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DIET IN RELATION TO REPRODUCTION AND REARING OF YOUNG

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Any information which aids in securing an answer to the question as to what constitutes an optimum diet is of the utmost importance. The employment of the biological method of investigation has been exceedingly fruitful of results in this field. By this method the diet is submitted to actual feeding tests with experimental animals and their behavior thereon is observed. For this purpose the rat is most frequently employed on account of its relatively small food consumption, its omniverous habit, and its comparatively short reproductive cycle and span of life. It is thus possible to get the life histories of these animals for several generations on a given diet in a comparatively short time. This is, moveover, a field of investigation in which the English speaking people are the acknowledged leaders. Instantly the names of Hopkins, Drummond, Osborne, Mendel, McCollum, Sherman, and others, are called to mind in connection with this line of work. Recognition and study of the substances designated vitamins have revealed relationships in nutrition hitherto unsuspected. It would have been quite impossible to disclose these newer fields of investigation by means of the usual methods of chemical experimentation.

For a time the growth of the young rat was extensively studied, because at this stage of very rapid metabolism much can be determined relative to the adequacy of the diet employed. It was soon observed (1) that a diet shown to be adequate by this test may in reality be inadequate, for on continuing the experiment abnormal results may become manifest at the reproductive stage. The females may not become pregnant, or becoming pregnant they may give birth to but few young and the young may be born dead. Again, the young may be apparently normal at birth and may then die within a few days or else at about the time of weaning. It appears that parturition, the first few days of life, and at weaning time, are critical periods in the life of the rat. The sum total of environmental conditions and diet may be considered optimum when growth of the animals is normal, when

reproduction is accomplished at the usual age and thereafter at normal intervals with the normal number of young to the litter, and, what is most important of all, when the young are successfully weaned and continue the cycle from generation to generation. At present we are very far from knowing, in detail, these optimum conditions.

A diet may be considered to consist of water, salts, fats, proteins, carbohydrates, and vitamins. The constituents may for the most part vary both quantitatively and qualitatively. Perhaps the most widely known salt mixtures in use are those of Osborne and Mendel (2) and of McCollum (3). The former has generally been added to the extent of 4 per cent of the diet, the latter in varying amounts up to 5 per cent. When protein is added to the diet this is usually in the form of casein, and when constituting the entire protein of the diet it is usually added to the extent of 18-20 per cent. It may be washed with acidulated water for the removal of vitamins. Fats, varying in kind and amount, may be added as a source of vitamin or to supply energy. Little has recently been done to determine whether fats vary in nutritive value aside from their vitamin content. Starch or dextrin are used as added carbohydrates. The latter is made from the former by autoclaving the material, moistened with dilute citric acid solution, for several hours. As concerns the vitamins, vitamin A and vitamin B have been most studied and it is clear that these are essential in the diet of the rat.

Mathew writes (4): "Milk is a food for the young and growing. It is a most interesting food, since it represents the answer Nature has given to the question as to the best food for developing mammals. After a long period of experimentation in the monotremes, marsupials and lower placental mammals, the milk of the higher placentals was evolved. It is probable that there are good reasons for the presence in milk of most if not all its constituents." The importance of milk, and of the various food products derived from milk, is such that a knowledge of the nutritive values in this field of food products is highly desirable.

We shall consider some observations that have to do with the nutritive value of whole milk powder as determined by the biological methods using the rat as the test animal. The results obtained not only add to the knowledge of the nutritive value of milk powder but also bring to light some general relationships in the field of nutrition. The milk powder was Merrell-Soule material and it is desired to render this company thanks for the very

generous supply accorded us during the progress of this investigation. With reference to its manufacture they state (5): "Natural milk is first pasteurized by the holding process and partially concentrated by a vacuum process. Then it is sprayed under heavy pressure into the chamber through which a current of filtered warm air is passing. The milk is broken up into almost invisible spray and upon reaching the warm air loses its moisture by evaporation. The humid air passes out through a vent and the solids fall as a dry powder of less than 3 per cent moisture. The important feature in the process is that the milk powder never reaches the boiling point and therefore does not carry the cooked flavor to the product, "Klim"; nor, even more important, does it lose one iota of its food value." After numerous analyses the following values have been selected for the whole milk powder used in this work: protein, 26.4; fat, 27.5; and ash, 6 per cent.

Other investigators have reported their observations on the nutritive value of whole milk powder. It may be said that in general the expressed opinions are that it is in some way inadequate. Recently Mattill and Stone (6) have reported results from the reproductive viewpoint especially. They studied the following diets:

Whole milk powder 50, Starch 38, Lard 10, Salts 2.0.
Whole milk powder 60, Starch 28.4, Lard 10, Salts 1.6.
Whole milk powder 70, Starch 18.8, Lard 10, Salts 1.2.
Whole milk powder 80, Starch 9.2, Lard 10, Salts .8.
Whole milk powder 100, Iron citrate 0.2 per cent.

As concerns growth it is stated that the animals grew for the most part at better than the normal rate for a period of 75 days on these diets but subsequently the rate was below the normal. The decline was proportionately greater for the females than for the males. Reproduction was not successful. Among some 30 females carried on these diets only 6 are reported as being pregnant and young were seen in but two instances and the young were either dead when found or else died in a few days. Moreover, there was not the manifestation of the usual maternal instinct, as the females prepared no nests but allowed the young to lie scattered about the cage.

At about this time the question as to the effect of the salt content of the diet upon the reproductive performance of the rat was receiving serious consideration in this laboratory. In studying the nutritive value of yeast it was observed (7) that when 5 per cent of salt mixture were included in the diet the animals were almost entirely sterile though the rate of growth was normal.

When the salt content was reduced to 3.7 per cent reproduction occurred but there was difficulty in rearing the young, as these tended to gradually wither away and die during the suckling period. As whole milk powder contains about 6 per cent of ash it is evident that in the diets of Mattill and Stone set forth above the salt content was for the most part greater than 5 per cent. It was now tentatively assumed that their failure with reproduction might be due to the high salt content of the diets. Diets 1 and 2 were therefore devised to determine whether or not this was the cause of their failure. A diet containing 60 per cent of whole milk powder was chosen as this contains about 3.6 per cent of ash, an amount which, in terms of salt, had been shown to produce comparatively good results. As milk is known to be low in iron 0.2 per cent of iron citrate was added, increasing the salt content to 3.8 per cent. To insure a generous amount of protein 6 per cent of water washed casein were added, 4 per cent of agar-agar served as roughage, and the remainder to 100 was composed of dextrin. Diet 2 analogous to diet 1 had a portion of the dextrin replaced by salt, McCollum's number 185 (3), to the extent of 2.4, thus raising the salt content of this diet to about 6.2 per cent. In order to forestall shortcomings on the part of the milk powder an additional lot of rats receiving daily a liberal allowance of fresh whole liquid milk was started on each of these diets. But as there appeared to be no advantage in this addition it was discontinued after the first litters of young had been weaned. The general results of the experiment were not entirely in accord with preceding expectations.

Every female on each diet became pregnant and gave birth to young, and young were successfully weaned on every one of the 4 diets (that is, diets 1 and 2 supplemented and unsupplemented with liquid whole milk) discussed. Also, the lots receiving the salt addition had pronouncedly better records as to reproduction and rearing of young than did the others. The animals in the lots whose diet carried 3.8 per cent of salt made more rapid growth and became heavier than did those with more salt in the diet, but as a whole those getting more salt mixture in the diet reproduced earlier, had more litters and more young, and were more successful in rearing them. In fact, diet 2 just discussed is one of the very best devised in this investigation. From the behavior of the animals on these diets it appears that optimum growth and optimum reproductive performance may not necessarily both be obtained on the same diet. Females to the number

of 25 in various lots have been carried on diet 2 for different lengths of time. Altogether 64 litters comprising 505 young were actually seen and of this number 214 were successfully weaned. It should be stated that frequently females and their young were discarded before the young were weaned. The best single record was of a female which produced 4 litters of 42 young and weaned 39 of them. Fourth generation young have been weaned on this diet. They were apparently entirely normal and in general appearance the equal of any young observed on these milk powder diets.

In order to further test the effect of added salts diet 3 was devised which is comparable to those discussed except that the salt content has been raised to about 8 per cent. The animals grew at considerably better than the normal rate. The 4 females on this diet became pregnant and produced young. Three of the females had 8 litters of 64 young of which number 58 were weaned. One female had only one litter of 5, and they were dead soon after birth. These animals were on the experminent only about 18 weeks. The second generation rats on this high salt diet grew well, and were remarkably thrifty looking, being unusually smooth and clean and free from all scabs. The 4 females each gave birth to a litter of young. Two of the litters were successfully weaned at which stage this experiment was discontinued. Clearly the salt content was not the limiting factor in the work of the other investigators. And the amount of salt which animals can tolerate in the diet is not absolute but is determined by the nature and perhaps by the relative proportions of the other constituents present.

It is known that given a selection from among a number of foods animals will select favorably to their own well-being. A lot of rats was placed in a cage where they had free access to butter, whole milk powder, casein, agar-agar, and salt mixture, contained in separate receptacles. It was observed that the young rats tend to consume a diet high in fat while with advancing age the consumption of butter and of whole milk powder decreased and considerable quantities of casein were consumed. This observation indicates that it is quite possible that the composition of an optimum diet for the rat varies with the age of the animal. The rats were kept on this diet for about 12 weeks and manifested growth at a little better than the normal rate. . Several of the females reproduced and from 2 litters of 5 and 6 young, 3 and 5, respectively, were successfully weaned. It now appeared that the

lard supplement employed by Mattill and Stone was responsible for their unsuccessful reproduction.

Lard was early used in experimental work of this nature particularly by Osborne and Mendel (8). These distinguished investigators were particularly interested in being able to determine the quantity of food eaten by the experimental animals and so desired to have the food in a pasty condition in order to prevent undue scattering. They clearly recognized that the inclusion of the lard in the diet might lead to undesirable conditions for they expressly state that "The necessity for the use of much fat to insure the requisite paste consistency (and thus avoid scattering of food) has put a distinct limitation on the range of choice. We have tried without success to avoid the use of so much fat." It is natural that persons employing essentially the technique developed by these pioneer investigators would include considerable lard in their experimental diets. The method of feeding employed in this laboratory does not necessitate the use of food in a pasty form, consequently the fats have usually been kept to the minimum of butter fat deemed necessary to furnish vitamin A. If the supposition that the lard is the deleterious factor is correct then the matter is capable of experimental proof.

For this purpose the diet containing 50 per cent of whole milk powder was selected. Diet 4 is exactly like the corresponding one of the other investigators while diet 5 has the lard removed and its weight replaced by agar-agar and dextrin. Though the animals were kept on diet 4 during a period of 32 weeks there was no evidence of a single advanced pregnancy. Copulation was observed several times when the animals had been on the diet from 4-8 weeks. The growth rate was perhaps a trifle below the normal, the maximum weight attained by the males being 250-300 grams, that of the females 200-250 grams. From the 32 to the 36 week the food was changed to that which is used in the stock colony. During this time there was no noticeable change in the behavior of these rats. Also, at about the 28th week on the ration 3 females were taken from the stock colony immediately after weaning their litters of young and were placed for a period of 7 days in a cage together with the males on diet 4. There was no evidence at all that would indicate that the males were fertile. The behavior of the animals on diet 5 was entirely different. The rate of growth was about the same as that of the animals on diet 4 but their reproductive performance was just the opposite. As shown in the table, 3 of the females had a total of 14 litters of

94 young, of which number 74 were weaned. Second generation animals on diet 5 grew at the normal rate but did not reproduce until they had been on the diet for a period of 20 weeks while normally reproduction occurs at 6-8 weeks after the animals are on the experimental diet. Each female produced a litter of young. There was a total of 24 young of which 22 were weaned when the experiment was discontinued. Since there were 2 males and 3 females in this lot it is probably not accidental that reproduction was so long delayed on this diet. The protein content of this diet is 13.2 per cent. This speaks for the quality of the protein in milk and for the presence in whole milk powder of the vitamins necessary for the metabolism of the rat.

In diet 2 the amount of fat is approximately three quarters that of the protein. It is therefore of interest to determine whether diet 4 while still retaining the 10 per cent of lard can be so modified as to bring about reproduction. Diet 6 is the result of such modification. On this diet the animals grew at very considerably above the normal rate, and the females reproduced and reared young. Only the first and second litters of young were weaned, the young of the third litters perishing a few days after birth. The second generation on this diet also grew at better than the normal rate but their record for reproduction was even less satisfactory. They became pregnant and produced young but these were few in number and seldom lived beyond a few days. Only one litter of 4 young was reared. These were apparently normal. It appears that undue concentration of fat and protein in the diet is deleterious.

Some differences were observed on diets composed almost exclusively of whole milk powder. It appears that when 0.2 per cent of iron citrate are added the animals barely get along, growth being about normal though the general appearance of the rats indicates abnormal conditions. There is some reproduction and a few young are reared. When, as in diet 8, the amount of iron citrate is increased there is a very decided improvement in the response of the animals. Growth is considerably better than normal, the appearance of the animals is good and reproduction and rearing of young are improved. Though the first generation, lot 154, has been on the diet only about 13 weeks first litters have been weaned and 2 of the females will have their second litters within a very few days. The second generation rats on diet 8 are about 7 weeks old and are fine appearing animals. These observations at once suggest the possibility of a relationship between

a polyvalent cation and the metabolism of a diet in which the fat and protein are comparatively high.

In all some 20 diets containing from 50-99.8 per cent of whole milk powder variously supplemented with salt mixture, fat, protein, agar-agar, starch, and dextrin have been studied. On nearly all of these diets growth has been better than normal. On many of them reproduction has been good though mortality of the young during the suckling period has been high. It is now pretty

TABLE I

COMPOSITION OF DIETS

No. of Ration	Percent of Whole Milk Powder	Percent Casein	Percent Salt Mixture	Percent Iron Citrate	Percent Agar-Agar	Percent Dextrin	Percent Starch	Percent Lard
1	60	6	0.0	0.2	4.0	29.8		
2	60	6	2.4	2.2	4.0	27.4		
3	60	6	4.2	0.2	4.0	25.6		
4	50	0	2.0	0.0	0.0	0.0	38	10
5	50	0	2.0	0.0	4.0	44.0		
6	50	20	2.0	0.0			18	10
7	99.8	0	0.0	0.2				
8	99	0	0.0	1.0				

TABLE II

RECORD OF GROWTH AND REPRODUCTION

Lot	Diet	Generation	Females	Weeks on Diet	Growth	Litters	Total Young Seen	Young Weaned	Young Dead 48 Hours After Birth
52	1	1	3	29	nn	11	57	45	12
53	2	1	3	29	n	11	94	87	7
91	1	2	3	25	nn	6	44	36	8
101	2	2	3	25	n	9	65	62	3
122	3	1	3	22	nn	8	64	58	6
152	3	2	3	15	nn	3	26	12	11
89	4	1	3	32	n-	0			
90	5	1	3	32	n	14	94	74	20
121	5	2	3	24	n	3	24	22	
114	6	1	3	24	nnn	9	65	50	2
138	6	2	3	19	nn	3	13	4	9
78	7	1	3	20	n	3	15	4	11
154	8	1	3	13	nn	3	15	13	2

n Represents growth at the normal rate, nn at better than the normal rate, etc.

well known that it is frequently difficult to rear the young on a diet on which the parents make very good growth. This may in turn depend upon the milk secretion of the mother for the milk may diminish in amount or may be qualitatively altered in such a way

as to be deleterious. Whether some definite substance must be supplied in the diet of the rat in addition to the usual food constituents and vitamins A and B for the secretion of normal milk is an open question. A survey of the records reveals that in this particular study over 200 females and about half that number of males have been carried on the different diets for periods varying from 12 to 32 weeks. 2085 young belonging to 286 litters were actually seen and of this number of young 1425 were weaned. As stated above, young were frequently discarded before there was opportunity for weaning them. It appears that the young which perished usually died within 48 hours after birth. The reason for this and the extent to which the deaths were accidental is not known. It has been shown (9) that in a large rat colony in which the diet was of wide selection the number of the young that was weaned in comparison to the number born was 70-80 per cent. These same investigators (9) further state: "Under the best of nutritional conditions instances are encountered in which all of the young are devoured by the mother, abandoned by her or for unknown reasons die before the day of weaning." Anyone familiar with the reproduction of domestic animals will realize that this is a matter of general application. In the table setting forth the reproductive records of the animals only those data applying to the 3 females having the best reproduction records are included. By so doing the degree of success attained in the rearing of young appears greater than is evident from the general average for all the animals included.

It is believed that the failures encountered in this investigation are not to be attributed to the lack of an essential factor, as concerns the metabolism of the rat, in whole milk powder, but is to be explained on the supposition that the constituents of the milk of the cow are not present in the relative amounts which constitute the optimum diet of the rat. In a number of diets devised wherein whole milk powder served as the sole source of protein and vitamins the experimental animals have made a record, as concerns growth and reproduction, which is entirely comparable with the behavior of these animals on a diet of wide selection.

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