A LONG TERM EXPERIMENT WITH RATS ON A HUMAN DIETARY

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(With Plate VI and 7 Graphs)

In spite of the conflicting claims of nature and nurture, few will deny that diet plays a prominent part in the health and well-being of man and animals alike. The work of McCulloch (1929–30) and the striking report of Orr and Gilks (1931) on two African tribes suggest that, physiologically and psychologically, human beings are more or less a function of the food they eat.

Nearer home, dietary surveys and experiments with the feeding of milk to children suggest that the diet of the industrial population may be deficient in several respects (Corry Mann, 1926; Orr, 1928; Leighton and Clark, 1929; Clark, 1929; Orr and Clark, 1930; McLaughlin *et al.*, 1931; Rosenberg, 1931; Turbott and Rolland, 1932; Davidson *et al.*, 1933; McGonigle, 1933*a*, 1933*b*).

Valuable as such surveys and experiments are, their interpretation is difficult, since factors other than diet may be operative at the same time to affect the general well-being of the people. Suboptimal conditions of health may exist in a human population and yet be difficult of assessment. Moreover, such suboptimal conditions may find expression in patent ill-health, not in the first but in succeeding generations.

In order to test such a possibility, lower animals must be used, and, by common consent, the rat is the animal of choice, since it is an omnivorous animal which breeds and grows rapidly.

The possibilities of such a method of experimentation have been most fully explored by McCarrison (1927*a*, 1927*b*, 1931) and by Guha (1934). Rats were fed on foodstuffs representing human dietaries in various parts of the world and at various social levels. These diets reproduced in rats the pathological conditions observed in the comparable human communities.

The present work

The present work differs in certain material respects from previous experiments.

First of all, the diet used is a close approximation to the average diet eaten by a working-class community in Scotland as ascertained by dietary survey (Davidson *et al.*, 1933). Not only is the same food used, but the food is so given that there is daily variation, thus mimicking the food habits of human beings. Sunday's ration differs from Monday's, and so on for all days of the week.

In addition, this regimen has continued for $2\frac{1}{4}$ years, and has fed four to five generations of rats, corresponding to 100 years or more of human life. This has been done deliberately in order to detect possible remote or cumulative effects of the dietary.

Since the addition of milk, with or without fresh green food, had been found in practice to improve the health and general well-being of children in the community concerned in the dietary survey (Leighton and Clark, 1929), an exactly comparable series of rats was fed on the same human diet *with additional* milk and green food.

The animals used

The animals were Lister Institute hooded rats, bred at this Institute for three to four generations before the start of the experiment. In December 1932, sixty animals, 30–40 days old, were arranged in two groups of twenty females and ten males each. These groups were strictly comparable as regards age, weight and breeding. No fresh stock was introduced. The data to be presented cover a period up to the end of February 1935.

The human survey dietary

Table I shows the composition of the human dietary as purchased.

Table I

Foodstuff	g.	Foodstuff	g.
Bread and cakes	28·80	Apples	0·56
Oatmeal	5·70	Meat (mince)	6·31
White flour	2·20	Fresh fish	3·00
Rice and barley	1·00	Smoked fish	4·38
Cremola	0·40	Butter	0·37
Sago	0·40	Margarine	1·25
Mealy puddings	0·25	Roast fat	0·41
Potatoes	29·90	Cheese	0·60
Turnips	1·30	Sugar	9·53
Carrots	0·53	Syrup	1·65
Onions	0·56	Eggs	2·50
Dried green peas	0·62		
Lentils	0·28	Raw whole milk	20·77 ml.

The rat dietaries

At the commencement of the experiment, one-half of the rats received the diet as eaten by the human population. This is referred to as the survey diet.

However, this diet seemed to be inadequate for breeding rats, six out of nineteen females of the parental generation dying while suckling their second litters. Doubling the amount of milk in the survey diet prevented the recurrence of this catastrophe. It was found necessary to retain this double quantity of milk throughout the remaining 21 months of the experiment. This is referred to as the experimental diet.

The other half of the rat colony received the survey diet with the addition of green food and as much milk as they cared to drink. This is referred to as the supplemented diet. The estimated milk consumption on this diet was 1·6 times the quantity in the experimental diet. 28 g. of fresh green food, kale,

thousand-headed kale, or outer cabbage leaves, were fed for every 100 g. of solid foodstuffs. The avidity with which the rats ate the green food was most noticeable.

The distribution of Calories between the proximate principles in the survey diet and in the experimental and supplemented diets is shown in Table II along with the standard for human beings recommended by the British Medical Association Committee on Nutrition (1933). Table III shows the mineral content per 1000 Calories in these diets and also includes a human standard for mineral requirements. This standard is based on the *per capita* diet of Stiebeling (1933), which allows for the age distribution of the human population of the United States. It is probable that the figure for iron in this standard is slightly on the high side.

Table II. *The percentage distribution of Calories between the proximate principles of food*

	First-class protein	Second-class protein	Fat	Carbohydrate
Brit. Med. Assoc. standard	6·0	6·0	27·0	61·0
Survey diet	4·9	6·6	22·4	66·1
Survey diet with double milk allowance, *i.e.* the experimental diet	5·7	6·2	23·8	64·3
Supplemented diet	6·3	6·4	24·5	62·8

Table III

	Mg. per 1000 Calories in diet				
	Ca	P	Fe	Cu	Ca:P ratio
Stiebeling standard	320	440	4·8	—	1:1·4
Survey diet	255	488	4·9	1·3	1:1·9
Survey diet with double milk allowance, *i.e.* the experimental diet	332	538	4·7	1·3	1:1·6
Supplemented diet	534	591	4·6	1·2	1:1·1

From these tables it is seen that the survey diet is low in first-class protein, and deficient in fat and in Ca, the Ca:P ratio being unsatisfactory. The experimental diet still shows a slight deficiency in first-class protein and in fat. The Ca content is now adequate and the Ca:P ratio improved. In the supplemented diet, the percentage of Calories derived from first-class protein is above the standard, but the percentage from fat is still below standard and that from carbohydrate slightly excessive. The Ca and P content is increased markedly and the Ca:P ratio approximates to 1:1.

In all the diets the Fe and Cu content is practically steady, although the enrichment with milk, which is poor in these elements, causes a slight fall per 1000 Calories in the experimental and supplemented diets.

An attempt was made to compute the vitamin content of the diets by use of the figures given in Faber and Norgaard (1934). The values given by them for B_1 and C in the various foodstuffs are in International units, and those for A in "American" units. Faber and Norgaard state that five such units are equivalent to two International units. It is probable that a more accurate equivalent is two American units equal one International unit (Fridericia,

personal communication). The figures for Vitamin A in Table IV are calculated on this assumption.

The standard vitamin requirement for man on the basis of a 1000 Calories is included for comparison. The standard for vitamins A and C is that of Stiebeling (1933) in terms of International units. The B_1 standard is based on the statement of Fridericia (Faber and Norgaard, 1934) that thirty International units of vitamin B_1 per man per day are required to protect against beri-beri, and ninety to one-hundred and fifty units are necessary during pregnancy and lactation.

Table IV. *Vitamin content per* 1000 *Calories in the diet*

	A (International units)	B_1 (International units)	C (International units)
Standard requirement for man	676	30–50	500
Survey diet	298	89	325
Experimental diet	359	92	338
Supplemented diet	3197	120	1270

Whatever may be thought of the absolute accuracy of the values given for the different foodstuffs, yet the differences between the diets, especially in content of vitamins A and C, is most manifest. The green food is almost wholly responsible for this difference. With regard to vitamins D and E, the supplemented diet is undoubtedly superior, but we have failed to find figures justifying numerical evaluation.

Food consumption

Although accurate determination was impossible, it is of interest to estimate roughly the quantity of food and the Calorie consumption per rat per day on these diets. From the amount of food fed and of food left uneaten, it was estimated that the amount of solid food eaten per rat was almost identical in both groups, but that the animals on the supplemented diet consumed 1·6 times as much milk as the animals on the experimental diet did. In addition, of course, the animals on the supplemented diet received green food. It is thus obvious that the animals on the supplemented diet were eating more than the animals on the experimental diet, although there was no restriction in the amount of food fed to either group.

On the experimental diet, each rat between the ages of 60 and 80 days ate, on the average, 34 g. per day yielding 65 Calories. On the supplemented diet each rat ate 47 g. per day yielding 72 Calories. These figures, of course, refer to grams total food, including milk, green food and moisture.

Further, taking males and females together, during the same period, the animals in the experimental group consumed 47 Calories per g. increase in weight, while the rats on the supplemented diet required 30 Calories for the same increase in weight. That these figures are not wide of the mark is shown by Slonaker's (1931*c*) statement that rats, 60–100 days old, ate daily 60 Calories, and by Kon's (1931) findings that rats, given free choice of food, used 47 Calories per g. increase in weight.

It should be noted that our animals, admittedly not on a rat dietary, compare very favourably with the rats of other workers; our rats, from 60 to 80 days of age, even on the experimental diet, put on 1·4 g. per day, while those on the supplemented diet gained 2·4 g. per day.

The management of the experiment

Method of preparing food. The diet was divided into seven portions, one portion for each day of the week and arranged to give as much variety as possible.

Diets fed on different days of the week

The experimental diet

A		B	
Meat (mince)	631 g.	Fish (cod or ling)	370 g.
Turnip	130	Potatoes	600
Potatoes	600	Onion	56
Flour	220	Fat (roast fat)	41
Carrot	53	Sugar	200
Sugar	100	Bread	400
Bread	300	Raw milk	700 ml.
Raw milk	700 ml.		

C		D	
Oatmeal	285 g.	Rice	50
Potatoes	600	Barley	50
Margarine	65	Sago	40
Syrup	165	Cremola	40
Mealy pudding	25	Peas	62
Eggs	2	Lentils	28
Bread	200		270 g. of mixture
Raw milk	700 ml.	Sugar	200g.
		Bread	400
		Whole milk boiled	654 ml.

E		F	
Fish (smoked or fresh)	370 g.	Potatoes	400 g.
Potatoes	600	Margarine	60
Sugar	200	Sugar	253
Butter	37	Oatmeal	285
Cheese	60	Eggs	3
Bread	400	Bread	200
Apple	56	Raw milk	700 ml.
Raw milk	700 ml.		

G

Bread 980 g.

These portions may not be equal in terms of dry matter, but, by experiment, were found to be equally well consumed by the animals. The quantities given above will feed, in our experience, approximately sixty rats 60–80 days old for 1 week.

All necessary cooking was by boiling. Meat, fish, eggs, oatmeal, flour and vegetables were boiled in water. Potatoes were peeled, boiled, strained and mashed. The pudding mixture (diet D) was boiled in the appropriate quantity of milk. These boiled portions were then poured over the remaining items of the diets, the bread being previously broken into small pieces. The whole was then thoroughly mixed.

Method of feeding. The requirement of the appropriate mixture for each group was weighed and the proportion of milk for the experimental group mixed with their food. Milk and other food for the supplemented group were

fed separately. Metal or enamel dishes, varying in size according to the number and age of rats in a cage, were used. To avoid under-feeding, the amount fed was regulated so that a small quantity remained in the cages each morning. Tap water was given *ad lib.*

Cages. Perforated zinc cages 12 in. × 12 in. × 6 in., with bottom tray for sawdust, were used for nursing mothers and litters, or for four or five growing rats, or for three adults. Galvanised wire mesh cages 18 in. × 16 in. × 22 in., with sleeping compartment raised off floor, carried fifteen to eighteen growing rats or twelve adults. This type of cage has a removable floor over a sawdust tray. Cages were carried on angle iron racks on tiers of three to five.

Housing. Both groups were always housed in the same room.

Temperature. The room temperature was kept at 68°–70° F.

Bedding. Wood-wool was used as bedding.

Cleaning. The smaller cages were cleaned twice or three times weekly according to the number of inmates. The removable floors of the larger cages were washed daily, the sawdust changed three or four times weekly, and the bedding (seldom soiled in this type of cage) changed twice weekly. All cages were thoroughly scrubbed and sterilised periodically. All feeding dishes were thoroughly washed daily and water bottles washed weekly. The colony house was washed out daily.

Handling. The handling of animals was such that they could be lifted from their cages at any time without being unduly alarmed.

Breeding. Mating was carried out in the large cages, three males to nine females. Inbreeding was not practised, but care was taken that the relationship of the mating males and females was the same in each group, and that they belonged to the same generation.

Age at first mating and series of litters in each generation are shown in the following table:

		Experimental group		Supplemented group	
		First mating days	Series of litters	First mating days	Series of litters
Parental generation		120	Until breeding ceased 9–10	120	Until breeding ceased 9–10
F_1	,,	147	4	147	4
F_2	,,	120–180	3	120	3
F_3	,,	Details not complete		160–170	1

Owing to lack of facilities only four series of litters were bred off the F_1 generation, and three series of litters off the F_2 generation. Remating took place immediately a litter was weaned. Succeeding generations were bred from the first litter of each dam, so that every dam in the parental generation was represented in succeeding generations. At birth the sex of all young was determined and numbers reduced to eight per litter, leaving, where possible, equal numbers of males and females. Sexes were separated at weaning or at least within 40 days of birth. In our experience no damage ever resulted to the litter by handling the young within an hour or two of birth.

Weaning. With certain exceptions, litters were weaned at 21 days. The exceptions were a few litters of the experimental group which were in too poor condition at 21 days. These were weaned at 25–28 days.

Weighing. At approximately 6 hours after birth all litters were weighed in bulk, that is, after the litters had suckled. At weaning, and every 7 days thereafter, all were weighed individually.

Haemoglobin estimations. In view of the recent report (Davidson *et al.*, 1933) that human beings on a very similar diet suffer from anaemia, haemoglobin estimations by the Haldane haemoglobinometer were carried out on (*a*) all breeding females in the first and second generations, at parturition and at weaning of litters; (*b*) all breeding males periodically; and (*c*) young animals in both groups at various ages.

Ear-numbering and record keeping. At weaning all rats were ear-numbered consecutively. The method employed is a modification of that cited by Dietrich (1910). Breeding performance of individual females was recorded by a special card index system, whilst general record keeping was such that the ancestry and all data concerning any rat could be traced in a few minutes.

Results

Reproductive capacity

In the experimental group, the time between introduction of the male and casting of the litter was, on the average for 213 litters, 38 days. In the supplemented group, however, the time was much shorter, averaging 28 days for 259 litters. Unfortunately, since no attempt was made to measure the frequency of recurrence of the oestrous cycle in these rats, we have no means of telling whether the delay was due to the male or female, or due to prolongation of the gestation period. This delay had the effect of leaving the experimental group progressively further behind the supplemented group in succeeding litters and generations. In spite of this the percentage of rats, over all generations, eventually conceiving, was very nearly the same, 82 per cent. of 262 matings on the experimental group, and 84 per cent. of 311 matings in the supplemented group.

On the supplemented diet, the numbers born per litter were slightly greater throughout all generations (Table V).

There was also a slight difference with regard to the nursing capabilities of the dams. Death of a complete litter during suckling is probably due to defective milk supply on the part of the mother. On the experimental diet, 13 per cent. of the litters did not reach weaning, on the supplemented diet, 10 per cent.

Table V

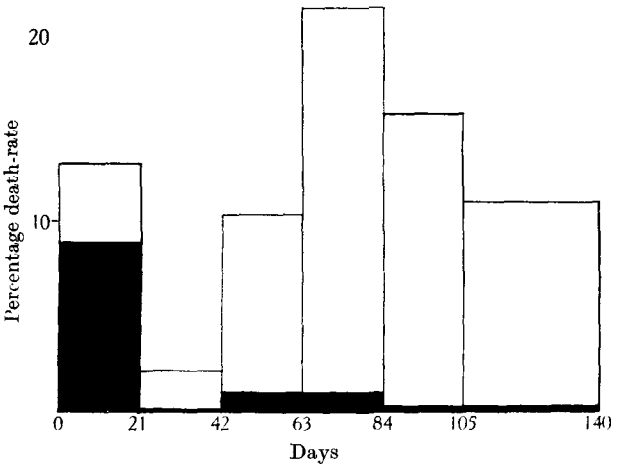
	Experimental	Supplemented
Time between introduction of male and birth of litter—average (days)	38	28
Females conceiving (%)	82	84
Number born per litter	7·29	8·15
Rats stillborn (%)	6·1	3·3
Death of entire litter during suckling (%)	13	10

Death-rate

(*a*) *At birth.* On the experimental diet, 6·1 per cent. were stillborn, on the supplemented diet, 3·3 per cent. (Table V).

(*b*) *Birth to weaning.* 13 per cent of the young on the experimental diet died during suckling, while 9 per cent. died on the supplemented diet. These figures are definitely significant when it is remembered that the number of young per litter was reduced to eight at birth in all cases.

(*c*) *From weaning onwards.* The rats of the experimental diet had a high death-rate compared with those on the supplemented diet, the highest incidence falling between 60 and 100 days of life, when growth was most rapid.



Graph I. Showing the death-rate from birth to 140 days calculated in 21-day periods up to 105 days. Black block—supplemented diet. Whole block—experimental diet.

The death-rate from birth to 140 days is shown in Graph I. In calculating this rate the following precautions were taken:

(1) Rats born dead, rats dying at birth, and rats destroyed to reduce litter number to eight were deducted from the total number born.

(2) At each appropriate point, the number of rats diverted to other experimental work, *e.g.* controlled infection experiments to be described elsewhere, was deducted. The rate therefore represents, at each age, the number of animals dying as a percentage of those exposed to risk. The graph does not extend beyond 140 days, since only animals for breeding were kept beyond this age.

The survival rate calculated from the same data is shown in Graph II.

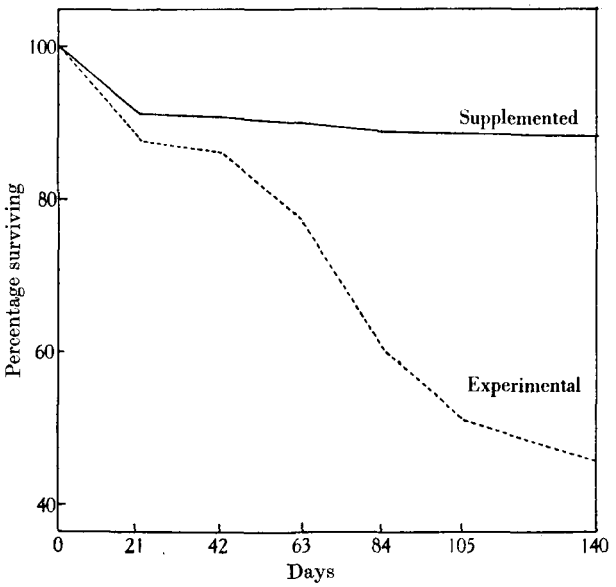
The heavy mortality among the experimental group rats affected the males more than the females.

Table VI shows the numbers of rats used in constructing the graphs. The numbers in the earlier periods are greater, but the numbers even at 105–140 days are adequate to make the results significant, and, at every period, all generations are represented.

Table VI. *Number of rats exposed to risk of death at various ages*

	Birth to weaning	21–42 days	42–63 days	63–84 days	84–105 days	105–140 days
Experimental	1211	899	742	616	473	347
Supplemented	1706	1160	1135	1097	616	325

The influence of diet on the death-rate was shown in spectacular fashion by rearing animals to weaning on one diet and then changing to the other diet at weaning. This was carried out by dividing the litters in two, one-half continuing on the parents' diet, the other half being changed to the diet of the other group. Table VII and Graph III present these results. Unfortunately, the numbers are rather small, but there seems to be little doubt about the



Graph II. Showing the survival rate from birth to 140 days calculated in 21-day periods up to 105 days. Continuous line—supplemented diet. Interrupted line—experimental diet.

effect of the different diets on the death-rate. In addition, there is a definite suggestion that the diet of the mother has an effect on the offspring which persists beyond actual weaning. Among the rats suckled by mothers on the supplemented diet and then transferred to the experimental diet at weaning, no deaths occurred until 63 days after weaning. On the other hand, among the rats reared by mothers on the experimental diet, and transferred at weaning to the supplemented diet, all the deaths occurred within 42 days of weaning.

Table VII. *The effect of diet on mortality*

	No. of rats	No. died 21–160 days	% death-rate
Bred and reared on supplemented diet	39	1	2·5
Bred experimental diet, reared supplemented diet from weaning	72	10	13·8
Bred supplemented diet, reared from weaning experimental diet	39	10	25·6
Bred and reared on experimental diet	73	44	60·3

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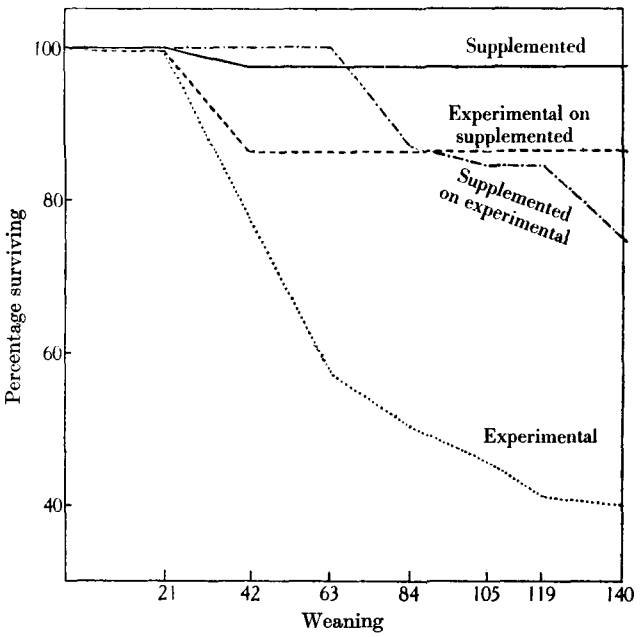
Causes of death

Throughout the whole experiment the great majority of animals which died were examined as soon after death as possible. 340 post-mortem examinations were made on animals from the experimental group, and 70 on animals from the supplemented group. With a few exceptions due to cannibalism, all dead animals were autopsied. Unfortunately, histological work could not be carried out, but, in many cases, a bacteriological examination was made.

The organ systems mainly concerned in the fatalities are shown in Table VIII. The deaths among the supplemented group were too few to allow analysis except in the age group 141 days and over.



Graph III. Showing the survival rate from 21 to 161 days. Rats changed from one group to the other at 21 days, that is at weaning. Continuous line—supplemented diet. Dash line—bred on experimental diet, reared on supplemented diet from 21 days. Dot-dash line—bred on supplemented diet, reared on experimental diet from 21 days. Dot line—experimental diet.

It is obvious that the most common fatal conditions were enteritis and pneumonia. From a large percentage of the cases an organism of the *Salmonella* group was recovered, both from the gut and from the heart blood and spleen. This organism was responsible for epidemics in the colony, and, although both groups were equally exposed to the infection, the fatalities were largely among the animals on the experimental diet. This organism was isolated later and used in controlled infection experiments, which will be described in detail elsewhere. In these controlled infection experiments also, a much larger percentage of the animals on the experimental diet succumbed than of the animals on the supplemented diet.

Table VIII. *Analysis of post-mortem findings*

Age group	Weaning to 63 days	64–84 days	85–105 days	106–140 days	141 days up	Supplemented group 141 days up
No. of rats examined	62	115	57	23	83	52
Intestinal tract						
Enteritis (%)	42	38	39	41	21	19
Nodular thickenings of gut wall (%)	—	—	4	13	54	19
Respiratory system						
Pneumonia (%)	35	62	63	64	12	27
Lung abscess (%)	—	—	—	—	12	11
Cardiovascular system						
Anaemia (%)	26	17	14	—	6	—
Pericarditis (%)	15	14	7	9	1	—
Heart rupture (%)	19	6	4	5	1	4

During the course of routine post-mortem examinations, it was found that many animals showed nodular swellings or thickenings in the distal part of the small intestine and in the proximal and intermediate colon. The incidence of these swellings was much greater among animals on the experimental diet. The nature of these swellings is obscure; they are, in the later stages at least, largely inflammatory, leading to local or general peritonitis. These intestinal nodules seldom occurred before 100 days, but, among the rats on the experimental diet, they were not infrequent from 120 to 140 days, and seemed to occur at a progressively earlier age in succeeding generations. The nature of these swellings is being investigated at the moment.

Affections of the respiratory system were even more common than gastro-intestinal conditions. These, again, were most frequent during the growing period. Associated with the lung conditions was a short-chain *Streptococcus* and, in other cases, a Gram negative bacillus. Lung abscesses, with inspissated pus, were found not infrequently in the older animals in both groups.

Anaemia was definitely present in a considerable proportion of the animals of the experimental group coming to post-mortem. Anaemia was not found in the supplemented group. Pericarditis was probably incidental to acute infection; heart rupture, a surprisingly frequent occurrence, was apparently associated with pyaemia.

Rate of growth

Excluding those born dead, the average weight of the whole litter at birth was 39 g. on the experimental diet and 44 g. on the supplemented diet. The average weights per rat, however, were 5·56 and 5·55 g. respectively. The difference in litter weight, then, was due to the greater number of rats per litter in the supplemented group—*vide supra*.

Table IX shows the numbers weaned and weaning weights, in all generations and series of litters. In the later litters of the F_1 generation the number weaned per litter on the experimental diet was small, and, in consequence, the weight per rat was, at weaning, sometimes higher than that in the supplemented group. Also, within the experimental group itself, the weight at weaning was greater

in the later litters. This suggests that mothers on the experimental diet were incapable of nursing efficiently the number of young which were usually born per litter. Apart from this, however, the weaning weights of the supplemented group were greater throughout all generations, and were remarkably constant. It has to be remembered that following the second pregnancy of the F_1 generation, there was a change from the survey to the experimental diet, and it is difficult to estimate how far this affected the weaning weights of later litters. Certainly, at the weaning stage, there is no suggestion that the experimental group is becoming progressively worse in succeeding generations.

Table IX. *Showing number weaned in the various generations and litters along with average weight of young at weaning*

	Experimental group					Supplemented group				
	Av. no. weaned per litter	No. of males	Av. wt. g.	No. of females	Av. wt. g.	Av. no. weaned per litter	No. of males	Av. wt. g.	No. of females	Av. wt. g.
F_1 generation Litter series										
1	6·7	54	30·5	53	29·5	6·2	50	37·8	56	37·8
2	6·6	52	29·9	48	28·6	6·8	64	41·3	65	38·9
	←	←	←	←	←					
3	7·0	29	30·9	34	29·7	7·4	63	40·3	62	38·0
4	7·1	17	29·3	26	29·9	6·7	57	40·6	51	40·1
5	5·4	23	35·7	26	35·4	6·2	50	39·8	38	39·3
6	4·4	17	41·5	14	36·0	6·5	36	40·5	29	37·4
7	6·5	12	38·5	14	39·0	5·7	19	43·8	27	42·9
8	3·0	5	45·6	4	48·3	7·1	18	40·8	25	38·9
9	3·5	4	47·2	3	46·6	5·0	5	48·8	5	39·4
F_2 generation										
1	6·1	35	31·3	44	31·5	7·1	46	35·6	47	35·5
2	6·2	49	34·9	55	34·6	6·2	43	41·1	56	39·3
3	6·5	80	35·6	77	34·2	6·2	37	41·8	31	41·3
4	6·2	50	34·7	56	36·3	4·3	17	50·0	9	53·5
F_3 generation										
1	6·0	30	37·3	30	37·4	7·4	80	41·2	76	40·2
2	5·5	42	35·8	38	33·3	7·7	66	40·1	73	40·5
3	7·4	20	34·9	17	33·9	7·2	65	42·8	58	40·7
F_4 generation										
1	—	—	—	—	—	6·12	50	40·0	42	39·2

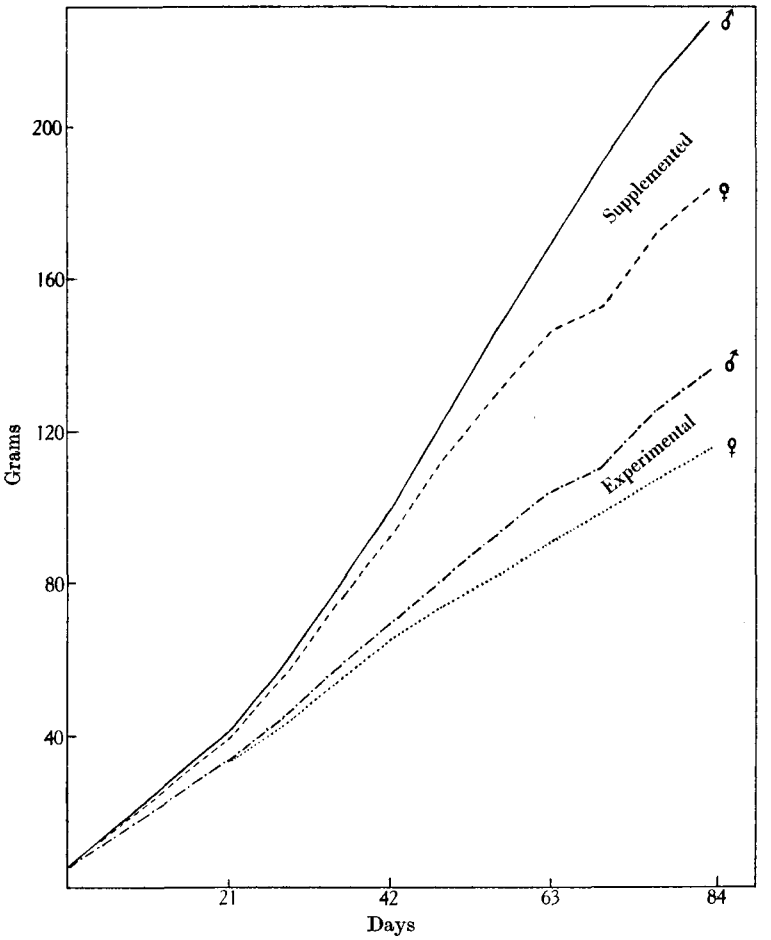
← Previous to this point the rats of this group received the survey diet; subsequently they received the experimental diet.

Graph IV shows a composite growth curve of all rats in both groups up to the age of 84 days. Subsequent to this age, the mortality in the experimental group was so high that graphic representation is not satisfactory. In addition, many rats, especially from the supplemented group, were drawn off at this stage, for other experiments.

Graph V, however, shows the weight curves for a few rats over a period of 392 days. The weights of twenty-two male rats were followed, eleven on the experimental diet representing six litters, and eleven on the supplemented diet representing ten litters. These rats belonged to the F_2 generation; that is, both parents and grandparents had been on the respective diets. The dips *A*, *B*, *C* in the curve of the supplemented group coincide with time of mating, and may be due to the restlessness of the male. For some reason, the experi-

mental group males did not suffer to the same extent. Within each group up to the age of 84 days, we made the somewhat surprising observation that the rate of growth increased progressively with succeeding generations, F_3 being better than F_2, and F_2 better than F_1. The cause of this change is obscure.

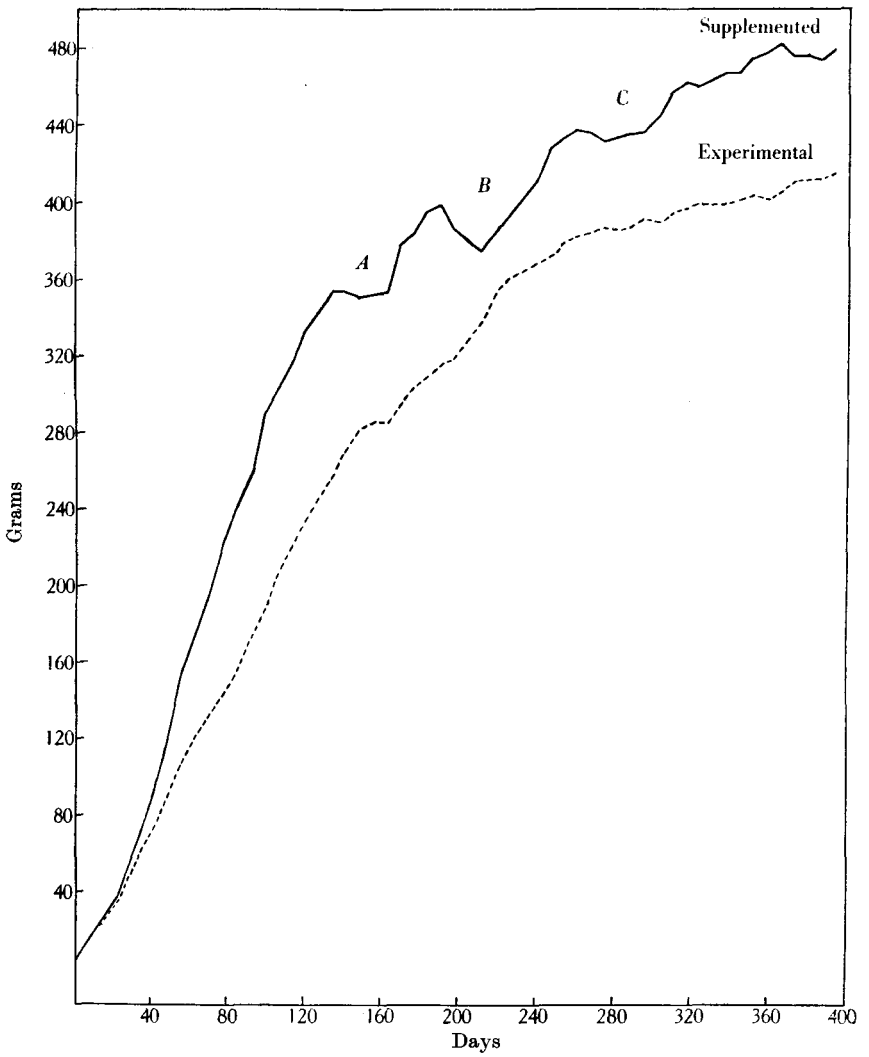
The paramount influence of diet is shown in Graph VI. As already described, this additional experiment was carried out by changing half of each



Graph IV. Composite growth curve of all rats up to the age of 84 days, males and females separately.

litter to the diet of the other group at weaning (see Table VII). The weight curves are constructed from the weights of those animals surviving to 160 days. The weights of animals dying during the course of the experiment are excluded, since these rats were in a sickly condition and usually lost weight some time before death. Males only are included; the females were fewer in number, and it is not justifiable to compare average figures from groups where the ratio of males to females differs since the sexes have very different growth curves.

The animals fed on the supplemented diet are, at the end of the period, at least 60 g. heavier per rat than the animals fed on the experimental diet. Again, as in the death-rate of the same animals (Graph III), there is a definite



Graph V. Growth curve of eleven males on supplemented diet and of eleven males on the experimental diet up to the age of 392 days. *A*, *B*, *C* show dips in curves coinciding with mating periods.

indication of persistence of the influence of the diet of the mother which suckled the animals. The animals from the experimental group fail to make up the leeway they have lost.

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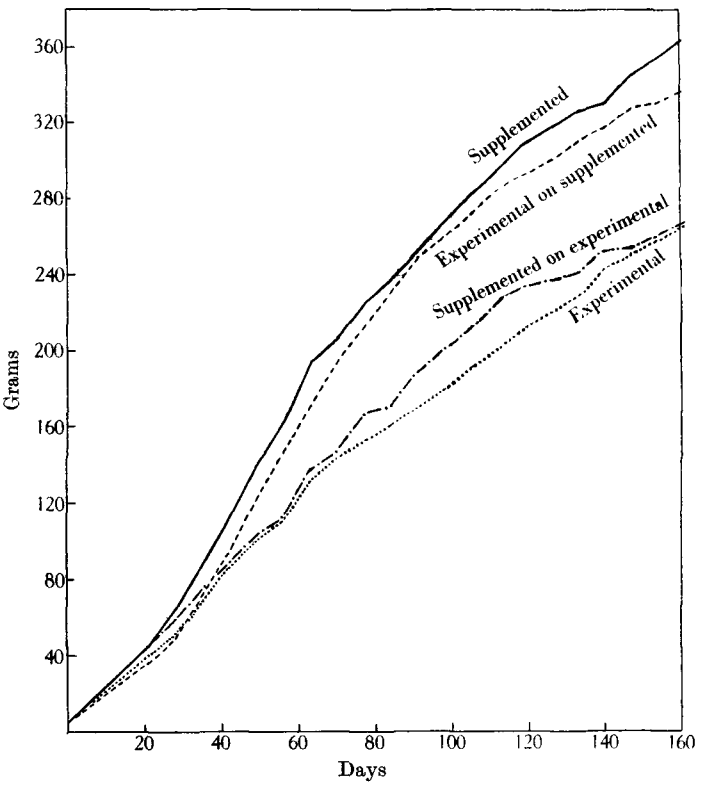
Haemoglobin

The percentage of haemoglobin in the blood, estimated from time to time as described above, showed a striking difference between the growing rats on the experimental and supplemented diets. At weaning, rats are known to have a low blood haemoglobin (Donaldson, 1924; Mitchell and Miller, 1931). Four to five weeks thereafter the Hb reaches 90–100 per cent. Our rats on the supplemented diet behaved in this fashion, but the rats on the experimental diet had at weaning a lower Hb than normal and took much longer, often with definite setbacks, to reach anything like the normal value for the adult.



Graph VI. Growth curves of rats changed from one diet to other diet at 21 days compared with the curves of those rats remaining on their original diets.

Haemoglobin estimations in females of both groups, at parturition and at weaning, in successive pregnancies showed no progressive change, nor was there any striking difference between the rats on the two diets. In both groups, parturition caused a very obvious fall in haemoglobin level, which rose rapidly even during the course of suckling. Graph VII shows the results obtained in the parental generation. On this graph the peaks represent the values at weaning and the depressions the values at parturition in successive pregnancies. The upper curves give the values for males of the same age.

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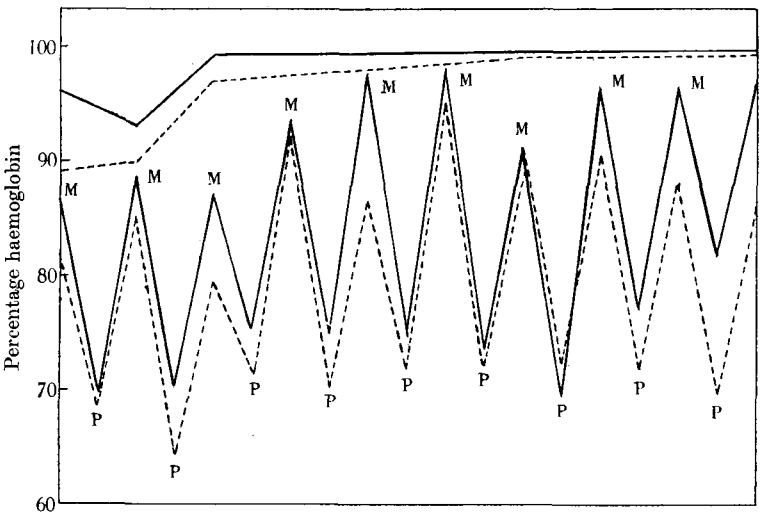
Clinical condition

It is possible in arbitrary fashion to divide the life span of a rat into three ages: (1) birth to weaning, (2) weaning to 100 days, which includes the period of maximum growth and puberty, and (3) over 100 days, the period of diminishing rate of growth and adult life.

Birth to weaning. In the experimental group, a few litters had a definitely unkempt appearance. Apart from this, and the difference in size, there was little to choose between the groups.

Weaning to 100 *days.* In a large number of animals on the experimental diet, the pigment of the coat changed from a dense black to a dirty grey. These animals looked anaemic, and their coats were staring. They were limp when



Graph VII. Showing average percentage haemoglobin of all rats in the parental generation commencing at 120 days of age. Record covers nine successive matings. Upper two lines—males. Lower two lines—females. Continuous lines—supplemented diet. Interrupted lines—experimental diet. *M*—mating; *P*—parturition. Mating occurred at weaning, at 21 days.

handled, not at all playful and often vicious. About the age of 70 days, there was frequently an accentuation of the general malaise, emaciation occurred, the abdominal wall was retracted and tense, and diarrhoea set in. In the majority of cases the illness ran an acute course, and the outcome was fatal. In some cases death followed on a more protracted illness, while a certain number of animals made a spontaneous recovery. Such conditions were of rare occurrence among the rats on the supplemented diet.

Over 100 *days.* Those animals on the experimental diet which survived the earlier critical period usually regained the black pigment in their fur to some extent, and the coat was less staring, but it never attained the glossy sleek appearance of the animals on the supplemented diet. They grew normally but failed to catch up with their companions on the supplemented diet.

In late life the animals on the supplemented diet often became extremely

obese. So great became the accumulation of abdominal fat in some cases, that the abdomen actually dragged along the floor of the cage. Pl. VI, figs. 1 and 2 show better than words can express the differences between the rats of the two groups.

Discussion

The diets

From the literature it is very difficult to find what is a "normal" rat diet, most authors being content to enumerate the proportions of the different foodstuffs in the diet without further analysis. The composition of rat's milk has been gauged by analysing the stomach contents of young rats immediately after suckling. Hatai (1917) finds 10·1 per cent. proteins, 2·4 per cent. carbohydrate and 31·5 per cent. fat; while Mayer (1933) finds 7·5 per cent. protein, 3·4 per cent. carbohydrate and 19·8 per cent. fat. Kellermann (1934) recommends that 16·8 per cent. of the Calories should come from protein, 68·2 per cent. from carbohydrate and 15 per cent. from fat; while Slonaker (1931*a*, 1931*b*, 1931*c*, 1931*d*, 1931*e*, 1931*f*) found that 14 per cent. of the Calories should be derived from protein to ensure optimal growth and development, but that a deviation of 4 per cent. on either side was not significant. The source of the protein, animal or vegetable, also seemed to be of no great moment. From this it seems that all our diets were at the lower limit of permissible protein content. Our data, too, are derived almost exclusively from rats during the phase of active growth and reproduction when protein of good quality is so necessary.

The fat content of both our diets was high, judged by the rat standards of other workers, but it seems unlikely, in view of the high level of fat in rat's milk, that this feature had any detrimental effect on the health of our animals.

The carbohydrate content of the experimental diet, and still more of the supplemented diet, was lower than that usually advocated for rats.

So far as the proximate principles are concerned, it is difficult *a priori* to take gross exception even to our experimental diet.

The mineral content plays a not unimportant part in the nutritive qualities of a diet. Too great a discrepancy in either direction between the Ca and P contents leads to disturbances in growth and in health. Working on rats, Simmonds (1924) recommended a Ca:P ratio of 1:0·63, and Bethke *et al.* (1927, 1932, 1933) found the permissible variation to be between 1:0·55 and 1:1. Kramer and Howland (1932) advocate a ratio of 1:0·66, and Kellermann (1934) has a ratio of 1:0·72 in his optimum stock diet. Cox and Imboden (1934*a*, 1934*b*) found the optimum ratio for gestation, lactation and weight of young, to be between 1:0·66 and 1:1. All workers agree that departure from such ratios increases the need for vitamin D in the diet.

Judged by these standards, the Ca:P ratio of the survey and experimental diets is unbalanced, and even the supplemented diet barely passes muster. The improvement in the ratio in the supplemented diet is due mainly to the

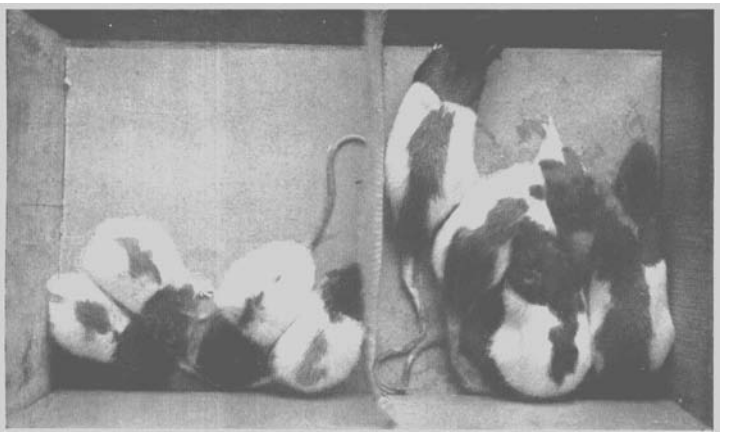


Fig. 1. Showing difference in general appearance between groups at 78 days old. Left, experimental diet; right, supplemented diet. Note the alert appearance of those on the supplemented diet.

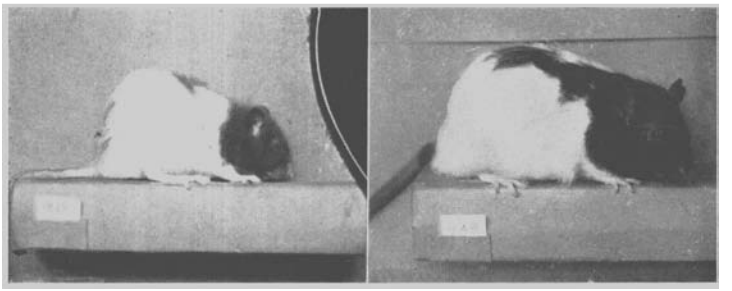


Fig. 2. Showing difference in coat pigment and general appearance at 78 days old. Left, experimental diet; right, supplemented diet. Note pale pigment and dull eye of rat on experimental diet compared with jet-black pigment and keen eye of rat on supplemented diet.

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green food. In our experience of these diets, macroscopic rickets was seen in one or two rats only at the very outset of the experiment when the survey diet was used.

None of our diets, either on rat or on human standards, was deficient in Fe or Cu.

The importance of vitamin content presents a thorny problem. The difference in vitamin C, since we were using rats, was probably of little moment, and the vitamin B complex was presumably adequate in both diets. D was quite possibly deficient, especially in the original survey diet. The vitamin A content of the supplemented diet as compared with the survey diet, or the experimental diet, is perhaps the most striking single differential feature of the diets. This difference would appear even more marked had we adopted the value for kale given by Sherman and Todhunter (1934). These authors find that 1 g. fresh curly kale is equivalent to 200γ carotene. However, no gross signs of vitamin A deficiency were observed on the experimental diet. Work is in progress to find if addition of carotene as a source of vitamin A is capable of approximating the biological value of the experimental diet to that of the supplemented diet.

Taking the diets as a whole, certain differences merit further discussion. Since the classical paper of Hopkins (1912) and of Hopkins and Neville (1913) it has been recognised that even small quantities of milk in the diet of rats play a very important part in nutrition. Sherman *et al.* (1921, 1922*a*, 1922*b*) and Steenbock (1923) have also shown the importance of milk in the ration. It seems justifiable to conclude from the experience of other workers, and from our own experience in the early days of the experiment, that milk is of great value, especially in a diet such as ours, with the protein content bordering on the lower permissible level. It is just under such circumstances that the addition of a protein of high biological value would be expected to exert its influence.

The importance of green food is more difficult to evaluate. The green food was responsible for the improvement in the Ca:P ratio, and for the marked increase in vitamin A. The roughage of the green food made no obvious difference to the faecal pellets. On both diets the faeces were somewhat bulky and soft.

An attempt has been made above to analyse and to discuss the differences between the survey, experimental and supplemented diets, but we feel that, with our present state of knowledge, it would be thoroughly unwise and unwarrantable to lay too great weight on such analysis, and, even more, to see in any one constituent the vital differentiating factor or factors. In some way or other, the addition of milk and of green food has converted a biologically poor into a biologically good diet.

Results

Reproductive capacity

So far as reproductive capacity is concerned, our rats, both on the experimental and supplemented diets, compare favourably with the rats used by other workers (Donaldson, 1924; Simmonds, 1924; Slonaker, 1931*a*, etc.). On this score no criticism can be levelled at our use of human diets for rats. The better nursing capability of the dams on the supplemented diet is due, we think, to the increased quantity of milk fed. When one considers the composition of rat's milk and the rate of growth of the suckling young, this is not surprising.

Death-rate

It so happened that, twice during the course of the experiment, large numbers of rats were bred on both diets simultaneously. When these animals reached 63–84 days, the period of maximum growth and of puberty, the death-rate was at its maximum. An organism of the *Salmonella* group was undoubtedly the most important causative agent. Although, from the nature of the housing and of the feeding of the rats, the animals on both groups were constantly exposed to infection, the fatalities occurred almost wholly among the rats on the experimental diet. The cause of this differential mortality rate is obscure. Fridericia (Faber and Norgaard, 1934), and other workers agree that two or three International units of vitamin A per rat per day suffice for normal growth, but that eight times as much vitamin A may be required to protect from infection. Even on the experimental diet the rats received about 23 International units of vitamin A per day and, on the supplemented diet, about 230 such units per rat per day. It would be attractive to see in the large vitamin A content of the supplemented diet the factor protecting the mucous membrane of the gut against what must have been a virulent strain of micro-organisms. But we feel that more work is required before we dare come to this conclusion, and the problem is being investigated at the moment by means of controlled infection experiments.

Growth

The difference in growth rate shows, in striking fashion, that the evaluation of a diet in terms of Calories is most untrustworthy. On neither diet was the intake of food restricted, and the experimental diet had actually a higher caloric value per g. food than the supplemented diet. The computed caloric intake per rat per day in both groups was very close. Yet the rats on the supplemented diet used 30 Calories per g. increase in weight, while the rats on the experimental diet required 47 Calories per g. increase in weight.

The prevalence of anaemia among the rats on the experimental diet cannot be attributed to lack of Fe and Cu as such. It is known, however, that the form in which the iron is presented is of importance. Green food is of value in forming haemoglobin in rats already anaemic (Levine *et al.*, 1932). Gastro-intestinal

infection, with consequent defective absorption of iron, is another possible factor.

The effect of pregnancy on the haemoglobin of rats has been studied by Sure *et al.* (1928), Mitchell and Miller (1931), Beard and Myers (1933), and by Van Donk *et al.* (1934). There is surprisingly little difference between our two groups of rats, and no significant departure from the standards set by other workers.

Clinical condition

We are unable to explain the depigmentation of the rats on the experimental diet. Gorter (1934) attributes depigmentation in hooded rats to a diet excessively rich in fat and carbohydrate, but our two diets are not sufficiently dissimilar in this respect to warrant such an explanation in this case.

The excessive obesity of the adult rats on our supplemented diet suggests that a human dietary, in this minor respect, is not suitable for rats. Our stock rats, on a stock rat ration, do not show such obesity, although they take no more exercise than did the rats in this experiment. It is possible, of course, that the unhealthy and unsightly obesity which afflicted the older rats on the supplemented diet is not so much an indictment of the use of a human dietary for rats, as an indictment of the diet consumed by human beings adopting more and more a sedentary occupation.

This leads directly to the most controversial aspect of the whole problem: to what extent can results, obtained from rats nourished on a human dietary, be used to elucidate problems of human nutrition?

Although the experimental diet used by us approximated closely to the food eaten by human beings, yet there were differences. The milk in the rat experimental diet was double the average quantity consumed by the population investigated in the human dietary survey. In addition, the human population did eat, on the average, a small amount of green food, but the amount was so small that it could not conveniently be fed to the rats on the experimental diet.

Since there is no reason to believe that rats are more dependent on green food than are human beings, and, since the human diet represents an average of the food consumed by over sixty families, there are good grounds for suspecting a definite deficiency of green food in some, if not in all, of the diets consumed by the households studied in the human dietary survey. The spectacular result from doubling the milk allowance also suggests the need for a greater milk consumption by human beings.

It may be objected that there is no evidence at the present time for such gross malnutrition with heavy mortality among human beings, even on the poorest diets, as we found in our rats on the experimental diet. It must be remembered, however, that our rats were exposed to an environment, in the hygienic sense, and to a risk of infection, which can seldom be operative in a human community at the present day. Nevertheless, if one can trust the records of the past, human beings once lived under conditions which can be

likened, without too great a stretch of the imagination, to the environment of our rats. It was then that the infant mortality and the death-rate in early life reached proportions not unlike those found in our rats on the experimental diet.

Until comparatively recent times, preventive medicine has been concerned chiefly with restriction of the spread of the organismal seeds of disease. Without belittling the importance of better environment and of better hygiene for public health, it does seem that too little attention has been paid in the past to the state of nutrition of the bodies of human beings, the soil in which micro-organisms grow.

In our rat colony the environmental conditions were the same for both groups of rats, all rats alike were undoubtedly exposed to risk of infection, yet the animals receiving the supplemented diet remained healthy. Thus we feel it is justifiable to suggest that diet is as important as, if not more important than, environment from the point of view of public health.

If our results are applicable, even to some extent, to human beings, they suggest that a large section of the human population is still far from the optimum state of nutrition, and that much could still be done, by means of improved food supply, to raise the resistance to infection, and to improve the physique of human beings.

Summary

For $2\frac{1}{4}$ years a large colony of rats was maintained on a diet based on a dietary survey of a human population. One-half of the rats was fed on the human survey diet, or this diet with a small increase in milk, the other half on the same diet supplemented with additional milk and green food. Four generations of animals were reared, all from the same stock.

The rats on the human diet with additional milk and green food were healthy in all respects so far as can be judged from our own rats on a stock diet and from the data of other workers.

On the other hand, in spite of an exactly similar environment and heredity, the animals without additional milk and green food, showed:

(1) a slightly impaired reproductive capacity;

(2) a very markedly increased death-rate due to increased susceptibility to an infection to which all rats were equally exposed;

(3) a definitely slower rate of growth;

(4) a lower haemoglobin content in the blood; and,

(5) a clinically poorer condition as judged by behaviour and state of the coat.

These findings are discussed and the possibility of applying them to human dietetic problems briefly touched upon.

REFERENCES

Beard, H. H. and Myers, V. C. (1933). *Amer. J. Physiol.* **106**, 449.
Bethke, R. M. and Edgington, B. H. (1927). *Proc. Amer. Soc. Animal Prod.* 13.
Bethke, R. M. *et al.* (1932). *J. Biol. Chem.* **98**, 389.
—— (1933). *J. Agri. Res.* **47**, 331.
Clark, M. L. (1929). *Lancet*, i, 1270.

CORRY MANN, H. C. (1926). *Med. Res. Counc. Sp. Rep. Ser.* 105.
COX, W. M. and IMBODEN, M. (1934*a*). *J. Biol. Chem.* **105**, Proc. 18.
—— —— (1934*b*). *Proc. Soc. Exp. Biol.*, N.Y., **32**, 313.
DAVIDSON, L. S. P. *et al.* (1933). *Brit. Med. J.* i, 685.
DIETRICH, W. (1910). *Swine.* Sanders Pub. Co., Chicago, Ill.
DONALDSON, H. H. (1924). *The Rat.* Wistar Institute, Philadelphia.
FABER, A. and NORGAARD, A. (1934). *Haandbog I. Diætetik.* Levin and Munksgaard, Copenhagen.
GORTER, F. J. (1934). *Nature*, **134**, 382.
GUHA, B. C. (1934). *Ind. J. Ped.* **2**, 30.
HATAI, S. (1917). *Amer. J. Anat.* **21**, 23.
HOPKINS, F. GOWLAND (1912). *J. Physiol.* **44**, 425.
HOPKINS, F. GOWLAND and NEVILLE, A. (1913). *Biochem. J.* **7**, 97.
KELLERMANN, J. H. (1934). *Onderstepoort J. Vet. Sci. and Animal Industr.* **2**, 649.
KON, S. K. (1931). *Biochem. J.* **25**, 473.
KRAMER, B. and HOWLAND, J. (1932). *J. Nutrition*, **5**, 39.
LEIGHTON, G. and CLARK, M. L. (1929). *Lancet*, i, 40.
LEIGHTON, G. and MCKINLEY, P. L. (1930). *Milk Consumption and the Growth of School Children.* H.M. Stationery Office, Edinburgh.
LEVINE, H. *et al.* (1932). *J. Nutrition*, **5**, 295.
MAYER, D. T. (1933). *Amer. J. Physiol.* **105**, Proc. 71.
MCCARRISON, R. (1927*a*). *Ind. J. Med. Res.* **14**, No. 3.
—— (1927*b*). *Ibid.* **14**, No. 4.
—— (1931). *Brit. Med. J.* i, 966.
MCCULLOCH, W. E. (1929–30). *West African Med. J.* **3**.
MCGONIGLE, G. C. M. (1933*a*). *Proc. Roy. Soc. Med.* **26**, 677.
—— (1933*b*). *Med. Officer*, **49**, 215, 225.
MCLAUGHLIN, L. *et al.* (1931). *J. Nutrition*, **4**, 115.
MITCHELL, H. S. and MILLER, L. (1931). *Amer. J. Physiol.* **98**, 311.
ORR, J. B. (1928). *Lancet*, i, 202.
ORR, J. B. and CLARK, M. L. (1930). *Ibid.* ii, 594.
ORR, J. B. and GILKS, J. L. (1931). *Med. Res. Counc. Sp. Rep. Ser.* 155.
Report of Committee on Nutrition (1933). Brit. Med. Assoc., London.
ROSENBERG, L. C. (1931). *Amer. J. Dis. Child.* **41**, 303.
SHERMAN, H. C. *et al.* (1921). *J. Biol. Chem.* **46**, 503.
SHERMAN, H. C. and CROCKER, J. (1922*a*). *Ibid.* **53**, 49.
SHERMAN, H. C. and MUHLFELD, M. (1922*b*). *Ibid.* **53**, 41.
SHERMAN, H. C. and TODHUNTER, E. N. (1934). *J. Nutrition*, **8**, 347.
SIMMONDS, N. (1924). *Amer. J. Hyg.* **4**, 1–108 (suppl.).
SLONAKER, J. R. (1931*a*). *Amer. J. Physiol.* **96**, 547.
—— (1931*b*). *Ibid.* **96**, 557.
—— (1931*c*). *Ibid.* **97**, 15.
—— (1931*d*). *Ibid.* **97**, 322.
—— (1931*e*). *Ibid.* **97**, 573.
—— (1931*f*). *Ibid.* **97**, 626.
STEENBOCK, H. (1923). *Science*, **58**, 449.
STIEBELING, H. K. (1933). *U.S. Dept. Agri. Misc. Publ.* No. 183.
SURE, B. *et al.* (1928). *J. Nutrition*, **1**, 299.
TURBOTT, H. B. and ROLLAND, A. F. (1932). *New Zealand Med. J.* **31**, 109.
VAN DONK, E. C. *et al.* (1934). *Amer. J. Physiol.* **107**, 616.

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