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Growth, Reproductive Productivity and Longevity of Mice Fed Polyunsaturated Ruminant-derived Foodstuffs

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Abstract

The effects on the well-being of mice of feeding linoleic acid-enriched foodstuffs derived from ruminants receiving protected polyunsaturated oil were determined. Growth, reproductive productivity and longevity were compared in mice fed freeze-dried human diets containing either these products or the corresponding conventional ruminant-derived foods. A laboratory mouse pellet diet was used as a standard for the comparison.

Growth rates and life spans were found to be similar in all three groups. Although the reproductive productivity of the mice on the polyunsaturated diet was never significantly different from that of mice on the conventional human diet, productivities of both groups on human diets were usually significantly below those of mice eating pellets. By the third generation of the second reproduction study, productivities of both these groups of mice had returned to the same level as those of mice eating pellets. Possible reasons for the depression in reproductive productivity in mice on the human diets are discussed.

It was concluded that the polyunsaturated human diet neither impaired nor improved the lives of the animals eating the diet relative to the lives of animals eating the conventional human diet.

Introduction

Ruminant-derived foodstuffs, such as beef, lamb, milk and dairy products, which contain elevated levels of linoleic acid, have recently become available (McDonald and Scott 1977). These foods are produced by feeding ruminants a supplement in which the polyunsaturated fat is encapsulated in a protein shell modified by reaction with formaldehyde (Scott *et al.* 1971; Pan *et al.* 1972). The fat is protected against hydrogenation by rumen microflora, and the polyunsaturated fat is subsequently released in the abomasum for absorption into the tissues of the animals.

It can justifiably be claimed that these new foods have not been previously available to man and that some nutritional evaluation would be desirable. This is particularly so as the foods result from the use of a novel supplement, the production of which involves the application to oilseeds of alkali, formaldehyde and elevated temperatures. The possibility exists that new and unusual compounds with possible side effects could be produced during the production of the supplement, or after its consumption by the ruminant, or during the processing, storage, cooking or consumption of the derived meat or milk products. This paper describes some experiments in which mice were fed two freeze-dried human diets based either on conventional meat and dairy products, or on their linoleic acid-enriched equivalents. The formulation of the diets was the same, being essentially that of a typical Australian diet based largely on foodstuffs derived from ruminants. Other normal components of a typical human diet were included in the freeze-dried mixtures, so that the remote possibility of a

deleterious interaction between the ruminant foods and other dietary items could be allowed for. The proportions of all the ingredients were the same in both diets. The experiments afforded an opportunity of comparing the effects on mice of two human diets essentially identical except for their fatty acid composition, although it was anticipated that the human diets, with their relatively high fat content and lack of cereal fibre, might not be as suitable for mice as a normal laboratory mouse-cube diet. The latter was included in some experiments as an absolute control. Previous experience had shown that high-fat diets containing beef and milk could be used successfully in reproductive studies for feeding rats and mice (Brown *et al.* 1959; Johnson 1965).

The intake of nutrients by mice consuming the human diets was estimated by reference to food composition tables. Both the human diets should have provided adequate supplies of nutrients in terms of the N.R.C. requirements for laboratory mice (Bell 1962).

The results of three experiments are reported here; firstly a preliminary study to ascertain the effects of the diets on the growth rate of the mice, and then two larger studies on the effects of the diets on growth, reproductive productivity and longevity of the mice.

Materials and Methods

Experimental Animals and their Management

Mice used were albino animals from a strain selected for large litter size (Quackenbush). Young animals and adult females were housed in groups of five, breeding animals were housed two per cage, and adult males kept for longevity trials were housed singly. Cages were of the shoebox type, wood wool or shredded paper was provided for nesting, and food and water were supplied *ad libitum*. The animal room was maintained at 21 ± 2°C and day length was regulated to 14 h light, 10 h dark. Due to circumstances beyond our control it was necessary to move the animals to new premises with new cages and equipment at the commencement of the third experiment, which led to some temporary management problems in the early part of that experiment.

Diets

Three basic diets were used, namely two diets based on either conventional ruminant-derived foodstuffs (*C*) or their linoleic acid-enriched counterparts (*P*), and a commercial pelleted diet (*N*).

The diet *C* represented a normal human diet (Table 1) based on foodstuffs derived from ruminants, the ingredients of which were cooked (if required), homogenized, blended, freeze-dried and broken up to provide a uniform biscuit-like product for the mice. The diet *P* contained the same ingredients in the same proportions as in the diet *C*, except that the meat and dairy products used in the formulation were derived from ruminant animals fed a formaldehyde-treated sunflower seed–casein supplement, and contained about 20% linoleic acid in their fat as compared to the 2–3% present in the fat of the conventional meat and dairy products (Scott *et al.* 1971; Pan *et al.* 1972). The freeze-dried diets were both manufactured in the same way. Handling procedures for both diets were such as to minimize the possibility of auto-oxidation.

All the processing operations up to the final wet mix stage were carried out at the laboratory. Freeze-drying of the final wet mix was carried out by a commercial organization. The dry product was packed in cans which were evacuated and flushed with nitrogen before sealing. The cans were held at -15°C until required.

In the third experiment there were some modifications in the method of manufacture and composition of the freeze-dried diets *C* and *P* (Table 1). The diets were prepared and freeze-dried at the Armed Forces Food Science Establishment at Scottsdale, Tasmania, and packed under vacuum. The entire operation from raw material to final packed product was completed within 48 h, and the product was held in an atmosphere of nitrogen after freeze-drying. Due to logistics problems it was not possible to obtain linoleic acid-rich milk and butter for the production of the *P* diet, and consequently both *C* and *P* batches were formulated using the appropriate type of cheese, together

with lactose, to replace the milk and butter while maintaining the same level of milk-fat present in the earlier diets. A major advantage of using cheese to replace milk was that the freeze-dried diets could be prepared without the antioxidant butylated hydroxyanisole (BHA), since linoleic acid-enriched cheese can be prepared without BHA (Czulak *et al.* 1974).

The commercial pelleted diet (*N*) was supplied by Allied Feeds (Sydney). The ingredients used in this diet were as follows: wheat, hominy meal, coconut meal, milk powder, lucerne meal, meat meal, dried yeast, bone flour, salt and antioxidant (ethoxyquin). The pellets contained about 23% protein, but had less fat (5%) and more crude fibre (6%) than the human diets (Table 1).

In additon to these basic diets, vitamin supplements were given to some of the animals in generation 2 of the second experiment and to two of the four groups in the third experiment. In the second experiment these were vitamin B1, vitamin B6 and multivitamin supplements (Janos Chemicals, Sydney). In the third experiment two of the four groups were given tocopherol acetate (Rovimix E, type 20W, water-dispersible, Hoffmann La Roche). Supplements were given in the drinking water, dosages being estimated on the basis of a water intake of 3 ml per mouse per day. Details of dosages, etc., are described on page 4.

Fatty Acid Analysis

Representative samples (5 g) of the diets were analysed for fat content, the lipid being extracted by the procedure of Bligh and Dyer (1959). The recovered lipid was transesterified using the method of Glass and Christopherson (1969). The methyl esters were analysed by gas–liquid chromatography (g.l.c.) [Packard model 7401, 170°C, glass U-tube columns 180 mm long by 2 mm i.d., packed with 10% SP-222PS (Supelco Inc., Pennsylvania)]. Abdominal fat samples from mice were extracted with chloroform (2 ml) by macerating the tissue (up to 100 mg) with a glass rod and allowing the mixture to stand overnight, with anhydrous sodium sulfate added to remove water from the extract. An aliquot of the filtered extract was taken and the dissolved lipid was transesterified as above for analysis by g.l.c.

Tocopherol Assay

Lipid extracts of the dietary components, or of the *N*, *C* or *P* diets, were assayed for tocopherols by saponification followed by spectrofluorometric assay using essentially the procedure of Hansen and Warwick (1970). An Aminco–Bowman spectrofluorometer was used, with an excitation wavelength of 295 nm and emission either at 327 or 340 nm (Duggan 1959). Appropriate standard solutions of tocopherol were used. Mouse adipose tissue was weighed and saponified directly for the assay rather than extracting the lipid first. Milk, cheese and butter were extracted with hexane using the procedure of Low and Dunkley (1971) to obtain the fat for the assay.

BHA Content and Peroxide Value

BHA in the diet *P* and its components was determined by a standard steam distillation procedure (Eastman Chemicals 1969), and peroxide values on the test diets were determined by the American Oil Chemists' Society (1974) method.

Experimental Design

Experiment 1. Growth rate and food intake

When 90 mice born to mothers fed on the mouse pellet diet (*N*) reached 3 weeks of age they were divided into three groups of 30 animals (15 males, 15 females). One group was fed diet *N*, the second the control diet *C*, and the third the polyunsaturated diet *P*. Immediately before being transferred to these diets the mice were weighed. Weighings were repeated at weekly intervals thereafter until the animals reached 7 weeks of age. Food intakes were measured for each week from the time the animals began to consume the diet until they were 7 weeks old, when they were killed and the fatty acid composition of the abdominal fat of each mouse was estimated.

Experiment 2. Reproductive productivity, growth rate and longevity

Generation 0. Three-week-old offspring of 27 pairs of mice fed on mouse pellets were divided into two groups of 100 mice (50 males, 50 females). One group was placed on diet *C* and the other

group on diet *P*. When the animals reached 10 weeks of age they were paired; sib matings were avoided. Young from the first-born litters were kept as parents for the next generation; young from second and subsequent litters were discarded at weaning.

At 19 weeks half the males from each group were transferred to individual cages for longevity studies. The remaining males were discarded. When the final litters were weaned, females were grouped five per cage for longevity studies. These mice were examined about once a week for survivors.

Generation 1. Reproductive productivities of three groups of animals were tested in this generation: (i) mice born to parents fed diet *N* and receiving diet *N* throughout the trial, (ii) mice born to generation 0 parents fed the control diet *C* and fed the diet *C* themselves throughout their lives, and (iii) mice born to generation 0 parents fed the polyunsaturated diet *P* and fed the diet *P* throughout their lives.

Reproductive productivity was measured by pairing the mice for 9 weeks; again, sib matings were avoided. The following records were kept for each pair: the number of litters born, litter size at birth, and litter size at 3 weeks. Numbers of young reared to 3 weeks by each pair were calculated by adding all the young in all litters born to that pair which survived to 3 weeks of age. All young in the firstborn litter of each pair were weighed at 3 and at 6 weeks. Second and subsequent litters were usually discarded at weaning. Parents for the next generation were drawn from the firstborn litters. After the males and females were separated about half the males and all the females were set aside for longevity studies.

Generation 2. In the two groups receiving the test diets *C* and *P*, 50 male and 50 female mice were selected from the offspring of 20 pairs of generation 1. In each dietary treatment these were divided into five lots of 10 pairs of mice, and the diets of these mice were supplemented daily with various vitamins as follows: sub-group 1, no vitamin supplementation; 2, vitamin B1 (10 μg per mouse); 3, vitamin B6 (12·5 μg per mouse); 4, vitamins B1 and B6 (10 and 12·5 μg per mouse respectively); 5, multivitamin mixture which provided the following intakes of vitamins per mouse per day: 25 i.u. vitamin A; 2·5 i.u. vitamin D3; 0·0125 i.u. vitamin E; 8·69 μg vitamin K; 10 μg vitamin B1; 20 μg vitamin B2; 75 μg niacin; 25 μg calcium d-pantothenate; 12·5 μg vitamin B6; 0·05 μg vitamin B12; 100 μg vitamin C. The reproductive capacity of this generation was measured as before, but this time all the progeny were discarded. Only the female and male parents whose diet had not been supplemented with vitamins were retained for histopathological study.

Ethane evolution was measured in male mice from generation 1 in an attempt to detect *in vivo* lipid peroxidation (Riely *et al.* 1974; Hafeman and Hoekstra 1975). Mice on each of the three test diets were each given an injection of carbon tetrachloride and placed in metabolism jars. The ethane content of the exhaled air was determined by g.l.c. (McGlasson 1969).

Experiment 3. Reproductive productivity, growth rate and longevity

During the second experiment (outlined above) litter sizes in the third generation of mice on the diet *P* were lower than expected, and it was concluded (see Results, below) that this was due to a deficiency of vitamin E in the diet, probably caused by faulty manufacture. The experiment was repeated using the modified *C* and *P* diets (formula 2) prepared by the Armed Forces Food Science Establishment (see above). The husbandry and experimental design of experiment 3 were similar to those of experiment 2 and three generations of mice were examined. One hundred male and 100 female mice were randomly allocated to either the diet *C* or the diet *P* at weaning, and the *C* and *P* groups were further divided into subgroups which were or were not receiving tocopherol acetate in the drinking water (110 μg per mouse per day). The subgroups are designated *C* and *CE* or *P* and *PE*, the suffix *E* (for vitamin E) referring to the subgroup receiving tocopherol acetate. About 23–25 pairs were in each group. The fertility of the male mice in each group was checked prior to commencement of the experiment by mating them with females on a conventional mouse cube diet.

Statistical Analysis

Values for mortality between birth and 3 weeks in the reproductive productivity data are expressed as percentages, but for the statistical analyses these percentages were transformed using the standard arc sine transformation of binomial data

$$\sin^{-1}\sqrt{[(\text{number of deaths}+0\cdot375)/(\text{number born}+0\cdot75)]}$$

(Anscombe 1948).

Fertile matings are expressed as a percentage of total matings. For the analysis of these results a generalized linear model with binomial errors (Nelder and Wedderburn 1972) was postulated. The systematic effects (e.g. diet, generation) were added in turn to the model, and the deviance from the model, which is analogous to residual sum of squares in regression, was calculated at each addition. Thus the change in deviance, as each term is added, measures the variation in fertility which is 'explained' by that term. The change in deviance is distributed approximately as χ^2, so that the probability of achieving a change in deviance as great or greater can be calculated.

The following procedure was adopted for analysing the longevity of the mice. Using the 5-week age intervals 11–15 weeks, 16–20 weeks, ..., the numbers of mice that died during each age interval in each treatment group were formed into a mortality table for each sex separately. The mortality was assumed to have a binomial distribution. The following model was fitted to the data:

$$y = \text{age} + \text{diet} + \text{generation} + (\text{diet} \times \text{age}) + (\text{generation} \times \text{age}) + (\text{diet} \times \text{generation} \times \text{age}),$$

where $y = \log[-\log(1-p)]$ and p is the mortality as a proportion of the number alive at the beginning of each age interval. This complementary log log transformation of the mortality was proposed by Mather (1949). Each term of this model was added in turn, beginning with age, and, as in the analysis of the fertility data above, the change in deviance due to each addition, and the associated probability were calculated.

Results and Discussion

Composition of Diets

For each formula (Table 1) the two human diets *C* and *P* had approximately the same composition, the difference being only in the fatty acid composition of the lipids. The fatty acids of diet *C* contained 2–3% of linoleic acid, while those of diet *P* contained about 20% linoleic acid. There was about 28% of linoleic acid in the fatty acids of the mouse pellets (*N*). Mice on diet *P* received 40–50 mg of linoleic acid/g diet, those on diet *C* received 5–10 mg linoleic acid/g diet, and those on pellets received 10–15 mg linoleic acid/g diet. These ranges take into account the formula differences and batch-to-batch variations in the fat and linoleic acid contents of the diets and their components.

Food Intake, Growth Rate and Fatty Acid Composition of Fat Depots of Mice Eating Diets N, C *and* P (*Experiment 1*)

The food intake of the mice eating pellets (*N*) (mean 5·95 g per mouse per day) was significantly greater than the intakes of mice on diets *C* and *P* (2·98 and 3·22 g per mouse per day respectively). There was no significant difference between the intakes of the human diets. Male mice ate more than females. The difference in intakes of mice on the pellet diet versus the two human diets could not be attributed solely to the higher caloric content per unit of the human foods (about 21 J/g compared with about 17 J/g for the pellets); other factors which could be involved include the difference in fibre contents of the diets, a tendency for the mice to discard unpalatable parts of the pellets (i.e. consumption overestimated), and the somewhat hygroscopic nature of the *C* and *P* diets (increase in weight, hence consumption underestimated).

Although male mice were always significantly heavier than female mice at the final weighing (7 weeks), the differences between the weights of males (or females) eating the *N*, *C* and *P* diets were not significant. Weights of males at 7 weeks from the three dietary groups were as follows: *N*, 32·7 g; *C*, 33·6 g; *P*, 32·1 g. In the reproductive studies described in the next section, young born to mothers eating the *N*, *C* and *P* diets were weighed at 3, 6 and 20 weeks of age. In these groups, too, no significant differences in growth rate were observed between the three dietary groups.

The linoleic acid contents of the fatty acids in the abdominal fat of the mice eating the *N*, *C* and *P* diets from weaning to 7 weeks of age were as follows: *N*, 17·2%; *C*, 4·3%; *P*, 20·5%.

Table 1. Composition of human diet used in experiments

Ingredient	Amount (g)[A]	
	Formula 1	Formula 2[B]
Milk[C]	615	—
Meat (50 : 50, lamb : beef)[D]	350	350
Cheese[C]	25	255
Butter[C]	55	—
Lactose	—	27
Cereal (Cornflakes)	27	27
Bread	120	120
Fruit (canned)	300	300
Fruit (fresh)	100	100
Vegetables (green)	200	200
Potatoes[D]	200	200
Dripping[E]	20	—
Sugar	30	30
Jam	50	50
Salt	7	7
Total (wet weight)	2099	1666
Approx. solids content	575	593
Protein (% dry weight)	19·7	24·6
Fat (% dry weight)	27·7	23·1
Crude fibre (% dry weight)	1·0	1·0

[A] Suggested daily intake of a human (formula 1).
[B] Cheese-based formula used in experiment 3 (see text).
[C] Butylated hydroxy anisole (BHA) and tocopherol acetate were each added at 10 mg/l to the linoleic acid-enriched milk.
[D] Boiled for 30 min prior to inclusion in mixture.
[E] Linoleic acid-enriched dripping contains BHA (0·02% w/w).

Reproductive Productivity

(i) *Reproductive productivity of mice fed* N, C *and* P *diets* (*experiment 2*)

Table 2 summarizes both the total productivity and the contribution of its components of mice from generation 1 of the first reproductive trial, i.e. the generation in which the animals eating the human diets had been exposed to the *C* and *P* diets from conception and in which a control group eating the *N* diet was examined. Differences between the productivities of the animals eating the *P* and *C* diets were small, suggesting that polyunsaturated fats had little or no effect on reproduction. However, differences between the productivities of animals eating the human diets and those eating the *N* diet were large, the main factor contributing to this difference being the high mortality among the nestlings in the groups eating the human diets. The contribution of diet to these mortality differences between the three experimental groups was highly significant ($P < 0{\cdot}001$).

There was some evidence that the high mortality among the nestlings of animals eating the human diets may have been due to neurological disturbances since a small number of young, particularly those on the *C* diet, exhibited gait disturbances. Electrophysiological and histopathological studies were therefore carried out on affected and normal mice. Although some affected animals showed a mild neuropathy, most showed normal nerve conduction and only minor pathological abnormalities—not enough to cause the severe gait disturbance observed. It was concluded that the deleterious effects of the diet might be due to disturbances of the central nervous system rather than to disturbances of the peripheral nervous system.

Table 2. Total reproductive productivity, together with the components of total productivity, of mice fed mouse pellets (*N*), control human diet (*C*), or polyunsaturated human diet (*P*) (generation 1 of experiment 2)

	Diet		
	N	*C*	*P*
Number of matings[A]	25	49	48
Fertile matings (%)	92	94	92
Number of litters born[B]	59	112	104
Mean litters per fertile pair	2·6	2·4	2·4
Mean litter size[C]	9·6	8·7	8·5
Number of young born	564	973	858
Mortality 0–3 weeks (%)	7·1	37·0	26·5
Mean number of pups reared to 3 weeks per mating[D]	21·0	12·5	13·1

[A] Matings in which the male survived for at least 9 weeks after the mice were paired, and in which the female survived for at least 15 weeks.
[B] Includes litters, born to obviously pregnant females, which disappeared *in toto* before the cages were examined for newborn litters.
[C] Mean of all litters in which some young were found. Litters which disappeared *in toto* were excluded.
[D] This value includes the infertile pairs.

(ii) *Reproductive productivity of mice fed* C *and* P *diets supplemented with vitamins (experiment 2, generation 2)*

Because vitamin deficiencies could have been responsible for the neurological disturbances and high mortalities observed among mice eating the human diets, the next generation of this trial (generation 2) was divided into five lots of 10 pairs within each dietary group (*C* and *P*); subgroup 1 received no supplements, whilst subgroups 2–5 received vitamin B1, vitamin B6, vitamin B1+B6, and a multivitamin mixture respectively. A variance analysis of the results showed that these supplements had no effect on mortalities among pups born to animals eating either the *C* diet or the *P* diet, and the incidence of mice exhibiting gait disturbances was of the same order as that observed in generation 1, namely 7 affected litters out of 96 among animals on the *C* diet and 1 affected litter out of 72 among animals on the *P* diet.

Unexpectedly, the variance analysis of these data showed that in generation 2, unlike generation 1, the animals eating the *P* diet had significantly fewer litters per fertile pair than animals eating the *C* diet ($P < 0{\cdot}001$) and total productivity was therefore lower in the *P* group than in the *C* group.

(iii) *Reproductive productivity over three generations of mice fed modified (formula 2) C and P diets, with or without supplementary vitamin E (experiment 3)*

The unexpected results from the reproductive study of the second experiment ultimately led to the whole study being repeated in this third experiment.

The number of litters per pair (1·9) from mice on diet *P* in generation 2 was not consistent with the number per pair from mice on diet *P* in the preceding generation of experiment 2 (2·4 litters per pair). The symptoms of a drop in reproductive capacity, but with no indication of decreased growth rate, were indicative of a deficiency of vitamin E. This was initially discounted because tocopherol acetate in sufficient quantity to maintain a concentration of approximately 0·6 mg vitamin E per gram of linoleic acid had been added to the milk, and indirectly (via the milk used in manufacture) to the cheese and butter used in preparing the formula 1 diet *P* (Table 1). An attempt was also made to detect vitamin E deficiency in the mice indirectly by testing for *in vivo* lipid peroxidation by measuring ethane in the air exhaled by the mice after injection of carbon tetrachloride. No differences in ethane production between the three groups was observed, indicating no differences in *in vivo* peroxidation, and hence presumably no inadequacy of vitamin E in any of the groups.

In order to clarify the situation, the tocopherol contents of the diets and their components, and of the adipose tissue of the mice receiving the *C* and *P* diets, were determined. The tocopherol contents of the linoleic acid-rich butter and cheese were considerably less than expected on the basis of tocopherol acetate added to the milk, but still higher than those of the conventional butter and cheese, and the level in diet *P* was higher than that of diet *C*. Both BHA and tocopherol acetate were added to the linoleic acid-rich milk on the assumption that they would partition into the fat phase and be carried through into the butter and cheese. This proved to be the case with BHA, but a considerable proportion of the tocopherol acetate remained in the aqueous phase, resulting in the level of tocopherol in the cheese and butter being considerably lower than expected. However, the tocopherol content of abdominal adipose tissue was similar in *C* and *P* mice of the same age, and a second factor must have been involved in the reduction of litters from the *P* mice.

No oxidation should have occurred in the diets *P* and *C* during storage as the commercial organization responsible for the freeze-drying and packaging of these diets had undertaken to exclude oxygen from the products by packing them under nitrogen. However, oxidation must have occurred in three of the eleven batches of diet, because while the antioxidant BHA was shown to be present in the linoleic acid-rich dairy products, no BHA could be detected in three batches of diet *P* after 6 months storage. In addition, peroxides could be detected in each of the last three batches of both diets *C* and *P* which had been used for feeding the last generation of mice in experiment 2. Analysis of the headspace gases in unopened cans of these batches revealed that air was present, showing that the products had not been packed under nitrogen as specified. The previous eight batches of diet *C* and eight batches of diet *P* appeared to have been correctly packed under nitrogen. These first eight batches of each type had been used shortly after production and it was only the last three batches which were subjected to a lengthy storage period.

The evidence presented above strongly suggested that, in the second experiment, the impaired reproductive capacity of the generation 2 mice on diet *P* was a result of an imbalance between the level of dietary linoleic acid and tocopherol, which arose because of two factors, namely the loss of added tocopherol acetate from the linoleic

acid-rich butter and cheese, and the slow oxidation which occurred due to the long storage of the last three batches of freeze-dried diets in air instead of in nitrogen.

The second reproductive study was therefore carried out using diets with a formula slightly different from that used in experiments 1 and 2 (see Table 1). Because of the possibility of imbalance between levels of dietary linoleate and tocopherol, the 50 pairs of animals in each dietary group (*C* and *P*) in each generation were divided into two sub-groups, one fed the unsupplemented diet, the other fed the same diet supplemented with vitamin E.

Table 3 summarizes the total reproductive productivity and the components of productivity of mice fed *C*, *CE*, *P* and *PE* diets. Table 4 shows the significances of differences between the groups.

Table 3. Total reproductive productivity of mice fed either unsupplemented control human diet (*C*) or polyunsaturated human diet (*P*), or the same diets supplemented with vitamin E (experiment 3). Three generations of mice were examined

Diet		Unsupplemented			Vitamin E-supplemented		
		Gen. 0	Gen. 1	Gen. 2	Gen. 0	Gen. 1	Gen. 2
C	Number of matings[A]	25	25	25	23	25	24
	Fertile matings (%)	92	80	80	87	72	83
	No. of litters born[B]	52	47	52	41	39	49
	Mean litters per fertile pair	2·3	2·4	2·6	2·0	2·2	2·4
	Mean litter size[C]	8·8	8·4	10·4	8·2	8·6	10·8
	Number of young born	459	384	542	327	337	528
	Mortality 0–3 weeks (%)	40·1	6·8	9·2	47·1	16·3	14·8
	Mean No. of pups reared to 3 weeks per mating[D]	11·0	14·3	19·7	7·5	11·3	18·8
P	Number of matings[A]	24	25	25	25	23	25
	Fertile matings (%)	92	88	80	96	65	60
	No of litters born[B]	48	44	49	50	32	33
	Mean litters per fertile pair	2·2	2·0	2·4	2·1	2·1	2·2
	Mean litter size[C]	8·5	9·4	9·9	7·8	8·5	8·9
	Number of young born	400	414	483	367	264	294
	Mortality 0–3 weeks (%)	32·5	22·5	6·4	41·7	24·6	7·5
	Mean No. of pups reared to 3 weeks per mating[D]	11·3	12·8	18·1	8·6	8·7	10·9

[A,B,C,D] See footnotes A, B, C and D of Table 2.

It is apparent from Table 4 that diet was without effect on any of the components of productivity, suggesting that substitution of polyunsaturated ruminant-derived foodstuffs for conventional ruminant products is unlikely to be detrimental to fertility. Contrary to expectation, the vitamin E supplement caused a small but significant rise in pup mortality. The reason for this is not clear, but it is unlikely to be due to vitamin E *per se*; it is almost certainly associated with one of the minor components of the water-dispersible supplement used.

Quite unexpectedly, generation was found to have a marked effect on all components of productivity: in generation 0 the total productivities of mice eating unsupplemented *C* and *P* diets were comparable with those of mice eating unsupplemented *C* and *P* diets in the first reproductive trial, but, by generation 2, the pro-

Table 4. Analysis of deviance from models fitted to reproductive productivity data of mice fed either unsupplemented control or polyunsaturated human diets, or the same diets supplemented with vitamin E (experiment 3). Three generations of mice were examined

Model term	Fertile matings per total matings			No. of litters per fertile mating			Litter size			% Mortality (transformed)		
	d.f.	Change in deviance	$P(\chi^2)$	d.f.	Mean square	F	d.f.	Mean square	F	d.f.	Mean square	F
Generation	2	11·6815	0·003**	2	2·1318	4·23*	2	152·54	11·53***	2	5·7131	42·29***
Diet	1	0·2294	0·632	1	1·0837	2·15	1	16·25	1·23	1	0·0064	—
Supplement (E) (Suppl.)	1	3·2435	0·072	1	0·8951	1·78	1	19·08	1·44	1	0·7362	5·45*
Diet × suppl.	1	1·0347	0·309	1	0·2045	—	1	23·44	1·77	1	0·1691	1·25
Gen. × diet				2	0·2144	—	2	30·09	2·27	2	0·3149	2·33
Gen. × suppl.				2	0·1375	—	2	2·29	—	2	0·0344	—
Gen. × diet × suppl.				2	0·1999	—	2	5·35	—	2	0·0093	—
Terms involving litter order							27	10·61	—	27	0·1352	—
Residual	6	5·2320	0·514	227	0·5038		490	13·227		490	0·1351	

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

ductivities of animals eating the unsupplemented human diets were as high as those of mice eating the *N* diet (compare Tables 2 and 3). This improvement in total productivity was due largely to a decline in pup losses during the nursing period although increases in the number of litters per fertile pair and increases in litter size also contributed to the improvement. These changes with increasing generation number may have been due to abnormally high pup losses in generation 0 resulting from management problems arising from the change in premises and equipment at the start of the experiment, but selection for mice which could do well on human diets may also have contributed, for only about $\frac{1}{2}$ to $\frac{2}{3}$ of the pairs from one generation were used to provide offspring for the next generation.

Longevities of Males and Females Eating Diets N, C *and* P

Longevity data were obtained on mice pooled from generations 0 to 1 of the first reproductive productivity trial (experiment 2). Percentage survivals in each age class of male and female mice eating *N*, *C* and *P* diets were calculated (in this context only survival beyond 10 weeks of age was considered). Numbers in each group were: males, *N* 25, *C* 51 and *P* 54; females, *N* 25, *C* 99 and *P* 95. An analysis of deviance showed that, after the effects of age were eliminated, none of the other factors examined had an effect on mortality rates, i.e. diet had no effect on mortality rate.

The longevity data of experiment 3 were analysed in a similar manner and the same conclusion was reached.

Conclusions

In Quackenbush mice receiving freeze-dried human diets based either on conventional ruminant-derived foodstuffs or on their linoleic acid-enriched counterparts, the difference in fat composition between the two diets had no effect on growth, reproductive productivity or mortality rate. In the first reproductive productivity trial (experiment 2), mice fed human diets reproduced less well than mice fed mouse pellets. In generation 0 of the second reproductive productivity trial (experiment 3) mice on human diets also had relatively low productivities but by generation 2 of this trial the reproductive productivity of animals on the human diets was as good as that of animals eating mouse pellets.

Depressed productivity in the first trial and in generation 0 of the second trial was due to high mortality among nestlings. In the first trial the mortality among *C* and *P* nestlings was associated with an unsteady gait and with scouring, but high mortality among *C*, *CE*, *P* and *PE* nestlings in generation 0 of the second trial was associated with high losses soon after birth. These may have been due, to some extent, to management.

At least some of the high mortality among nestlings was probably dietary in origin. Possible causes could have been:

(1) The high fat content of the human diets. The reproductive productivity of some strains of mice is known to be affected by the amount of fat in the diet (Knapka *et al.* 1977) and diets high in fat are known to cause hyperlipaemic neuropathies (Sandbank and Bubis 1973). However, mice in the last generation of experiment 3 did as well as mice on the pellet diet in experiment 2, but this could have been due to selection for animals which could tolerate high fat.

(2) A deficiency of B vitamins. There is no evidence for this from the second experiment where the diets were supplemented with B vitamins.

(3) Vitamin E deficiency. While this may have been a contributing factor in impaired reproductive capacity of generation 2 mice on the diet *P* in the second experiment, there is no evidence to suggest that lack of vitamin E was responsible for mortality among nestlings. In fact in experiment 3, mice on supplements of vitamin E had somewhat higher mortality in nestlings than those not on supplements.

It seems possible that the vitamin E supplement was in some way responsible for the lower performance of the supplemented animals, and the matter has been referred to the manufacturers of the supplement for investigation.

The data for longevity of female Quackenbush mice in both studies shows that there is essentially no difference in longevity between mice on the *P* diet and mice on the *C* diet, in both cases the longevity being similar to that of mice fed on conventional laboratory cubes.

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References

American Oil Chemists' Society (1974). 'Official and Tentative Methods'. 3rd edn. Method Cd. 8–53.

Anscombe, F. J. (1948). The transformation of Poisson, binomial, and negative binomial data. *Biometrics* **35**, 246.

Bell, J. M. (1962). Nutrient requirements of the laboratory mouse. NAS–NRC, Publ. 990, pp. 39–49.

Bligh, E. G., and Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–17.

Brown, W. D., Johnson, A. R., and O'Halloran, M. W. (1959). The effect of the level of dietary fat on the toxicity of phenolic antioxidants. *Aust. J. Exp. Biol. Med. Sci.* **37**, 533–48.

Czulak, J., Hammond, L. A., and Horwood, J. F. (1974). Cheese and cultured dairy products from milk with high linoleic acid content. *Aust. J. Dairy Technol.* **29**, 124–8.

Duggan, D. E. (1959). Spectrofluorometric determination of tocopherols. *Arch. Biochem. Biophys.* **84**, 116–22.

Eastman Chemicals (1969). Extraction of high-fat-content food products for BHA and/or BHT. Eastman Food Laboratory Standard Procedures 19 and 19A. Publication number ZG.163.

Glass, R. L., and Christopherson, S. W. (1969). A method for the differential analysis of mixtures of esterified and free fatty acids. *Chem. Phys. Lipids* **3**, 405–8.

Hafeman, D. G., and Hoekstra, W. G. (1975). Protection by vitamin E and selenium against lipid peroxidation *in vivo* as measured by ethane evolution. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 939.

Hansen, L. G., and Warwick, W. J. (1970). A fluorometric micro method for fat tocopherol. *Clin. Biochem.* **3**, 225–9.

Johnson, A. R. (1965). A re-examination of the possible teratogenic effects of butylated hydroxytoluene (BHT) and its effect on the reproductive capacity of the mouse. *Food Cosmet. Toxicol.* **3**, 371–5.

Knapka, J. J., Smith, K. P., and Judge, F. J. (1977). Effect of crude fat and crude protein on reproduction and weanling growth in four strains of inbred mice. *J. Nutr.* **107**, 61–9.

Low, E., and Dunkley, W. L. (1971). Separation of interfering compounds in the determination of tocopherol in milk. *J. Dairy Sci.* **54**, 1699–701.

McDonald, I. W., and Scott, T. W. (1977). Foods of ruminant origin with elevated content of polyunsaturated fatty acids. *World Rev. Nutr. Diet.* **26**, 144–207.

McGlasson, W. B. (1969). Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. *Aust. J. Biol. Sci.* **22**, 489–91.

Mather, K. (1949). The analysis of extinction time data in bioassay. *Biometrics* **5**, 127–43.

Nelder, J. A., and Wedderburn, R. W. M. (1972). Generalized linear models. *J. R. Stat. Soc., Ser. A* **135**, 370–84.

Pan, Y. S., Cook, L. J., and Scott, T. W. (1972). Formaldehyde-treated casein-safflower oil supplement for dairy cows. I. Effect on milk composition. *J. Dairy Res.* **39**, 203–10.

Riely, C. A., Cohen, G., and Liebermann, M. (1974). Ethane evolution: a new index of lipid peroxidation. *Science* **183**, 208–10.

Sandbank, U., and Bubis, J. J. (1973). Hyperlipaemic neuropathy: experimental study. *Brain* **96**, 355–8.

Scott, T. W., Cook, L. J., and Mills, S. C. (1971). Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. *J. Am. Oil Chem. Soc.* **48**, 358–64.

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