CCLVIII. OBSERVATIONS ON SPECIFIC NUTRITIONAL FACTORS IN LACTATION

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It is well known that diet has a profound effect on lactation and that the satisfactory secretion of milk is only possible in the presence of certain known dietary factors in adequate quantities. There is also general agreement that a diet good enough to support the physiological strain of optimal lactation would also satisfy the requirements for optimal growth and reproduction.

The converse, however, does not seem to be necessarily true; in experiments on rats several authors have found that diets made up from natural foodstuffs which allowed, under stock colony conditions, of excellent growth and reproduction were very unsatisfactory with regard to the lactational performance of the animals [e.g. Smith & Anderson, 1929; Kon, 1931; Kon & Peskett, 1933; Coward, 1938]. Such findings obviously suggest the existence of separate dietary factors specifically connected with lactation, and publications to this effect are not lacking. A number of workers, who used so-called synthetic diets and included in them all dietary factors known by them to be required by the rat, have reported lactational failures, though otherwise the performance of the animals was very good. Efforts to find out the cause of the trouble have shown in several instances that this was nutritional and could be cured by supplying the rats with certain natural substances or with extracts from them which apparently contained a distinct new dietary principle capable of stimulating lactation. Thus, Mapson [1932; 1933] showed that a supposedly complete synthetic ration could be much improved as regards its value in lactation by the addition of fresh ox liver or of aqueous extracts thereof. It should be noted that liver exerted also a marked stimulating effect on the growth of young rats fed on the synthetic diet, but it is not known whether the two actions were due to the same or to different substances.

Similar observations on the favourable effect of liver in conjunction with semi-synthetic diets containing varying proportions of soya-beans were made by Wilkinson & Nelson [1931] and studies by Feaster & Nelson [1935] also showed the value of liver and led the authors to assume the existence of a new dietary lactational factor.

Nakahara and his colleagues [Nakahara & Inukai, 1933; 1934; Nakahara *et al.* 1934; 1935; 1936; 1937; 1938] have published a comprehensive series of papers in which they reported failure of lactation in rats kept on a presumably complete synthetic diet and marked improvement following the administration of fresh liver or of carefully fractionated liver extracts.

In addition to liver, other natural foods have been found to supply factors capable of stimulating lactation. Thus Tso [1927] and Outhouse [1937] found these present in lettuce, and the latter and also Meyer [1936] found them in

oats and to a lesser extent in hydrogenated cotton seed oil. It was shown that vitamin E was not involved.

Swanson & Nelson [1937], who used synthetic diets containing dried tinned pork muscle as the sole source of protein, were able to cut down considerably the number of lactational failures on such diets by the administration of fresh liver, but found another substance, the pancreatic hormone "lipocaic" of Dragstedt *et al.* [1936], to be extremely active in this respect.

These admittedly incomplete quotations from the literature show that many students of nutrition have had little success with their purified diets when lactational performance was considered, even though an effort was made to include in such diets all known essential food constituents.

When weighing up the evidence, however, the remarkable experiments of Cox & Imboden [1936, 1] should be placed on the other side of the scale. These authors fed in the course of 2 years, to three generations of rats, exclusively a conventional "synthetic" diet and obtained remarkable success in growth, reproduction and lactation, all of which were as satisfactory as on their good stock diet. A large group of female rats brought to maturity on the stock diet was put through 10 reproductive cycles on the synthetic diet and lactation in this case was also very satisfactory [Cox & Imboden, 1936, 2]. The diet consisted of casein, dextrin, lard, rice-cellulose and salts. The vitamin B complex was supplied as an aqueous, protein-free extract from yeast and the other addenda were wheat germ oil and an oily solution of carotene. Vitamin D was not supplied separately but it seems probable that the 9% of lard contained enough of the factor [Kon & Booth, 1934]. Richter *et al.* [1938] also noticed successful lactation when rats were offered a free selection of purified food constituents.

In planning our own investigations on the lactational performance of rats on purified diets we decided to use a diet similar to that of Nakahara & Inukai [1933] which has the advantage of simplicity and gave in the hands of these authors a high incidence of lactational failures.

EXPERIMENTAL

First series of experiments

Forty female rats (hooded Norwegian), born in the first half of April 1936, were reared on the stock colony diet until they were about 3 months old. This diet has the following composition:

Whole wheat, milled	69	Butter	5
Linseed cake	14	CaCO ₃	0.5
Crude casein	6	NaCl	0.5
Dried brewer's yeast	5		

In addition the stock rats receive fresh whole milk *ad libitum*, carrots or watercress daily, liver twice a week, about 5 g. each time. Pregnant and lactating females receive liver 3 times a week. The experimental rats were then placed on the following diet:

Ground rice (Patna polished)	75
Casein (New Zealand, repeatedly washed at the isoelectric point)	10
Butter (Empire)	10
Salts (Steenbock's 40)	5
Dried yeast (D.C.L.)	5

Table I. *Lactational performance of rats on the experimental (casein) diet and on the same diet supplemented with liver, liver extract and additional yeast*

Control group													
2nd lactation. Experimental diet only													
Rat no.	1st lactation. Experimental diet only				Gain or loss in mother's wt. during lactation	2nd lactation. Experimental diet only				Gain or loss in mother's wt. during lactation			
	No. of pups		Av. wt. at weaning	No. of pups		Av. wt. at weaning							
	Original	Weaned					Original	Weaned					
	♂	♀	♂	♀		♂	♀	♂	♀				
5328	4	4	4	2	+ 4	4	4	—	—	—	—		
5351	5	3	3	3	— 2	3	2	45.0	41.0	+ 19	0		
5364	5	3	3	3	+ 7	3	1	35.0	29.0	— 13	+ 9		
5397	3	5	3	5	+ 31	3 (+5)	0	41.2	0	—	—		
5412	4	4	3	3	+ 12	3	5	39.0	37.4	—	—		
5427	4	4	4	4	— 8	—*	—	—	—	—	—		
5444	4	4	4	4	— 13	4	4	41.0	37.5	—	—		
5460	4	4	4	0	—	—†	—	—	—	—	—		
5464	4	4	4	0	—	5	3	—	—	—	—		
5485	4	4	4	4	+ 5	4	4	43.2	42.2	+ 19	—		
Average	4.1	3.9	3.1	2.8	+ 3.3	4.1	3.5	40.8	37.4	+ 4.3	—		
% of pups weaned	75.6		71.8		80	69.7		57.1		75	63.4		
	73.7												
Liver group													
2nd lactation. Experimental diet only													
Rat no.	1st lactation. Experimental diet + liver				Gain or loss in mother's wt. during lactation	2nd lactation. Experimental diet only				Gain or loss in mother's wt. during lactation			
	No. of pups		Av. wt. at weaning	No. of pups		Av. wt. at weaning							
	Original	Weaned					Original	Weaned					
	♂	♀	♂	♀		♂	♀	♂	♀				
5325	4	4	3	3	+ 14	3	5	44.3	42.2	+ 9	—		
5388	4	4	4	4	+ 15	4	4	41.0	37.0	+ 19	—		
5413	5	3	5	3	— 4	4	4	43.5	39.5	+ 2	—		
5428	4	4	2	4	+ 20	3 (+1)	4	40.0	40.0	— 3	—		
5434	4	4	4	4	+ 14	5	2	50.2	46.0	— 40	—		
5437	4	4	0	0	—	3	4 (+1)	—	—	—	—		
5443	—†	—	—	—	—	—†	—	—	—	—	—		
5452	4	4	3	3	+ 19	3	5	—	—	—	—		
5471	5	3	0	0	—	1 (+1)	1 (+5)	37.5	38.2	— 8	—		
5484	4	4	3	4	+ 12	3.5	4.4	42.8	40.5	— 3.5	—		
Average	4.2	3.7	2.6	2.7	+ 12.9	3.5	4.4	42.8	40.5	— 3.5	—		
% of pups weaned	63.2		73.5		78	75.0		68.6		75	71.8		
	68.4												

% of does lactating successfully: 78

Liver extract group

	1st lactation.				Experimental diet + liver extract				2nd lactation.				Experimental diet only			
	0	2	4	4	0	2	4	4	3	5	0	0	—	—	—	—
5323	0	2	4	4	0	2	4	4	—	—	—	—	—	—	—	—
5352	4	4	4	4	1	2	32.0	34.5	+ 4	5	0	0	—	—	—	—
5356	2	6	2	5	2	5	37.5	37.2	-11	3	5	3	47.2	43.3	+17	+17
5365	—	—	—	—	—	—	—	—	2 (+1)	5	0	0	—	—	—	—
5395	4	4	4	4	0	0	—	—	—	5	0	0	—	—	—	—
5436	4	4	4	4	4	4	38.0	36.2	- 5	4	4	4	45.2	42.0	+13	+13
5445	4	4	4	4	3	3	38.5	35.7	- 9	2	6	2	42.0	39.0	+ 5	+ 5
5459	4	4	4	4	3	3	39.0	39.3	- 3	4	4	4	47.5	—	+13	+13
5473	4	4	4	4	0	0	—	—	—	3	5	0	—	—	—	—
5478	4	4	4	4	4	4	39.0	34.2	-17	4	4	3	44.3	43.25	+ 6	+ 6
Average	3.3	4.0	2.1	2.3	63.3	58.3	37.3	36.2	- 6.8	3.3	4.6	1.8	45.3	41.9	+10.8	+10.8
% of pups weaned	60.8				54.5				30.4				42.5			

% of does lactating successfully: 67

50

Yeast group

	1st lactation.				Experimental diet + yeast				2nd lactation.				Experimental diet only			
	4	4	4	4	2	3	42.0	39.7	4	4	4	0	—	—	—	—
5321	4	4	4	4	0	2	—	36.0	4	4	4	4	45.5	44.0	0	0
5350	4	4	4	4	0	2	—	43.0	4	4	4	4	45.0	41.2	-10	-10
5394	5	3	5	3	5	3	45.2	40.5	3	4 (+1)	3	4 (+1)	40.7	41.3	- 2	- 2
5396	4	4	4	4	3	4	42.0	—	4	4	4	3	37.0	35.5	-41	-41
5404	3	5	3	5	3	5	37.3	38.4	4	5	3	4	—	—	—	—
5435	7	1	6	1	6	1	42.7	43.0	6	2	0	0	—	—	—	—
5443	4	4	4	4	0	0	—	—	—	—	—	—	—	—	—	—
5451	2	3	0	0	0	0	—	—	—	—	—	—	—	—	—	—
5472	4	4	3	3	2	2	40.7	40.0	—	4	0	0	—	—	—	—
5474	5	3	0	0	0	0	—	—	2	2	0	0	—	—	—	—
Average	4.2	3.5	2.2	2.0	52.4	57.1	41.7	40.1	3.8	3.8	1.8	2.0	42.1	40.5	-13.3	-13.3
% of pups weaned	54.8				46.7				53.3				50.0			

% of does lactating successfully: 70

50

After birth the no. of pups in the litter was reduced to 8, the 4 heaviest of each sex being kept. If the no. of live pups was under 8, substitutes were given when possible to make up this number.

* Resorption. † Doe not pregnant—too late to be remated.

† Died during pregnancy.

N.B. In the "no. of pups" column + indicates substituted offspring.

This diet differs from that of the Japanese workers in that casein replaces an equivalent quantity of "fish protein". Mating was started after the does had been 1 week on the diet. Females in oestrus were paired with stock colony bucks, positive matings were detected on the following morning by the vaginal smear technique and inseminated does were placed at once under the various experimental treatments. The matings were completed within a week. The distribution of the rats was as follows: 10 does were kept on the experimental diet alone and formed the control group; 10 received, in addition to the diet, 1 g. daily of fresh beef liver (liver group); 10 were similarly given daily 1 ml. of liver extract equivalent to 5 g. of fresh liver (liver extract group). The extract was prepared by the method described by Nakahara *et al.* [1935] save that the preparation was stopped short at the fractionation by phosphotungstic acid. Finally, a last (yeast) group of 10 rats was given additional yeast to ensure that the vitamin B complex was not a limiting factor in the other diets. The yeast was supplied by mixing 90 parts of the basal diet with 10 parts of a potent brewer's yeast. On the 20th day of pregnancy the rats were placed in individual cages. If more numerous, the litters were reduced to 8 on the day of birth. In the case of smaller litters this number was, if possible, made up by using young from other mothers. The young rats were weaned at 21 days. All mothers were then transferred to the basal diet only and were kept on it to the end of the experiment. They were remated about 2 months after weaning and were allowed to produce and nurse a second litter. The results are given in Table I.

It is evident from the table that we were unable to repeat the results of the Japanese workers. Not only was lactation quite satisfactory on the basal diet alone, inasmuch as 8 out of 10 does nursed their young and 74% of the available young were weaned, but none of the additions tried (fresh liver, liver extract and yeast) improved their performance. If anything, the performance of the rats in the supplemented groups was below that of the controls.

With the second matings the results were very similar. This time the rats which had previously received fresh liver fared best, but there was little to choose between them and the group which was on the control diet throughout. The "liver extract" and "yeast" groups again lagged behind.

Second series of experiments

As casein was used in our diet instead of the "fish protein" fed by Nakahara & Inukai [1933], it was thought advisable to attempt to repeat the findings of these workers by using a diet as similar to theirs as possible. For this purpose white fish ("rock salmon") was obtained from a fishmonger. The muscle was freed from visible fat and skin and, after mincing, 10 lb. were boiled for 10 min. with 9 gal. of water containing a few drops of glacial acetic acid. The solids were allowed to settle, the supernatant liquid was poured off and the process was repeated with three further lots of water. On the last occasion the solids were strained off through muslin, dried in a hot air oven and milled. This protein was used in the experimental diet, which now had the same gross composition as that of Nakahara & Inukai [1933].

Twenty-six young does born in the stock colony towards the end of December 1936 were used for this experiment. They were placed at weaning on the experimental diet described on p. 1989 and 3 weeks later were given the fish protein diet. They were thereafter kept on this diet to the end of the experiment.

As our first aim was to find out whether we could obtain a satisfactory percentage of lactational failures on a synthetic diet, no curative or protective treatments were attempted, but all the available animals were put through

Table II. *Lactational performance of rats on the experimental (fish protein) diet*

Rat no.	1st lactation										2nd lactation									
	No. of pups					Av. wt. at weaning					No. of pups					Av. wt. at weaning				
	Original		Weaned			♂		♀			Original		Weaned			♂		♀		
6041	3	2 (+3)	3	2 (+1)	3	42.7	41.3	4	4	4	4	4	3	4	4	45.6	42.2	4	4	+20
6056	5	3	0	0	0	—	—	—	—	—	4	4	3	4	4	41.0	40.2	—	—	-8
6065	2	6	2	3	3	56.0	53.3	4	4	4	4	4	4	4	4	44.2	41.7	—	—	-9
6068	4	4	0	0	0	—	—	—	—	—	4	4	0	0	0	—	—	—	—	—
6081	2	3	2	3	3	46.0	44.6	4	4	4	5	3	2	1	1	29.0*	37.0	—	—	+12
6084	4	4	2	4	4	47.5	43.5	4	4	4	5	3	3	5	3	36.0	34.6	—	—	-14
6099	8	0	0	0	0	—	—	—	—	—	Some still-born					—	—	—	—	—
6119	4	4	2	3	3	44.5	43.0	4	4	4	3	5	3	5	3	37.3	36.0	—	—	+12
6142	4	4	4	4	4	41.2	39.5	4	4	4	4	4	4	4	4	42.2	36.5	—	—	+11
6143	5	3	5	3	3	44.6	42.0	4	4	4	3	5	2	5	5	46.5	43.0	—	—	+26
6153	4	4	0	0	0	—	—	—	—	—	3 (+1)	3 (+1)	0	0	0	—	—	—	—	—
6159	4	4	4	4	4	37.5	30.3	4	4	4	4	4	0	0	0	—	—	—	—	—
6162	4	4	0	0	0	—	—	—	—	—	5	3	0	0	0	—	—	—	—	—
6163	4	4	4	4	4	40.0	40.0	4	4	4	4	4	0	0	0	—	—	—	—	—
6173	4	4	4	4	4	44.2	44.2	4	4	4	5	1 (+2)	2	1 (+2)	3	46.0	48.0	—	—	+4
6174	1	7	1	6	6	40.0	42.1	4	4	4	4	4	4	4	4	44.7	44.3	—	—	+9
6183	5	3	0	0	0	—	—	—	—	—	4	3	0	0	0	—	—	—	—	—
6189	2	6	0	0	0	—	—	—	—	—	1	2	1	2	2	50.0	53.0	—	—	-6
6190	0 (+3)	1 (+2)	0	0	0	—	—	—	—	—	Not pregnant					—	—	—	—	—
6197	4	4	2	3	3	51.5	45.6	4	4	4	4	4	4	4	4	41.5	41.0	—	—	+2
6212	0	0 (+7)	0	0	0	—	—	—	—	—	Ate pups at birth					—	—	—	—	—
6213	4	4	0	0	0	—	—	—	—	—	4	4	4	4	3	35.5	38.6	—	—	-2
6221	4	4	4	4	4	38.7	36.0	4	4	4	0 (+4)	4	4	0 (+4)	0	46.2	36.3	—	—	+12
6224	4	4	0	0	0	—	—	—	—	—	2	0	0	0	0	—	—	—	—	—
6236	4	4	3	2	2	42.6	44.0	4	4	4	4	3	4	3	3	40.5	39.6	—	—	+13
6237	5	3	0	0	0	—	—	—	—	—	5	3	3	3	3	51.6	46.0	—	—	+14
Average	3.7	4.0	1.6	1.9	44.1	42.1	3.8	3.6	2.2	2.5	42.4	41.1	56.8	68.7	62.8	42.4	41.1	—	—	+6.0
% of pups weaned	43.3					45.0					43.3					43.3				

% of does lactating successfully:

54

64

N.B. In the "no. of pups" column + indicates substituted offspring.

* One ♂ weaned at weaning only 20 g.

two reproductive cycles on the basal diet alone. Two consecutive matings were carried out in March and June 1937 and the results are given in Table II. The methods were as previously described.

This time again the results were not encouraging, the number of successful lactations being too high. Thus, 54% of the females at the first mating and 64% at the second mating reared young and 45 and 63% of the available young were weaned. Though the percentage of successful lactations in this experiment was a little lower than that usual in our stock colony (see Table IV below), the percentage of lactational failures was very much lower than that reported by Nakahara & Inukai [1933]. For reasons more fully set out in the discussion we do not believe that this basal diet could be used by us satisfactorily in the study of specific lactational factors.

Experiment involving hormonal treatment

An interesting point which emerges not only from this work but also from our stock colony records has been investigated further. We find, namely, that a female which fails to raise her young after her first parturition is more likely to fail after subsequent ones than a female which raises her first litter.

For example, in the first experiment (Table I) 7 rats failed twice, while out of 26 which raised first litters and produced second litters 21 weaned them successfully. Again, in the second experiment (Table II) 7 rats failed twice, but out of 14 which suckled once 13 were successful for the second time. It should be added, however, that a successful lactation may quite well follow an unsuccessful one, as for example in the second experiment where 4 rats reared pups from a second pregnancy, having failed to do so from the first one. Conversely, 2 rats which nursed their first litter normally failed to bring up the second. Such individual fluctuations in the lactational performance when the diet apparently remains unchanged have led us to suspect that the failure to rear young under normal (stock colony) conditions may be due to failure at some point of the hormonal mechanism now known to be involved in lactation [cf. Nelson, 1936; Folley, 1938]. In view of the importance of the anterior pituitary in lactation, it is reasonable to suspect that such failures might be due to lack of an anterior pituitary hormone. An attempt to explore this possibility was therefore made. As already mentioned, a certain proportion of the rats belonging to our stock colony fail to rear their litters. 21 such females, all of which had experienced their first parturition but had failed to rear their young, were used for an experiment on the possible connexion between lactational failures and the anterior pituitary hormones. In most cases living young were born to these females but all had died within a few days of birth. In three cases, marked with an asterisk in Table III, the young, as far as could be ascertained, were born dead, so that it is not really certain that the mother could not have reared them had they been alive.

The rats were divided at random into two groups and one group of 11 received on the day after their second parturition a subcutaneous implantation of one compressed tablet weighing about 80 mg. of acetone-dried and powdered pig anterior pituitary. The remaining 10 females were given no treatment.

The litters were treated at birth as already described on p. 1992 and the surviving young were weaned at 21 days. The results of the experiment are presented in Table III, from which it is evident that on this occasion the treated rats lactated no better than the controls. The marked incidence (over 75%) of failures confirms our view that female rats which fail once to rear their young are likely to fail again, but the experiment throws no light on the cause of the failures.

Table III. *Effect of one treatment with anterior pituitary powder on the ability to rear their second litters of rats which failed to rear their first litters on the stock diet*

Rat no.		Treated rats						Av. wt. at weaning	Gain or loss in mother's wt. during lactation
		No. of pups							
		Born		Substitutes		Weaned			
♂	♀	♂	♀	♂	♀	♂	♀		
6390	5	5	—	—	4	4	41.7	42.5	+ 22
6480	1	3	—	—	1	3	52.0	48.0	- 20
6562	4	6	—	—	0	0	—	—	—
6460	1	7	—	—	0	0	—	—	—
6438*	1	2	—	—	0	0	—	—	—
6463*	Some still-born		2	4	0	0	—	—	—
6527	1	1	—	2	0	0	—	—	—
6362*	1	0	—	5	0	0	—	—	—
6410	Some still-born		2	2	0	0	—	—	—
6590	4	4	—	—	0	0	—	—	—
6499	4	3	—	—	0	0	—	—	—
Av. % of pups weaned	2.9	4.9	—	—	0.6	0.8	46.9	45.3	+ 1
					19.2	15.9			
					17.5				
% of does lactating successfully:						18			
Control rats									
6513	8	5	—	—	7	0	43.5	—	+ 27
6420	6	2	—	—	4	2	49.5	47.5	+ 3
6422	0	3	4	—	0	0	—	—	—
6391	5	1	—	—	0	0	—	—	—
6579	Some still-born		3	3	0	0	—	—	—
6432	2	3	—	—	0	3	—	59.0	+ 4
6455	2	3	1	1	0	0	—	—	—
6447	2	5	—	—	0	0	—	—	—
6596	5	7	—	—	0	0	—	—	—
6445	6	1	—	1	0	0	—	—	—
Average	4.9	3.9	—	—	1.2	0.6	46.5	53.3	+ 11.3
					25.0	14.3			
					19.6				
% of does lactating successfully:						30			

* Only dead pups in first pregnancy.

N.B. Average of "pups born" includes substituted offspring.

Lactational performance of stock colony

The stock diet, the composition of which is given on p. 1989, was introduced into the laboratory in 1931 as the best of several diets tried and has been used ever since. This diet, containing a generous allowance of fresh liver, gives excellent fertility and growth. Nevertheless, a certain percentage of stock colony females fail to rear their young. A record of such failures for the years 1932-8 is set out in Table IV. It is hoped to present later a more detailed statistical analysis of the extensive data which we possess relating to the lactational performance of our stock colony rats. It will be seen that while in 1932 and 1933 some 90% of does weaned young, this percentage has been about 70% since 1934. The better performance in the first 2 years is possibly connected with more intensive breeding during that period, when the females were allowed only 2 weeks rest between weaning of young and next mating. Females are normally first mated at 3 months old and on those occasions when they were mated later

Table IV. *Record of litters weaned and of litters lost in the stock colony for the years 1932-8*

+ denotes weaned litters; - denotes that no pups were weaned

	1932		1933		1934		1935		1936		1937		1938		% of failures 1932-8	Total litters	% of failures 1934-8	Total litters
	+	-	+	-	+	-	+	-	+	-	+	-	+	-				
Jan.	19	0	20	3	29	22	6	7	11	5	-	-	45	30	34.01	197	41.29	155
Feb.	10	2	25	3	8	8	-	-	2	1	12	14	-	1	33.72	86	52.17	46
March	16	1	5	2	11	8	24	12	2	2	41	10	10	16	31.88	160	35.30	136
April	17	1	38	1	1	8	1	0	22	7	6	5	31	6	19.44	144	29.89	87
May	12	4	46	3	8	7	-	-	29	4	18	7	56	17	19.91	211	15.75	146
June	21	2	-	-	31	7	29	8	-	-	30	8	11	0	17.01	147	18.55	124
July	10	5	21	1	4	0	4	6	-	-	-	-	0	4	29.09	55	55.56	18
Aug.	34	2	20	2	-	-	12	6	30	8	-	-	-	-	15.79	114	25.00	56
Sept.	8	1	31	8	32	5	11	4	3	0	73	36	-	-	25.47	212	27.44	164
Oct.	18	2	2	3	2	0	-	-	14	11	-	-	-	-	30.77	52	40.74	27
Nov.	1	2	-	-	21	7	2	1	-	-	-	-	-	-	29.41	34	25.81	31
Dec.	31	4	7	4	6	0	9	4	28	9	29	11	-	-	22.54	142	25.00	96
Totals	197	26	215	30	153	72	98	48	141	47	209	91	153	74	-	1554	-	1086
% of failures	11.66		12.77		32.00		32.88		25.00		30.33		32.60					

The high percentage of failures in Jan./Feb. 1934, Sept. 1937 and Jan. 1938 may possibly be due to the fact that the majority of rats used were 5 months old when first mated. Further, since 1933 the does have had longer rests between litters.

(Jan.-Feb. 1934, Sept. 1937 and Jan. 1938) there was a high percentage of failures.

A comparison of stock colony females and of the animals on the "synthetic" diets (Tables I, II and IV) shows that there is little difference between them regarding lactational performance if the criterion of successful lactation is the ability to rear young. Whether or not the synthetic diets used by us would prove in the long run adequate for growth and reproduction is a matter of conjecture. What remains certain is that the simplified diets satisfied, in these experiments at least, the lactational requirements of the nursing mothers almost as well as a stock diet of natural foodstuffs. It is significant that the stock diet supplies to lactating mothers over 1 g. of fresh liver daily and that the addition of such liver to the simplified diet was without effect.

The secretion of milk by rats which fail to rear litters

When litters are examined and weighed after birth it is our custom to see whether by gentle massage milk can be expressed from the mother's nipples. We have almost invariably been able to obtain a drop or two of milk in this way irrespective of whether the mother failed later to feed and rear her litter or reared it successfully.

DISCUSSION

In considering the question of dietary requirements for lactation, specific dietary lactational factors (if such exist) should be carefully distinguished from dietary constituents which are essential whether or not the animal is lactating and the requirements for which may be greatly increased during lactation. Specific dietary lactational factors may be defined as dietary constituents which are essential for lactation alone of physiological processes. In order to prove the existence of such specific "lactation vitamins" the first need, as Nakahara & Inukai [1933] recognized, is to discover a diet which, while allowing satisfactory growth and reproduction, causes complete failure of lactation without any other pathological effects. Our experiments, contrary to the findings of the Nakahara school, fail to provide any evidence of the existence of such dietary factors, since, on a diet which was as far as we could make it similar to the "Factor L"-deficient diet of the Japanese workers, a satisfactory proportion of our rats was able to suckle and wean two successive litters. It is of course possible that our diet might not prove so satisfactory over a longer period and it must further be borne in mind in connexion with our failure to confirm the results of Nakahara & Inukai [1933] that diets of the same gross composition often give varying results in different laboratories. Nakahara *et al.* [1938] have recently reported that rats experiencing their second lactations on the "Factor L"-deficient diet after having lactated once on a normal diet do not fail so signally to lactate as rats undergoing their first lactation on the "Factor L"-deficient diet. This would not explain our failure to confirm their original results, however, since we used rats experiencing their first lactation.

Incidentally, it is of interest to note that our experimental diet gave satisfactory growth, since the post-weaning growth rate of a group of rats fed solely on our version of the Japanese diet was as good as that of our stock colony rats (see Fig. 1).

The failure to rear their young which, as we have seen, is normally exhibited by about 30% of our stock colony mothers, is probably not due to lack of specific dietary lactational factors, since milk can almost invariably be expressed from the teats of such rats. Nakahara & Inukai [1933; 1934], on the other hand,

reported that the mammae of rats which had failed to lactate on their "Factor L" deficient diet usually showed no signs of milk secretion on histological examination. We have not yet carried out histological studies on the

Fig. 1. Growth of female rats on the stock colony diet and and on the experimental (fish protein) diet ———. Fish protein diet, December 1936. ———. Stock diet, August 1936. Stock diet, March 1937.

mammary glands of our stock colony mothers which fail to rear their young, but we believe that in such cases the failure to wean young may possibly be due to factors of a psychological nature. It is evident that failure to rear young may be due to other causes than lack of milk secretion. Thus Hain [1935] attributed the death of the young of oestrogen-treated rats to the unwillingness of the mother to allow the young to suckle rather than to inhibition of milk formation, while Daniels & Everson [1935] found that a high percentage of the young of rats fed on diets deficient in manganese died because they were too weak to suckle.

As far as they go, our experiments support the findings of Cox & Imboden [1936, 1, 2] that rats can lactate normally on simplified diets and we have not obtained any evidence of the existence of specific dietary factors stimulating lactation qualitatively different from the dietary principles normally required by the non-lactating rat.

SUMMARY

1. A simplified diet of ground rice, casein, butter, salts and yeast was found to produce satisfactory lactation in rats. Additions of fresh liver, liver extract or yeast were without effect on the lactational performance.

2. Fairly satisfactory lactation was also obtained on a similar diet in which casein was replaced by fish protein.

3. Stock colony rats which failed to rear their first litters were found to be more likely to lose their second litters than rats which successfully raised their first litters. Subcutaneous implantation of tablets of pig anterior pituitary into such rats just after the second parturition had no effect on their ability to rear their young.

Note added 29 October 1938. In view of the findings reported on p. 1992 concerning the effect of additional yeast it is of interest that Perla [1937] believes that an excess of vitamin B₁ has an untoward effect on lactation and the nursing instinct of rats.

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