FEEDING AND BREEDING OF LABORATORY ANIMALS

XI. VITAMIN E DEFICIENCY IN MICE ON A DIET CONTAINING 85% OF WHOLE-GRAIN CEREALS, AFTER THE ADDITION OF 2% OF COD-LIVER OIL

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(With 5 Figures in the Text)

INTRODUCTION

A cubed diet for rats and mice is described in Part IX (Bruce & Parkes, 1949) of this series which, with drinking water but no other supplement, was excellent for rapid growth and intensive reproduction. The first batch, diet 39, was prepared without a specific source of fat; the cubes proved crumbly and wasteful, and in order to give them cohesion 2 % of cod-liver oil was added to the second batch. This also served to ensure an adequate content of vitamins A and D which would otherwise be dependent on products such as fish meal liable to vary greatly from sample to sample. On these diets rats and mice successfully reared six or more litters during the period of study (6–7 months); and the first generation of mice bred on diet 39, although reared from weaning on a diet known to be inadequate for reproduction, were in turn bred successfully (Bruce & Parkes, 1949).

The cube containing 2 % of cod-liver oil (diet 41, original formula) became the standard diet for all rats and mice maintained at this Institute. About 7 months after its introduction paralysis shortly after weaning was observed in a single litter of young rats. The animals so affected died. A fortnight later the same thing occurred in another litter, and thereafter the condition appeared sporadically. The symptoms were highly suggestive of vitamin E deficiency. All the females which had given birth to these litters were over 1 year old, and their requirement for vitamin E would therefore be particularly high (Evans & Emerson, 1939). Thus the diet became suspect.

Soon after the appearance of these symptoms in young rats a high mortality of litters was recorded among cross-breeding mice in another experiment. From 100 mating pairs only thirty-seven first litters were weaned, forty-three died from neglect or were eaten by the parents shortly after birth, and twenty were aborted. Such a mortality was far greater than could be attributed to seasonal variation. Moreover, it took place in the spring when, under normal conditions, reproduction would be expected to improve. This further demonstrated that the addition of 2 % of cod-liver oil to the cubes had made a profound difference to their efficacy as a breeding diet.

The destructive action of cod-liver oil on vitamin E is well known. It has been the subject of much experimental work (Burr & Barnes, 1943; Mason, 1944), and

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as mentioned in the previous paper (Bruce & Parkes, 1949), the cod-liver oil in diet 41 was reduced to 1 % in all batches made after 1 March 1949. Nevertheless, for several months both rats and mice had received the diet containing 2 % of cod-liver oil. A further study of the effect of this alteration in the cod-liver oil content of the cubes is reported here.

MATERIAL AND METHODS

The housing and general care have been described in detail elsewhere (Parkes, 1946; Bruce, 1947; Bruce & Emmens, 1948). The mice were mated as monogamous pairs, the male being left continuously with the female so that mating could take place at post-partum oestrus.

It was not possible to distinguish between true resorption gestations and pseudopregnancy with bleeding, because the females were not sacrificed at the end of an apparent pregnancy from which no young were born. The danger of confusing these two conditions in rats has been discussed by Mason (1942), and if the latter condition occurs in mice, it was not separately identified in these experiments. In the description which follows, these two possible events and litters which were killed at birth before the young could be counted have been grouped together under the general term 'pregnancy failures'.

The animals were from the same stocks as described in Part IX (Bruce & Parkes, 1949) of this series, with the addition of two closely inbred strains of mice; Champagne mice obtained originally from Glaxo Laboratories Ltd., and Wild Type mice of the Grey Lethal strain described by Grüneberg (1943).

Vitamin E was supplied as a 1 % solution of α-tocopherol acetate in olive oil given by mouth at a level of about 25 mg. solution per mouse per week.

The cubed diets were prepared by Messrs J. and H. Robinson Ltd., Deptford Bridge Mills, London, S.E. 8, according to the composition given in Table 1.

The average weight of young at weaning is closely related to the number of young in the litter. For this reason only litters contributing weaned young have been included in calculating average size of litter weaned.

EXPERIMENTAL RESULTS

Failure of lactation in an established colony

As soon as the diet became suspect attention was given to the behaviour of a small mouse colony for which detailed records were available. Previously the colony had been maintained on diet 32 (Table 1). It was composed of two mating groups: (*a*) thirty pairs which were 6 months old when the change to diet 41 took place, no replacements being made as the females died or failed to become pregnant, and (*b*) a younger group of twenty-four pairs in which replacements were made when necessary to keep the number constant.

The proportion of litters killed in the whole colony in successive 2-monthly periods was first calculated. The results are shown in Fig. 1. Once the change of diet had been made, a steady rise had taken place in the proportion of litters killed until after 7–8 months from 70 % of the pregnancies no young were weaned. But,

Table 1. *Percentage constituents of diets* 32, 39 *and* 41 *with theoretical composition*
(Calculated from *Bull. Minist. Agric., Lond.*, no. 124.)

	Diet no.			
	32	39	41 First batch	41 Final formula
Wholemeal flour	58	45	45	46
Sussex ground oats	—	40	40	40
Dried meat and bone meal	6	—	—	—
Fish meal	—	11	8	8
Dried yeast	12	0·5	1	1
Full-cream dried milk	20	—	—	—
Dried skimmed milk	—	3	3	3
Cod-liver oil	2	—	2	1
Sodium chloride	1	0·5	1	1
Calcium carbonate	1	—	—	—
Digestible protein	18·4	15·0	13·6	13·7
Soluble carbohydrate	47·8	48·3	48·4	49·0
Fat	8·9	2·5	4·5	3·5
Fibre	0·5	1·4	1·4	1·5

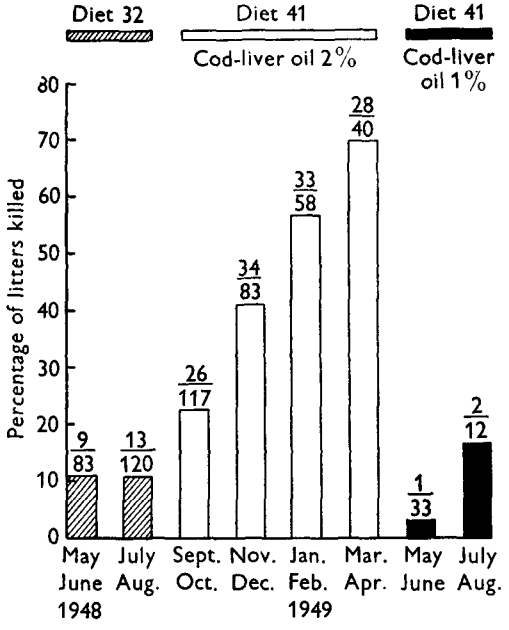

Fig. 1. Failure of lactation in mice on a diet containing 2 % of cod-liver oil, and recovery after reduction of the cod-liver oil to 1 %. Proportion of litters killed in successive 2-monthly periods.

by this time, more than half the females in the colony were over a year old and on these grounds alone, a fall in the production of young weaned was to be expected (Bruce, 1947). With the reduction of the cod-liver oil content of the cubes to 1 % the proportion of litters killed immediately fell, but as this was coincident with the withdrawal of the older females from the colony, both factors might have contributed to the result.

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To distinguish between them the average production of young per female was tabulated for the two groups separately. The results are shown in Fig. 2. In the older females production was maintained for about 2 months after the change of diet and thereafter it fell precipitously. In younger females production rose for

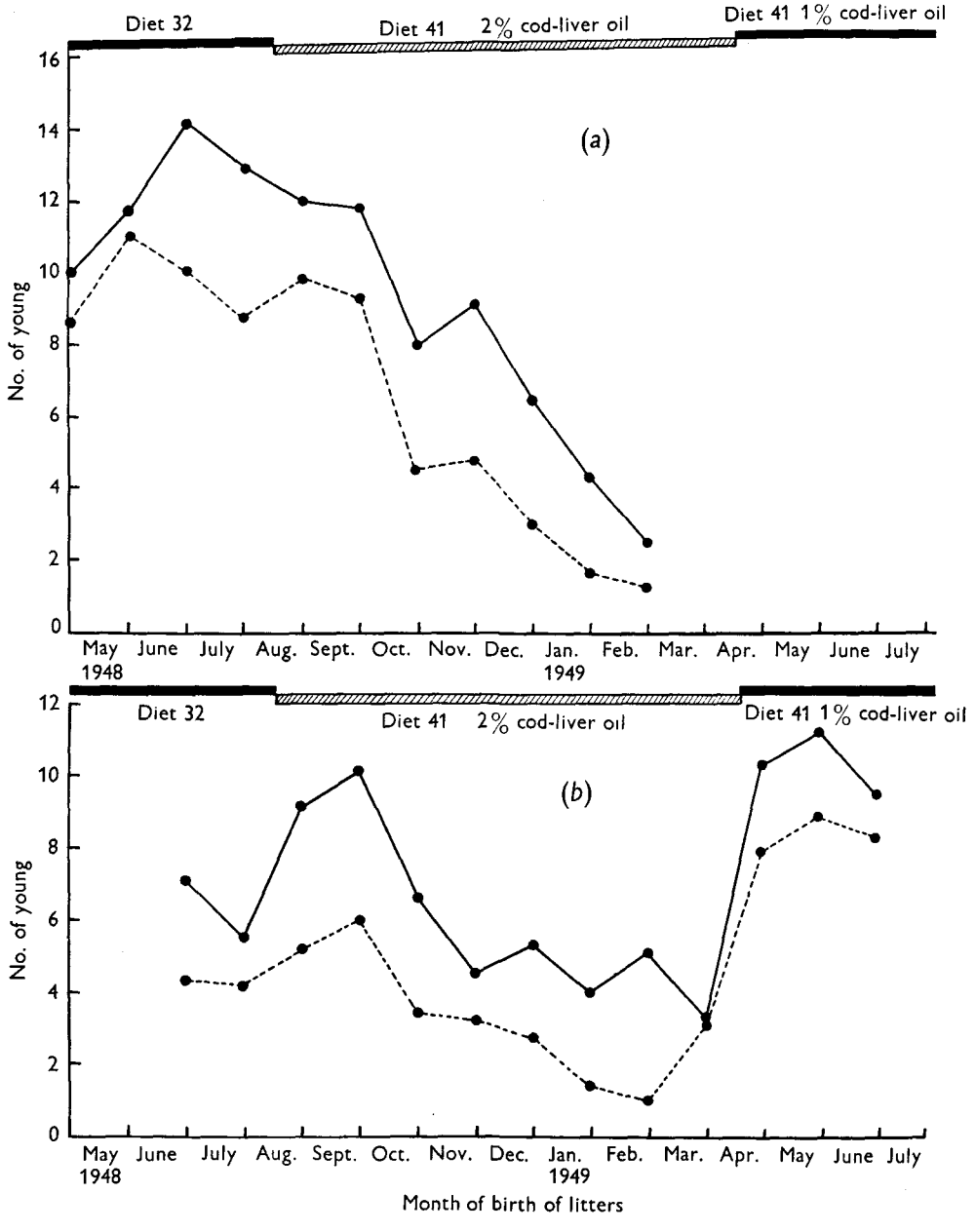

Fig. 2. The gradual failure of reproduction in mice on a diet containing 2 % of cod-liver oil, and recovery after reduction of the cod-liver oil to 1 %. Average production of young per female per month: (*a*) thirty monogamously mated pairs. No replacements. (*b*) Twenty-four monogamously mated pairs. Replacements made as required. ●—● number of young born; ●---● number of young weaned.

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their first few litters which covered the period of the change of diet. This was to be expected because, in mice, there is an increase in litter size with parity for early litters (King, 1916–17; Parkes, 1924; Bittner, 1936). However, production was not maintained, and it ultimately fell to the same low level as that of the older group. Reducing the cod-liver oil in diet 41 to 1 % resulted in immediate recovery, and the monthly production rose to a higher level than before. The original diet was clearly at fault.

Finally, the average number of young from first and second pregnancies was related to the month in which the female was born and hence to the diet which she had received during growth as well as for reproduction (Fig. 3). For this

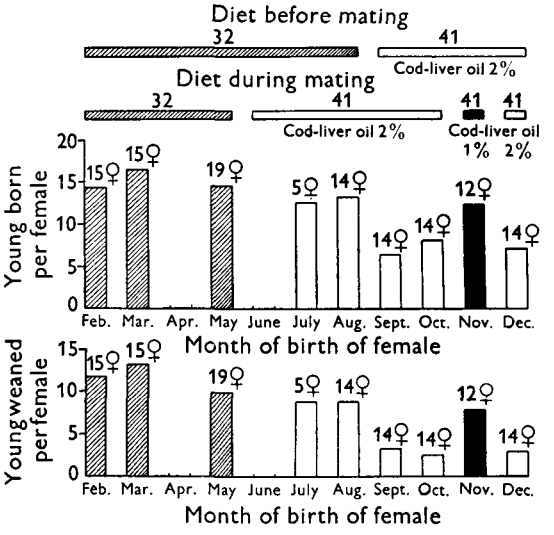

Fig. 3. Average production of young per female from first and second pregnancies.

illustration the records of still younger females, from the same stock, have been added to those of the two main groups. Serious impairment of litter size and the failure of lactation, as shown by the inability to rear the young, did not appear in early pregnancies until the females had been reared on diet 41, 2 % cod-liver oil, as well as receiving this diet during the period of reproduction. There was an immediate improvement when the cod-liver oil content of the cubes was reduced for the period of mating.

These findings suggested experiments to confirm the identity of the deficiency, to examine still further the influence of the cod-liver oil content of the cubes and to seek assurance that similar troubles would be unlikely with the continued use of diet 41 now containing 1 % of cod-liver oil.

Identification of the deficiency

The symptoms being highly suggestive of vitamin E deficiency, attention was first given to α-tocopherol.

The effect of α-tocopherol supplement on the second pregnancies of mice which had failed to rear young from first pregnancies, and on first and second litter

production when given from mating, is shown in Tables 2 and 3. In all three experiments a similar group of mice receiving the diet alone served as control.

Table 2. *Recovery of lactation in second parities with α-tocopherol*

	First parity	Second parity	
	Diet alone	Diet alone	With α-tocopherol
No. of mating pairs	50	28	22
Pregnancies: Failures	17	18	4
Litters killed	33	5	5
Litters weaned	0	5	13
Proportion of pregnancy failures (%)	34	64	18
Proportion of pregnancies from which young were weaned (%)	0	18	59

Table 3. *Prevention of lactation failure in first and second parities with α-tocopherol*

	Test 1		Test 2	
	Diet alone	With α-tocopherol	Diet alone	With α-tocopherol
No. of mating pairs	14	12	18	16
Average litter-size born (young)	4·8	6·6	6·5	7·2
Average litter-size weaned (young)	4·6	6·9	6·1	6·9
Average weight of young weaned (g.)	9·0	9·3	8·5	8·7
Proportion of pregnancies from which young were weaned (%)	32	58	61	84
Proportion of young weaned (%)	43	73	60	84

When α-tocopherol was given for second pregnancies, thirteen out of eighteen litters were weaned and the proportion of pregnancy failures was nearly halved (18 % as compared with 34 % for first litters). In the control group which continued on the diet alone the deficiency became more severe. Even though a few litters were weaned, second pregnancies had nearly double the proportion of pregnancy failures (64 % as compared with 34 % for first litters) (Table 2).

When α-tocopherol was given as a supplement from mating there was a big improvement in reproduction; both litter size and weaning rate increased.

Thus, the failure of lactation in mice which developed when 2 % of cod-liver oil was added to the diet was a straightforward vitamin E deficiency. It could be corrected with α-tocopherol and the subsequent reproduction (Table 3, test 2) was about normal for mice. There was no reason to postulate any other fault in the diet than that of vitamin E deficiency.

The effect of reducing the cod-liver oil to 1 %

The influence of the cod-liver oil content of the diet was examined by direct comparison of the two diets containing 2 and 1 % respectively.

Recovery of lactation with 1 % *of cod-liver oil.* Two tests were made. In the first test females which had failed to rear young from a first pregnancy when the diet

contained 2 % of cod-liver oil were at once given the diet with 1 % of cod-liver oil. In the second test, to ensure that vitamin E reserves were depleted, and to avoid the error arising from the inclusion of any females which might have suckled a second litter without a change of regime (cf. Table 2), the reduced cod-liver oil content was not given until a female had failed to rear young from two successive pregnancies. There were no control groups, but observations were continued for some time and even included up to four pregnancies after the change of diet, i.e. fifth or sixth parities, of the females concerned. The results are given in Table 4. Three females failed to become pregnant again after the change of diet; all were exceptionally fat and weighed about 50 g.; they have been excluded from the results. Of the remaining thirty-five females, thirty-three showed recovery of lactation in subsequent pregnancies.

Table 4. *Recovery of lactation after reduction of the cod-liver oil content of the diet to* 1 %

	Test 1 Females failed to rear young from their first pregnancy	Test 2 Females failed to rear young from two successive pregnancies
No. of mating pairs	13	22
Pregnancies observed after change of diet: Failures	3	4
Litters killed	6	10
Litters weaned	21	32
Proportion of pregnancy failures (%)	10	9
Proportion of pregnancies from which young were weaned (%)	70	70
Proportion of young weaned (%)	80	75

Table 5. *Comparison of reproduction on diets with* 2 *and* 1 % *of cod-liver oil*

	Cod-liver oil 2 %	Cod-liver oil 1 %	
	Parity 1 + 2	Parity 1 + 2	Parity 3 + 4
Number of mating pairs	20	19	13
Average litter-size born (young)	4·5	8·4	10·2
Average litter-size weaned (young)	4·1	7·7	8·2
Average weight of young weaned (g.)	9·6	10·1	9·5
Proportion of pregnancies from which young were weaned (%)	35	95	92
Proportion of young weaned (%)	40	92	80

Prevention of the deficiency with 1 % *of cod-liver oil.*

The results of a direct comparison of reproduction on the two diets are given in Table 5. With 1 % of cod-liver oil normal reproduction was maintained up to fourth litters, when the experiment was stopped. With 2 % of cod-liver oil reproduction failed from the start and observations on this group were stopped after the second parity. The vitamin E reserves of the mice used in this test were therefore low, and

the success of the diet with the reduced cod-liver oil content could not be attributed to previous stores of the vitamin.

Thus, alterations in the cod-liver oil content had twice altered the character of the diet. The addition of 2 % of cod-liver oil to the original cube produced a vitamin E deficiency; the subsequent reduction of the cod-liver oil to 1 % made the diet again suitable for reproduction even under conditions of intensive production when the requirements of all essential factors would be maximal.

α-tocopherol and 1 % *of cod-liver oil*

Although reducing the cod-liver oil content of the diet had enabled reproduction to return to normal limits, there still remained the possibility of further improvement with α-tocopherol. A direct comparison was therefore made of the total reproduction of two groups of mice receiving the diet containing 1 % of cod-liver oil, one of which also received α-tocopherol supplement. The weaning rate of both groups for first parity was low (Fig. 4), because the only females available were

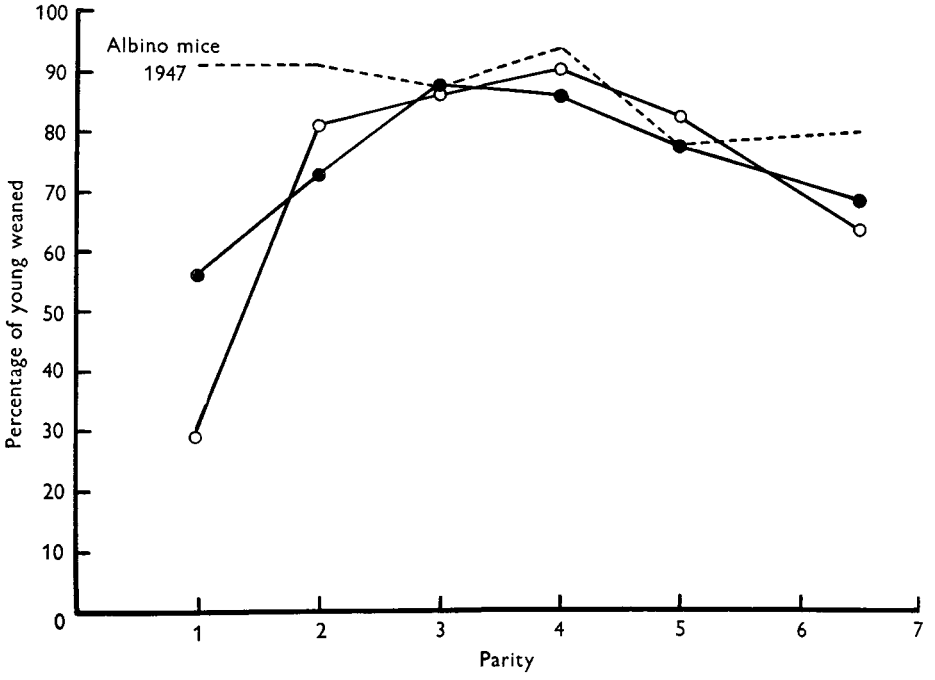

Fig. 4. The effect of α-tocopherol on the proportion of young weaned from successive pregnancies. ● diet 41 alone; ○ diet 41 plus α-tocopherol; --- previously recorded for albino mice, 1947.

already about 5 months old at the start of the test; but it rose to a normal level for third and subsequent litters. For comparison, the weaning rate previously found for a closely recorded colony of albino mice (Bruce, 1947) is also shown. At the end of the period of observation (6 months), the females were nearly a year old, and the apparent drop for sixth and seventh litters, only a few of which were produced, is not significant. There is clearly no difference between the groups.

The mice used in this comparison came from a stock known to have very low reserves of vitamin E. If the vitamin E content of the diet were marginal even after the reduction of the cod-liver oil to 1 %, successive pregnancies might reveal differences not apparent in an average figure calculated for the whole period, especially as in mice and in rats the vitamin E requirements increase with the age of the female (Emerson & Evans, 1939; Goettsch, 1942). Reproduction has therefore been tabulated for two successive periods: (i) all pregnancies terminating within 3 months of the start of the test, (ii) the remainder. Even in the second period there is no difference between the groups (Table 6). It must be concluded that reproduction in mice on diet 41 containing 1 % of cod-liver oil has not been improved by the addition of α-tocopherol.

Table 6. *Comparison of reproduction on diet with* 1 % *of cod-liver oil alone and with α-tocopherol*

	Period 1		Period 2		Totals	
	Diet alone	With α-toco-pherol	Diet alone	With α-toco-pherol	Diet alone	With α-toco-pherol
No. of mating pairs	12	12	10	12	—	—
Average litter-size born (young)	6·4	7·3	7·1	7·9	6·6	7·7
Average litter-size weaned (young)	6·0	6·4	8·8	7·5	6·3	7·0
Average weight of young weaned (g.)	8·2	8·3	7·7	8·2	8·0	8·3
Proportion of pregnancies from which young were weaned (%)	77	71	73	80	75	75
Proportion of young weaned (%)	74	72	78	81	75	77

Reproduction in rats

There was no established rat-breeding colony in existence here over this period, and the effect of alterations in the cod-liver oil content of the diet can only be judged by records from a few breeding pairs. Early sterility was exhibited by the F_1 generation of male rats reared on the diet with 2 % of cod-liver oil, about one-third of the animals being affected from puberty. Histological sections of the testes of the affected animals showed degeneration typical of vitamin E deficiency (Evans & Burr, 1927).

After reduction of the cod-liver oil to 1 %, litter production was about normal. A few pregnancy failures, however, have been recorded recently, and it seems possible that the rat requirement for vitamin E may not yet be completely satisfied. The rat is notably susceptible to seasonal variation, and reproduction may fail spontaneously for several months during the winter. This effect, without extensive checking by histological sections of the testes, might easily be confused with that of male sterility.

The continued use of diet 41 *as a stock diet*

The ultimate test of adequacy for a diet suitable for breeding animals must be its continued use for successive generations, close records being kept to ensure that minor variations in production represent only normal fluctuations and are

not trends developing within the colony in any particular. Such a check is being kept with mice for diet 41.

At the time of writing, the third generation in two stocks has just started to breed, and first litters are being suckled. A small group which was given the original batch of diet containing 2 % of cod-liver oil from mating is also suckling first litters. Thus, in the course of three generations, reserves of vitamin E have been built up. Even if lactation on the vitamin E-deficient batch of diet is not maintained there has been no reduction in litter size for first litters (diet 41, 1 % cod-liver oil, 24 litters average 6·7 young born per litter, range 3–11 young; original batch of diet, 2 % cod-liver oil, 9 litters, average 6·4 young born per litter, range 4–9 young).

A comparison of weaning rates in successive generations of two stocks is given in Table 7. The fall in the first generation of both stocks has been corrected in the

Table 7. *Weaning rate in successive generations of mice on diet* 41

Strain	Generation	Month of birth of first litters	No. of pregnancies observed	No. of litters weaned	Proportion of pregnancies from which young were weaned (%)
F.F.	*O*	May 1949	61	46	75
	F_1 (i)	Aug. 1949	27	17	
	F_1 (ii)	Oct. 1949	36 — 63	23 — 40	63
	F_2 (i)	Nov. 1949	14	10	
	F_2 (ii)	Jan. 1950	11 — 25	10 — 20	80
Cross-bred	*O*	May 1949	66	64	94
	F_1	Aug. 1949	46	37	80
	F_2	Nov. 1949	73	62	85

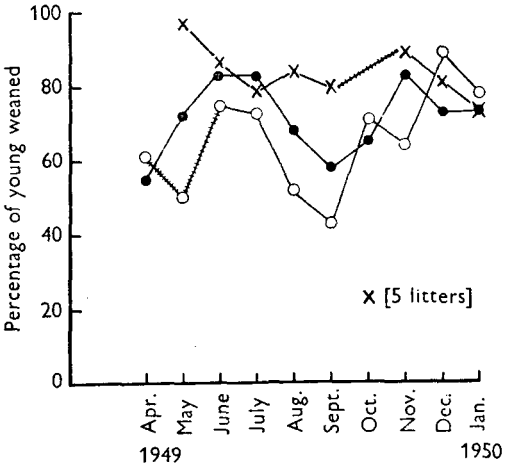

Fig. 5. Seasonal variation in the proportion of young weaned. ● F.F. colony (223 litters); ○ W.T. colony (151 litters); +++++ α-tocopherol given; × cross-bred mice (170 litters).

second generation. It can be attributed to seasonal variation, as the former was in production in the late summer when a fall is to be expected. The effect of season on the proportion of young weaned is shown in Fig. 5. The prolonged and gradual

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depletion of vitamin E reserves, to which the mice were subjected while they received the deficient original batch of diet, had seriously reduced the vigour of the stocks. This was particularly noticeable in the Wild Type colony; and to help to build up reserves again in this stock, α-tocopherol was given for the first 2 months after the change of diet. The fall in production in the late summer and recovery at the end of the year of the two inbred stocks is marked. But, it is interesting to note that the more vigorous cross-bred mice do not show the same fall in weaning rate for August and September. Only five litters from this stock were born in October, and the low value found for this month cannot be regarded as significant.

From these results it can be concluded that diet 41 is entirely adequate for breeding mice.

DISCUSSION

The results described above are summarized in Table 8 in which is shown the average production of young weaned per pregnancy in all tests. This is an arbitrary

Table 8. *Summary of results: Vitamin E deficiency and diet* 41

				Average production of young weaned per pregnancy
1. α-Tocopherol and 2 % of cod-liver oil:				
Recovery:	First pregnancy	Diet alone		0·00
	Second pregnancy	Diet alone		1·41
	Second pregnancy	Diet + α-tocopherol		2·91
Prevention:	Test 1	Diet alone		1·46
		Diet + α-tocopherol		4·04
	Test 2	Diet alone		3·72
		Diet + α-tocopherol		5·81
2. Reduction of the cod-liver oil to 1 %:				
Recovery:	Test 1	First pregnancy	2 % cod-liver oil	0·00
		Subsequent pregnancies	1 % cod-liver oil	4·80
	Test 2	First and second pregnancies	2 % cod-liver oil	0·00
		Subsequent pregnancies	1 % cod-liver oil	4·80
Prevention:		First and second pregnancies	2 % cod-liver oil	1·45
		First and second pregnancies	1 % cod-liver oil	7·29
		Third and fourth pregnancies	1 % cod-liver oil	8·15
3. α-Tocopherol and 1 % of cod-liver oil:				
	Period 1	Diet alone		4·59
		Diet + α-tocopherol		4·57
	Period 2	Diet alone		5·00*
		Diet + α-tocopherol		6·03*

* This difference is not statistically significant. $P = > 0{\cdot}05$.

but comprehensive criterion taking into account all three effects of vitamin E deficiency in mice as found under the experimental conditions: pregnancy failure, low weaning rate and reduced litter size. On this basis, the difference in production resulting either from supplementary α-tocopherol when the diet contained 2 % of cod-liver oil or from the reduction of the cod-liver oil content of the diet to 1 % is striking in both curative and prophylactic tests; but no direct comparison was made of the relative efficacy of the two factors.

The absence of improvement when α-tocopherol was given with the diet containing 1 % of cod-liver oil is equally apparent.

The pioneer work on the identification of vitamin E (Evans & Burr, 1927) concerned severely deficient diets and rats whose stores of vitamin E were deliberately depleted. Under such conditions the young are not carried to term. In less severe deficiency, as in the work reported here, pregnancies terminate normally, but lactation fails and the young are not reared. Such an effect was first recorded for mice by Goettsch (1942), who bred seven generations on a vitamin E-deficient diet, the females being given just enough vitamin E as wheat germ oil or as α-tocopherol, to allow the young to be reared.

Muscular dystrophy is rarely seen in mice (Goettsch, 1942). It was not observed in these experiments.

The distribution of vitamin E in plant and animal tissues is known to be wide, but few figures are available for the exact tocopherol content of most feeding stuffs. The biological assay is laborious and there are difficulties in the biochemical analyses. The germ of cereal grains is considered one of the richest natural sources of vitamin E, and it was expected that a diet containing 85 % of whole-ground cereals would amply supply the vitamin E requirements. That the addition of 2% of cod-liver oil would produce such a marked deficiency of vitamin E was wholly unexpected. Diet 32, which also contains 2 % of cod-liver oil (Table 1), had been in use as a stock diet for rats and mice for over 18 months, three generations of Wistar strain rats had been successfully bred on this diet. There is evidently considerable danger in the intimate admixture of even a relatively small amount of cod-liver oil in a diet which is not freshly prepared at short intervals. In a cubed diet made in bulk only, the destructive action of the cod-liver oil on the vitamin E content has ample opportunity to be fully effective, but these experiments do not show whether 2 % of cod-liver oil would be as fatal to diet 41 if added freshly at frequent intervals. Hickman & Harris (1946), in an extensive review of tocopherol interrelationships, stress the variation in the requirements of different species for anti-oxidants such as vitamin E according to the nature of the diet. These authors state: 'Much misunderstanding exists concerning the quantity of an anti-oxidant needed to protect a given quantity of carotene, vitamin A, and other labile substances. As far as we know, there is no fixed interrelation. The quantitative relationship is with the medium, not the substance protected. Every time a labile substance passes to a new medium it has a new requirement of anti-oxidant.

The two questions so frequently asked are: "How much vitamin E does it take to stabilize vitamin A?" and "How much to synergize vitamin A or carotene?" They can be answered by: "It depends on the medium" and "It depends on the animal".'

The differences in the value of diets 32, 39 and 41 first batch, with 2 % of cod-liver oil, for breeding rats and mice, lend support to this view.

SUMMARY

The appearance of vitamin E deficiency in rats and mice after the introduction of 2 % of cod-liver oil to a diet containing 85 % of whole-grain ground cereals is described. Evidence is given to show that the deficiency can be prevented by the reduction of the cod-liver oil content to 1 %.

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