but this is not so. It is because the precise objective of many animal studies has not clearly been defined that there is so much misleading information in the literature. All too often one reads claims, on the basis of an animal model, that a particular pathophysiological abnormality has been identified in kwashiorkor, when it is apparent that neither oedema—the cardinal feature of kwashiorkor—nor any of the other associated clinical signs have been reproduced, except severe growth retardation; merely the animal has been fed on a low-protein diet. This is why we in Uganda concluded the following. 'For our particular needs an animal model can only be considered suitable if a pathological state eventually develops that resembles kwashiorkor so closely that a paediatrician would have no difficulty in diagnosing the condition'. We also considered it important that 'the pathological changes should be produced under dietary and environmental circumstances as similar as possible to those of the Ugandan child who gets kwashiorkor' (Coward & Whitehead, 1972). I emphasize that my purpose is not to criticize fundamental studies on the response of animals to nutritional stress, rather it is to identify specific requirements for animal models which purport to provide a replica of the two clinical syndromes.

### *Dietary design*

An *a-priori* requirement is that the clinical and biochemical features of the type of kwashiorkor or the marasmus in which one is interested, plus the dietary and

other circumstances under which it develops, have been defined as accurately as possible. Both syndromes result from a multiplicity of environmental insults and one must recognize that the precise details of the experimental design will vary and it is thus not necessarily valid to copy exactly a methodological approach which has been worked out elsewhere.

In Uganda the principal features of kwashiorkor that we needed to reproduce were oedema, a failure of growth, sparse light coloured hair, wasted muscles, pallor of the skin, a flaky paint dermatosis, a fatty liver and mental apathy. It was necessary to reproduce these signs on diets that were based on local staples—plantain, sweet potato and cassava, varyingly supplemented with vegetables such as groundnuts and beans.

We ultimately adopted as an experimental animal the baboon, for a number of reasons. Being a primate it has, like man, an extended childhood, thus one can impose malnutrition for long periods of time without the complication of the onset of puberty. This is not possible with non-primates. The general physiological features and nutrient requirements are also more like those of the human baby.

In spite of the fact that baby baboons grow more quickly and have higher protein requirements than young children, it was soon found that the *ad lib.* feeding of mixed staples did not by itself result in the appearance of a kwashiorkor-like state and it became necessary, therefore, to think of modifications to the basic experimental design. The main problem was that after an initial fall, plasma albumin concentrations tended to stabilize at a level higher than that associated with the onset of oedema. The animals had in fact physiologically adapted to their new circumstances, a phenomenon Coward and his colleagues have subsequently studied in depth (Coward & Sawyer, 1977; Fiorotto & Coward, 1979).

Initially an attempt was made to create further protein stress for the young baboon by feeding only cassava, which has an even lower protein and higher carbohydrate content than the other staples. The justification was that for very poor children this would not be uncommon, but it had remarkably little effect. Results from a parallel longitudinal study in children had also alerted us to the importance of periodic energy deprivation, largely associated with episodes of infection, in the aetiology of malnutrition in Ugandan children. Continuing with our philosophy that it was important that the animals should be subjected to the same types of dietary stress as the children, the food intake of some of the baboons was consequently restricted over certain periods to simulate this condition experimentally. A further factor which had emerged from our food consumption studies in the Ugandan children was that they were drinking quite substantial amounts of very sweet tea. In fact, during periods of infection this was often their only source of food. The diet of other baboons was therefore modified to bring it more in line with that of the poorly fed child, by adding sucrose to the diet so that it constituted about 20% of the total energy intake.

I have gone into these details because they help to emphasize the difference in approach between an animal study, which is specifically designed to reproduce as exactly as possible a clinical syndrome, and a study which is designed to establish

the relationship between a single nutrient deficiency and the physiological response. The former type of animal design is almost inevitably going to be more complex and one has to face it, much less tidy from a scientific point of view. There are likely to be many nutrient deficiencies, not just protein and energy deficiencies.

#### *Pathological features of kwashiorkor in the baboon*

So much for methodology—how convincing were the results? The literature reveals that even in primates it has been exceedingly difficult to reproduce a really convincing animal model of kwashiorkor as it exists as a clinical syndrome.

Most investigators working with primates have observed oedema only around the eyes and this was our finding too, during the early stages of oedema development; although ultimately the oedema did spread to the jowls. It was tempting to relate this pattern of oedema to the moon face seen in preclinical cases of kwashiorkor. We did not achieve, however, oedema in other parts of the body such as the legs, feet and hands, as one would find in the malnourished child; although traces of oedema were seen in these areas. It is perhaps important that where fluid accumulates in oedema is at least partly determined by the posture of the individual. The posture of the severely malnourished baboon is such that the head frequently rests on the floor of the cage and thus virtually becomes the lowest part of the anatomy.

Edozien (1968) discussing the oedema which he had succeeded in producing in rats, considered that the earlier attempts had failed because the deficient diets had not been given for long enough, but this conclusion must be in doubt. Classical studies by Stewart *et al.* (1975) imposed malnutrition over generations of rats to achieve a better model, but no oedema developed. It is the opinion of my colleagues, Dr Coward and Mr Baker, at present developing models of kwashiorkor in baboons at Cambridge, that the degree of oedema and the ease with which it can be produced depends on how young the baby baboons are when the deficient diets are introduced. The earlier they are given, the greater is the extent of the oedema.

It must be emphasized that we completely failed to produce oedematous malnutrition just by feeding low protein–high carbohydrate staples. The animals had to be precipitated into the condition by being stressed in some way. In most of our animals this was by introducing a period of severe food deprivation which, if the previous malnutrition had existed for long enough, was sufficient to cause an acute drop of plasma albumin concentration down to values associated with oedema development. It is becoming apparent, however, that reductions in plasma protein concentrations are not just due to a reduced plasma protein synthesis. Protein loss, for example, into the gastrointestinal tract, can precipitate oedematous malnutrition in an otherwise marasmic child. Recent experimental work with malnourished rats infected with intestinal helminths has supported this view (Crompton *et al.* 1978). This finding is indicative of the complexity which might be required of a truly representative animal model—a disease entity as well as a nutritional one might need to be introduced.

Apart from weight faltering and oedema, the other clinical signs associated with

kwashiorkor in children are more variable. Deo *et al.* (1965) discussing their failure to produce skin lesions in their monkeys, concluded that the only skin change in kwashiorkor that could definitely be attributed to protein deficiency itself was an atrophy of the epidermis and the more dramatic changes, including pigmentary disturbances, were probably related to deficiencies of other nutrients. We did reproduce the flaky paint dermatosis which is such a common feature of kwashiorkor in Uganda, and our general conclusions about its cause would be in accord with those of Deo *et al.* (1965). In more straightforward baboon studies now being carried out in Cambridge, where a deficient 'artificial' diet is supplemented with essential vitamins and minerals, these skin lesions are by no means so serious. Precisely which nutrients might be implicated still needs to be investigated, but riboflavin would be a possibility and the work of Foy *et al.* (1964) is of particular relevance in this respect. The possibility of essential fatty acid deficiency should also not be ignored. This fact has been emphasized by Naismith (1964) and certainly the total fat content of the diet, and hence the essential fatty acids, was low.

Hair changes also constitute a variable pathological feature of kwashiorkor. In Uganda the usual picture is of pale sparse hair. Essentially a similar type of hair was reproduced in the experimental animals. These took a long time to develop and their appearance probably depended on the chronicity of the malnutrition.

An unexpected feature in our earlier studies on baboons was the virtual absence of fatty infiltration into the livers of those baboons just given the high starch staples. There was, however, a dramatic effect when supplementation with moderate amounts of sucrose was introduced. Not only did the addition of sucrose seem to potentiate the development of a fatty liver, it was also associated with a more rapid fall in serum albumin concentrations, and other pathological signs of kwashiorkor appeared more quickly too. We have not yet followed up this anomaly, but it does draw attention to the importance of over-all dietary composition. Clearly difficulties are presented to the malnourished baboon when he has to metabolize sucrose: problems which do not occur with starch (Patrick *et al.* 1973).

Perhaps the most depressing feature of human kwashiorkor is the severe mental apathy which affects almost all the children. It is perhaps wrong to try and draw too close a parallel between baboons and humans, especially in terms of mental attitudes, but the animals did become progressively quieter as the malnutrition proceeded and eventually they went into a recluse existence at the back of the cage. One difference between the animals at this stage, however, and other animals on which we have worked, such as the pig and the rat, is that anorexia is not a particular problem except in the terminal stages. Thus, with the baboon it is possible to maintain the stress of a low protein-high carbohydrate containing diet much more easily.

The animal model that we had thus produced showed stunting of growth, loss of weight and muscle, hypoalbuminaemia, oedema, skin and hair changes and a fatty liver. The final appearance of the animals, however, resembled marasmic

kwashiorkor much more than the more classical type of kwashiorkor for which we had hoped. During the development of classical kwashiorkor in children there is pronounced failure of growth, but actual loss of weight and severe muscle wasting is not a major feature until the final stages of the development of the nutritional syndrome; whereas our baboons lost weight throughout most of the period of their malnutrition.

It is clear that many factors have to be considered before a really adequate replica of human kwashiorkor is achieved. It can be categorically stated that it is only permissible to say that an animal has kwashiorkor when frank oedema can be demonstrated. How important the presence of the other pathological features is, depends on the particular geographical type of kwashiorkor that one is trying to reproduce. If marked hepatomegaly and severe fatty liver are a prime feature of that kwashiorkor, then surely it is essential that these, too, are reproduced.

The investigative approach which I have described provides the opportunity to study a number of important questions such as the relative contribution of different dietary and environmental factors. It should be possible to discover, for example, whether the skin lesions really are due to a vitamin or to essential fatty acid deficiency, by adding one or other of these nutrients to the diets of a selected group of young animals. It is also possible to study the pathology and pathophysiology of different organs with the confidence that they are probably very similar to those of the child.

It is important, however, not to be carried away by this type of approach. It is essential that one has a clear objective before one embarks on an animal study as complex as this. When there is no need to have an exact replica of the human condition a simpler approach may not only be adequate but preferable.

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*Printed in Great Britain*