LXXXV. A STOCK DIET FOR RATS BRED FOR VITAMIN TESTS.

BY KATHARINE HOPE COWARD, MARJORIE RUTH CAMBDEN
AND EVA MAUD LEE.

*From the Pharmacological Laboratories of the Pharmaceutical Society
of Great Britain.*

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THE diet of rats bred for vitamin tests should be rich enough in all dietary factors to allow normal growth of the young and free reproduction; but it must not be so rich that the young selected for experimental work do not exhaust their reserves of vitamins in a reasonable time when given vitamin-deficient diets. It is desirable also that there should be as little difference as possible between the reserves of members of the same litter and of different litters. It is necessary then, that the stock diet should vary as little as possible from day to day and also from season to season. The ideal diet for this purpose will only be available when all dietary essentials have been recognised and when they can be procured in a pure state. In the meantime a stock diet must be determined largely by trial of materials available in a reasonably stable and uniform state.

We have obtained evidence that a diet made from a given recipe may be satisfactory in one laboratory but unsatisfactory in another laboratory, although it may be very difficult to determine wherein the respective constituents of the diets differ. Kon [1931] has had a similar experience. After failure to improve two diets found to be satisfactory in various laboratories, but unsatisfactory in our own, we have devised a third one which has proved adequate for growth, reproduction and lactation of the colony. Evidence that the young rats of this colony are suitable for tests on vitamins A and D is given.

DIET A.

When the vitamin work of these laboratories was begun in 1927, it was decided to adopt the stock diet of the Wisconsin University laboratories of which one of the writers (K. H. C.) had had 10 months' working experience in 1925-6. The diet [Steenbock, 1923] had been in use there for about 9 years practically without modification. It consisted of:

Yellow corn (ground)	...	...	76.0 %
Linseed oil meal	...	...	16.0 „
Crude caseinogen	...	...	5.0 „
Ground alfalfa	...	...	2.0 „
Sodium chloride	...	...	0.5 „
Calcium carbonate	...	...	0.5 „

Table I. *The influence of various stock diets on the powers of reproduction of rats.*

Diet	No. of does on diet	Diet of parents	No. of litters		No. of rats weaned	Av. wt. of rats weaned per doe g.
			Born	Weaned		
1. Mixed diet from weaning, diet A from birth of first litter	12	Mixed	67	58	335	843.8
2. A from weaning	9	Mixed, A from birth of first litter	26	13	77	148.5
3. A from weaning, B after first litter	17	A from weaning	33	2	12	15.9
4. B from weaning, 5 % dried yeast added after birth of first litter	1	A from weaning	2	0	0	0
5. B from weaning, watercress added after birth of first litter	4	Mixed from weaning, A from birth of first litter	5	0	0	0
6. A from weaning, mixed before birth of first litter	6	Mixed	10	5	21	78.5
7. A + watercress	8	Mixed	15	3	7	19.4
8. B + 5 % dried yeast + watercress	3	Mixed	7	5	25	176.7
9. B + 5 % dried yeast + watercress and "light white casein" in place of skimmed milk	3	Mixed	8	5	28	254.7
10. A + 10 % "light white casein" + watercress	2	Mixed	4	0	0	0
11. A + 10 % dried yeast + watercress	2	Mixed	6	2	6	53
12. A + 8 % dried yeast + watercress + 10 % "light white casein"	2	Mixed	7	3	16	147
13. A	4	Mixed	5	0	0	0
14. A irradiated	5	Mixed	4	1	4	24
15. A + 0.04 g. cod-liver oil daily	3	Mixed	1	0	0	0
16. A + 10 % dried yeast	2	Mixed	3	3	13	170
17. A + 10 % "light white casein"	2	Mixed	3	0	0	0
18. A (1930) test	11	C, L, W, P	16	6	28	63.5
19. A + 4 g. lean beef per rat per week	13	C, L, W, P	15	5	28	48.7
20. C + liver and watercress	19	Mixed	58	48	252	441.8
21. C + liver and watercress	7	Mixed different source	18	11	44	63
22. C + liver and watercress	6	Do.	19	14	60	297.8
23. C + liver and watercress	B and W 5	Mixed, then B from birth of first litter	13	12	64	371
24. C + liver and watercress	11	C, L and W	21	12	56	141.3
25. C and porridge after weaning of first litter, mixed previously	8	Mixed	28	23	137	521.5
26. C and porridge from about 10 weeks of age (the first litters from 25)	37	Meal ration, green food, varying amounts of milk	89	44	208	151.9
27. C and porridge (second or subsequent litters from 25)	12	Do. till weaning of first litter, then C and P	47	28	123	278.2
28. C + liver and watercress with extra 5 % dried yeast and extra 6 % "light white casein"	8	C and porridge	31	27	140	474.4
29. Do.	3	Do.	17	12	65	533
30. Do.	B and W 3	C, L, W, P	18	11	53	505.7
31. Do.	3	Mixed	14	7	40	304.7
32. C + L, W and P, 1st generation	B and W 92	C, some P, some L, some W, some any two, none all three	280	205	1144	416.2
33. C + L, W and P, 2nd generation	71	C, L, W, P	219	137	768	309.7
34. C + L, W and P	21	C, L, W and mostly P	56	40	212	296.7
35. C + L, W and 20 % dried milk	21	Do.	72	56	321	426.6
36. C + L, W and P	12	Mixed	69	49	230	651.0
37. C + L, W and P, 1st generation	B and W 27	C, L, W	51	29	153	159.4
38. C + L, W and P, 2nd generation	B and W 22	C, L, W, P	56	28	131	170.4
39. C + L, W and 20 % dried milk	B and W 134	C, L, W, P or C with 20 % dried milk, L and W	458	310	1769	361.6*
40. High vitamin	30	C, L, W, P	40	24	122	116.8

* Nine of these does are still alive.

In addition to unlimited quantities of this mixture the rats were also given unlimited quantities of fresh milk from cows which were kept specially for the work on a winter feed at the University farm. For the young does, 5 % of butter was added to the diet, but for the older does (those which had already borne three litters) this was omitted.

In January 1927, this recipe, spoken of as diet A in this paper, was used for the first rats imported into these laboratories. Four bucks and twelve does (two litters) were obtained from the Glaxo Research laboratories. They were pure albinos of the Wistar strain, and all the does were either pregnant or suckling their first litters, the result of brother-sister mating. The result is shortly summarised in Table I, 1. The rats which had grown up on a mixed diet in the Glaxo laboratories thrive on the new diet and in time produced 5-6 litters each of good average weight. But, as certain of these litters were selected for breeding and allowed to grow up, it became evident that the later litters were growing more slowly than the earlier ones (Table II). Moreover,

Table II. *The decreasing rate of growth of successive litters taken for breeding from rats fed on diet A.*

Date of selection of litter for breeding	Litter	Weight at 12 weeks (g.)	
		♂	♀
17. iii. 27	Second from ♀ 15	204	149
			151
			141
			141
19. iii. 27	,, ♀ 6	197	158
			160
			147
			147
5. iv. 27	,, ♀ 8	187	144
			144
			148
			148
7. iv. 27	,, ♀ 10	177	135
			133
			118
			118
13. iv. 27	,, ♀ 2	171 171	134
			131
			133
			131
14. v. 27	Third from ♀ 6	155	133
			140
			136
			137
			124
25. v. 27	,, ♀ 16	169	131
			139
			131
			132
			131

as these litters grew up and reproduced, the number of litters weaned and the weight of each litter weaned became less and less (Table I, 2). It was painfully evident that the diet, as made with materials available in this laboratory, was inadequate for normal growth and reproduction.

Various experiments were then carried out to try to discover the deficiency. Litters of young rats freshly imported into the laboratories were divided into groups so that each litter was represented in each group. The groups were then fed from about the age of 4 weeks till death on some modification of diet A. The results are summarised in Table I, 10–17. The only improvement in the diet was that made by the addition of 5 % dried yeast, but even with this addition, it was obvious that some factor or factors other than those contained in dried yeast was lacking. The addition of extra protein in the form of 10 % “light white casein,” or of extra vitamin D by the irradiation of the diet, or of extra vitamins A and D by the giving of 40 mg. cod-liver oil (directly by mouth) each day did not effect any improvement. A recent experiment (1930, when other diets were giving good results) showed diet A still to be inadequate and not improved by the addition of about 4 g. lean beef per rat per week (Table I, 18 and 19).

Fresh tap-water is always supplied daily in inverted narrow-necked bulbs. Bedding consists of sawdust, and wood-wool is supplied to each doe when she is isolated a few days before the birth of a litter.

Each rat or group of rats is given a clean cage at least once a week. The dirty cages are scraped and washed with hot water, occasionally also with soap.

The colony is housed in a room with a north aspect. It is heated by one hot water radiator and two electric ones controlled by a thermostat so that the temperature remains nearly constant at about 70° F. Ventilation is arranged through door and window. There is no scabies in this colony.

DIET B.

Meantime a smaller experiment had been made with the Sherman diet referred to as diet B in this paper [Sherman and Campbell, 1924]. It consisted of

Whole wheat (ground) with 2 % NaCl	...	...	...	...	67 %
Dried whole milk (or skimmed milk with proportionate butter)					33 „

From Table I, 3–5, it may be seen that this also was unsatisfactory. Experiments (Table I, 8 and 9) were made to try to improve the diet by the addition of watercress and 5 % dried yeast and also by the substitution of “light white casein” for the dried skimmed milk. The young born from rats in these experiments were few in number and grew at a subnormal rate. No further attempts were made to improve either of these diets A and B, although some of the does which had been fed on one or other were later fed on a diet of kitchen scraps to see whether they were still capable of reproducing freely if properly fed (Table I, 6). No litters were produced.

Throughout the whole of this work, so far, the oestrous cycles of the rats had been examined by daily examination of the vagina from the time when they had reached a suitable weight for mating (about 140–150 g.). The does

were mated only at the cycles, *i.e.* when the contents of the vagina had been free from leucocytes and contained only nucleated and cornified cells.

With the rats which had been imported into this laboratory when mature, it was very seldom that copulation did not take place at the mating, and no instance of foetal resorption was noted, which would have been indicated by failure to produce a litter after red blood cells had been found in the vagina 14 days after copulation. With the young rats that grew up from our own breeding, cycles occurred regularly and copulation took place at mating, but very frequently this was not followed 14 days later by the finding of red blood cells in the vagina. For example one doe received a buck (sperm found in vagina) on 13 different occasions but only once produced a litter; this litter died within 4 days of birth. Brother-sister mating, and cross-mating were equally ineffective. Three healthy bucks of proven fertility were imported from the Glaxo Laboratories but were no more effective than the bucks from our own breeding. Hence it appeared certainly to be an impoverishment of the does and possibly also of the bucks.

A point of interest emerged at this stage of the breeding. In Table III it is shown that the longer the period of gestation the less chance is there of the litter being weaned.

Table III. *The correlation between the mortality of young rats and the length of their gestation period.*

Gestation period (days)	Litters born	Litters which lived at least 1 week	Litters weaned	% litters which lived at least 1 week	% litters weaned
21	48	40	35.5	83	74
22	206	139.5	116	68	56
23	76	40	30	53	39
24	20	8	7	40	35
25	7	2	1	29	14
26	1	0	0	0	0

DIET C.

Meanwhile, another diet (named the C stock diet, as it was the third one to be used in this laboratory) had been devised which for some time promised to give good results. It consisted of:

Yellow maize, ground ...	...	...	...	65	%
Whole wheat, ground ...	...	...	...	20	,,
"Casein, light white" (B.D.H.)	...	...	...	9	,,
Dried yeast ...	...	...	...	5	,,
Sodium chloride	...	...	...	0.5	,,
Calcium carbonate	...	...	...	0.5	,,

About 2 g. fresh liver and 2 g. fresh watercress were given to each rat twice a week. The result of this is seen in Table I (20-25). Reproduction was very much better than on the previous diets but still not satisfactory and a

modification of the diet was made. Since the addition of cod-liver oil, dried yeast and watercress to diet A had not given results equal to those obtained in the Wisconsin Laboratories, it seemed possible that the milk from the ordinary London supply was deficient in some hitherto unrecognised factor. As it was not easy to get a daily supply of fresh untreated milk, it was decided to try the C stock diet supplemented by porridge made with Plasmon oats (which contain some dried milk) mixed with the proportion of "fresh" milk usually taken by human beings. The liver and watercress were not given to the rats having the C stock diet and porridge. This diet was given to certain rats which had been sent to these laboratories together with their first litters, and which had previously been brought up on a diet of mixed meat, green food and varying amounts of milk. The result of this trial was more successful (Table I, 25). The first litters of these rats were fed on the C stock diet from about 4 weeks of age and on porridge also from about 10 weeks. The result was fairly bad (Table I, 26). Certain of the second and subsequent litters of the same rats were fed on the C stock diet and porridge from the time of weaning. The result was better (Table I, 27) but not so satisfactory as that obtained with the rats which had been brought up on a mixed ration and given diet C only after the weaning of the first litter.

Other modifications of the original C stock diet were also tried. A further 6 % "light white casein" (to increase the protein) and 5 % dried yeast (possibly required to balance the extra protein) were added and the usual quantities of liver and watercress were given. Good results were obtained (Table I, 28-31). Also, the combination of C stock diet, liver, watercress and porridge was tried on other litters. The result with the first generation of rats on this diet was satisfactory (Table I, 32) but when it became apparent that the second generation was not thriving on it as well as the first (Table I, 33), another attempt was made to improve it.

This was carried out as a definite experiment. Each of 14 litters was divided equally into two parts at 4 weeks of age. Half of each litter was brought up on C stock diet, liver, watercress and porridge and the other half was brought up on C stock diet containing 20 % dried milk, liver and watercress (no porridge). The result shows that the diet containing the dried milk is definitely better than the one that did not contain it but which was supplemented with porridge instead (Table I, 34, 35). Further litters on this modification (20 % dried milk added to the C stock diet, *plus* liver, *plus* watercress) also gave good results (Table I, 39).

Failure of black and white rats fed on diet C.

These tests were made almost wholly on albino rats. Our experience with a black and white strain of rats fed from weaning on C stock diet, liver, watercress and porridge is interesting. Litters from rats which had been brought up on a mixed diet reproduced well (Table I, 36). Litters from rats which had been brought up on C stock diet, liver and watercress (no porridge) did

much less well (Table I, 37), and the second generation did equally badly (Table I, 38). The result obtained with the black and white strain of rats was considerably worse than that obtained with the albino strain. The difference has not been investigated further. It is probable that the diet which is just good enough for the albino strain is not (somehow) good enough for the more vigorous black and white strain.

Vitamin-rich diet.

Still another trial was made to see how far the C stock diet could be improved by supplementing it with foods rich in vitamins. Nine litters were given the C stock diet in unlimited amount and, twice weekly, a double quantity of liver or beef, about 4 g. lettuce, 4 g. watercress and 3 g. hard-boiled egg, and, every day, porridge, about 0.5 g. marmite in water and 1 drop (0.02 g.) of a good sample of cod-liver oil. The result was distinctly poor. (Table I, 40 contrast with results 32-35 and 39. The parents of the three groups of rats had been brought up on the same diet C.)

Comparison of the main diets tried.

The results obtained from each of the main diets recently used are further summarised in Table IV in which are given (a) the average weight of a young

Table IV. *Summary of the more important results detailed in Table I.*

	Av. wt. of rat at 3 weeks g.	Av. No. of rats weaned per litter	Av. wt. of each litter at 3 weeks g.	Av. total wt. of young from each doe g.	% of litters weaned
<i>Albino rats.</i>					
C stock, liver and watercress	30.9	4.9	151.7	306.6	75.2
C stock, L, W and porridge: 1st generation	33.3	5.6	186.8	416.2	73.2
2nd generation	28.6	5.6	160.5	309.7	62.5
C stock, L, W and porridge } Litter com-	29.4	5.3	155.8	296.7	71.4
C stock, L, W, 20 % dried milk } parison	27.9	5.7	160.0	426.6	77.4
C stock, L, W, 20 % dried milk	27.3	5.7	156.1	361.6*	67.7
High vitamin diet	28.7	5.1	146.0	116.8	60.0
<i>Black and white rats.</i>					
C stock, L, W and porridge: 1st generation	28.1	5.3	148.4	159.4	24.2
2nd generation	28.6	4.7	133.8	170.4	50.0

* Nine rats still alive.

rat at 3 weeks, (b) the average number of rats in each litter weaned, (c) the average weight of a litter at 3 weeks, (d) the average total weight of young (3 weeks old) from each doe, and (e) the percentage of litters weaned.

We therefore decided to adopt the C stock diet with 20 % dried milk, liver or beef (see below) and watercress, twice a week for the whole of our colony; but being alarmed at the number of litters that died during lactation, we decided to add a further 5 % yeast to the diet of the lactating does. The result is seen in Table V. There is still a high mortality of first litters but,

Table V. *Diet. C stock plus 20 % dried milk, liver and watercress.*

	No extra source of vitamin B			Marmite given from birth of young			5 % dried yeast (extra) from birth of young		
	No. of litters born	No. of litters weaned	% litters weaned	No. of litters born	No. of litters weaned	% litters weaned	No. of litters born	No. of litters weaned	% litters weaned
First litters	49	24	49	95	48	50.5	100	55	55
Second litters	64	22	34.4	2	2	100	100	63	63
Third litters	11	4	36.4	1	1	100	93	59	63.4
Fourth litters	12	8	66.7	—	—	—	49	39	79.6
Fifth litters	7	6	85.7	—	—	—	18	17	94.4
Sixth litters	—	—	—	—	—	—	9	6	66.7

judging only from the groups which contain a reasonably high number of litters, it would appear to be advantageous to give the extra dried yeast to the lactating does. During the third week of lactation, no liver is given in order to prevent any possibility of the young eating it and thus varying their reserves of vitamin A.

The mating of the rats.

During the work on the C stock diet and its modifications, does were mated (two or three to one buck) when they reached 140–150 g. in weight, and 2 to 3 weeks' rest was given after the weaning of a litter before the doe was again mated. Thus we can trace the parentage (male and female) of all the rats at present in the colony. Every doe was weighed once a week and, when she was obviously pregnant, she was given a separate cage. The young were weighed weekly and weaned when 23 days old. Now, however, having gained the knowledge we desired concerning a suitable diet, we are no longer keeping records of matings. We have divided our breeding rats into four groups according to the quarter of the year in which they were born. Mating is arranged within each group, pregnant does are given separate cages and the only weighings taken are those of the litters when 3 weeks old.

We examined the vagina of 47 of the rats on the C stock diet with 20 % dried milk, daily from the date of opening of the vagina until they were about 9 months old, though only on the fourteenth day after the finding of sperm and not at all during lactation. We found the occurrence of the oestrous cycle normal but we have many times found sperm in the vagina without finding red blood cells 14 days later. Often the sperm has been scanty and though this might be due to the length of time intervening between copulation and the examination of the vaginal smear, yet it is also possible that our bucks are less than normally fertile and some improvement in their diet might be desirable. We do not, however, wish to modify the diet of the does as the young they produce are richly enough, and yet not too richly, stored with vitamins to be suitable for vitamin tests, and if the diet were modified to increase fertility, the condition of the young for vitamin tests might be less good.

Seasonal variation in the reproduction of the rats.

For the last two years, we have taken about 30 young does per month for breeding on various diets, and there has certainly been a greater number of births in the summer months than in the winter, November, December and January being the worst. This might so easily have been due to seasonal differences in the fresh food of the diet and in the poorness of certain modifications of the diet, that we have not attached any great importance to it. We have, however, analysed the records of the rats that have been brought up entirely on C stock diet with dried milk, liver and watercress and we have found a drop in the number of litters born during November, December and January. Table VI summarises the figures on this point.

Table VI. *The seasonal variation in the reproduction of rats receiving the C stock diet with 20 % dried milk plus liver and watercress.*

	No. of does*	No. of litters weaned	No. of litters weaned per doe per month
August, 1930	10	8	0.80
September, "	22	14	0.64
October, "	30	11	0.37
November, "	37	9	0.24
December, "	63	8	0.13
January, 1931	83	17	0.20
February, "	95	28	0.29
March, "	128	35	0.27
April, "	158	54	0.34
May, "	171	49	0.29
June, "	172	69	0.40
July, "	155	46	0.30
August, "	153	57	0.37

* These numbers refer to does which were first mated at least 2 months before the month signified.

The seasonal variation is evident even if the numbers for the first 2 months are ignored as they probably should be. But there is no reason to suppose that this variation is due to any other cause than the diet; for the seasonal variation in the liver, watercress and dried milk may easily be great enough to account for the variation in the reproduction of the rats.

Rate of growth of the rats of this colony.

Composite growth curves have been drawn for the bucks and does of three generations respectively fed on C diet *plus* 20 % dried milk *plus* liver and watercress. The results are summarised in Table VII. It is evident that there is no tendency for the later generations to grow more slowly than the earlier ones. It has similarly been calculated that the rats of first litters do not grow more slowly than do rats of subsequent litters. It has also been determined that there is no seasonal variation in the average weights of bucks or does at 13 weeks of age.

Table VII. *Growth of rats fed on C diet plus 20 % dried milk plus liver and watercress.*

		Average weight (g.) at										σ of weight at 13 weeks	
		No. of animals	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks	12 weeks	13 weeks	$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$
BUCKS													
1st generation	70	46.0	62.3	84.2	104.9	122.7	139.9	153.6	165.2	178.0	186.7	26.196	
2nd "	51	44.2	59.9	82.9	101.1	118.5	133.4	145.7	160.2	170.8	181.8	23.333	
3rd "	5	45.7	62.0	84.0	104.2	125.4	145.2	154.0	165.2	179.4	206.7*	39.249	
Weighted mean	126	45.3	61.3	83.7	103.4	121.1	137.5	150.4	163.1	175.0	185.3	26.0	
DOES													
1st generation	241	42.6	56.4	71.9	84.8	96.8	107.0	115.8	124.1	130.5	131.1	13.55	
2nd "	175	40.4	54.1	69.6	82.3	92.8	103.0	113.1	120.7	128.2	134.6	14.387	
3rd "	31	42.9	58.3	75.4	89.7	101.9	110.8	120.0	127.9	134.7	139.8	13.111	
Weighted mean	447	41.8	55.6	71.3	84.2	95.6	105.8	115.0	123.1	129.6	135.2	13.86	

* Four only.

Suitability of the young of this colony for vitamin tests.

We have used the young of this colony for tests for vitamins A, D, B₁ and B₂. So far we have carried out no work on the other components of vitamin B.

1. *Vitamin A work.* While we were giving the stock rats liver twice a week, we found that the young weighing 40–50 g. when given our vitamin A-free diet grew far beyond the weight at which they should cease to grow (80–100 g.). We tried giving the vitamin A-free diet to certain does during the third week of lactation in order to reduce the reserves of vitamin A in the young and this succeeded with the litters which survived but was apparently too drastic when the A-free diet was given from the 14th day of lactation, for some of the litters died. When, however, the diet was given from the 17th day of lactation, the young never died. We, therefore, now give beef to all the stock rats three times and liver only once in a fortnight. We choose rats weighing about 30 g. for vitamin A tests and we find that these cease to grow at about 80–100 g. weight. Occasional bucks grow above 120 g. and does above 110 g. but these we discard.

2. *Vitamin B tests.* Young rats from this colony are suitable for vitamin B tests (B₁ and B₂) at 55–60 g. weight.

3. *Vitamin D tests.* For prophylactic tests we use rats weighing about 40 g. For curative tests we use rats weighing about 60 g. at the beginning of the preparatory period. The room in which the tests are carried out faces east and has yellow blinds always drawn over the windows. In Fig. 1 we have indicated the average degree of rickets produced by the rachitogenic diet in litters of rats from does fed from weaning on the C stock with 20 % dried milk, liver and watercress. It is evident that we are getting a more and more nearly uniform, severe degree of rickets. There is no obvious seasonal variation in the degree of rickets developed. Some workers attribute variations in the degrees of rickets produced to a variation in the amount of vitamin D in their

stocks of yellow maize. We have no information on this point as we buy 1 cwt. of whole yellow maize per week (using it in the stock diet as well as in the rachitogenic diet) and grind it for use as required. Variations in such small quantities would not be noticeable. It seems to us that the fluctuations in severity of rickets developed in our rats can most probably be ascribed to variations in the vitamin D content of the dried milk of the stock diet. From May to July, 1930, we used a sample which had been specially dried for us by the Glaxo Co. in the previous February. From August to November we used a sample which the same firm dried for us in late July. We then used a dried milk, bought in the open market, until March, 1931, when we again had a sample of milk specially dried for us. We then used this sample and the one bought monthly in the open market during alternate weeks until October 1931,

Fig. 1. To show the degree of rickets developed in litters of rats from does fed on C diet + 20 % dried milk + liver and watercress, measured by the method described by Dyer [1931].

after which we used the one bought in the open market continuously. It seems to us, that having once reduced the vitamin D reserves of our colony, they can be kept low by the use of winter dried milk in the stock diet. It would appear to be safe to rely on the low vitamin D content of dried milk bought in the open market in winter, if it were known to be prepared from fresh English milk. New Zealand dried milk might be richer in this factor but we have no information on this point. Our use of English dried milk bought in the open market in the summer seems to raise the vitamin D reserves of the young litters even when it is used in alternate weeks only, dried winter milk being used in the other weeks. Even so, the degree of rickets is still severe, and it may be advantageous for the colony to have a limited supply of dried summer milk rather than dried winter milk continuously.

SUMMARY.

Evidence is given that a diet found to be suitable for a colony of rats in one laboratory was not satisfactory in this laboratory where it was made from the same recipe but necessarily of materials from different sources. The

deficiency was not determined though it was shown to be none of the recognised vitamins.

A second diet recommended by other workers was also found to be unsatisfactory in this laboratory though the nature of the deficiency was not as fully investigated as that of the first diet.

A fresh diet was devised which, with certain modifications, has proved to be satisfactory for at least two generations of rats. Furthermore, evidence is given that the young of the rats fed on this diet are suitable for tests for vitamins A, B₁, B₂ and D.

Reproduction on this diet shows a definite seasonal variation which may be accounted for by the variation in certain of the constituents of the diet (fresh liver, watercress and dried milk). There was, however, no seasonal variation in the growth of the young rats on this diet. Fluctuations in the degree of rickets produced in the young under certain conditions appear to be correlated with the source of the dried milk of the stock diet, and, with this controlled, the fluctuations are not serious, and gradually disappear.

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