CXL. A DEFICIENCY DISORDER INDUCED IN SUCKLING YOUNG RATS BRED ON A PURIFIED SYNTHETIC DIET WITH "GLAXO CASEIN" (CASEINOGEN) AS SOLE SOURCE OF PROTEIN.

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IN a former communication [Mapson, 1932] the stimulating effect on growth of small amounts of mammalian liver fed as supplement to a purified basal synthetic diet containing all hitherto known dietary principles was demonstrated. It was shown that young rats when transferred from the stock diet to the synthetic ration and fed in addition on small amounts of fresh ox-liver showed a greatly accelerated growth over control animals. Evidence was submitted of the non-identity of this growth-promoting factor with any of the better-known vitamins. This growth-promoting principle was provisionally named physin. During the course of this work the anomalous behaviour of a small number of litters was observed.

The addition of small amounts of liver to the diet failed to give the usual acceleration in growth seen in the majority of the litters. These variable results were in contradistinction to the very uniform and consistent responses obtained when liver was fed not directly to the offspring but by transmission from the parent animal.

These results were explicable on the assumption of a seasonal variation in the content of physin in the stock dietary. This assumption was supported by the fact that control offspring from litters showing little or no effect of the liver feeding displayed an absolute growth rate of a higher magnitude than that of control animals from litters in which the acceleration effect of the liver feeding was apparent. Moreover, the growth rate of control animals in the first group was approximately the growth rate given by the liver-fed offspring of the second group.

An attempt has been made in the more recent work to eliminate as far as possible the effect of a stock dietary and to render the young animals more uniformly deficient in this growth-promoting factor. The further possibility had to be borne in mind that the actual synthetic diet originally used was not entirely deficient in physin. The existence of such a dietary principle had been demonstrated in the former work merely by its stimulating effect on growth, it was hoped by using a completely deficient diet, that animals might show definite pathological symptoms of such a deficiency.

With the latter consideration in mind the synthetic diet used in the former work has been modified in various respects. The results of these experiments here recorded indicate in a striking manner the nutritional differences existing

between various caseinogen preparations. A definite deficiency disorder has been disclosed by a modification of the original synthetic diet by the substitution of a different brand of caseinogen in the ration.

This deficiency has been cured by the addition of active curative extracts containing physin to the basal ration.

EXPERIMENTAL.

It was considered that even though modifications of the former synthetic diet might not yield a ration entirely deficient in physin, and might therefore still sustain some growth in the young animal, such low physin-containing diets might yet be inadequate for normal breeding and lactation. Hence offspring from such parents might be expected to be relatively more deficient in physin and more uniform in this deficiency than experimental animals derived directly from the stock diet used in this laboratory.

The experimental procedure has thus been to breed animals on modifications of the original basal diet, and a twofold study of the lactating powers of the females and growth rate of the offspring on such diets has been attempted.

All the animals used were black and white rats from the stock strain bred in this laboratory. The young animals were separated at weaning from the stock diet at an age of approximately 4-5 weeks. Their weight at this age ranged from 40 to 60 g.

At this stage they were placed on the synthetic basal ration. All the synthetic diets were fed *ad libitum*.

Some 16-20 weeks were allowed to elapse before mating of the animals occurred. During gestation and lactation only the synthetic diets were used.

The principal modification of the original synthetic diet has been the substitution of "Glaxo casein" (caseinogen) for "light white casein B.D.H." (Na caseinate). The composition of the synthetic diets used is shown in Table I.

Table I. *Composition of diets (parts by wt.).*

Diet	E	E'	B	F
Protein				
"Glaxo casein"	—	—	—	23
"Light white casein"	23	23	23	—
Rice starch	40	40	46	40
Sugar	17	17	17	17
Arachis oil	15	15	15	15
Wheat embryo	8	8	2	8
Dried yeast	8	8	8	8
Cod-liver oil	2.5	2.5	2.5	2.5
Salt mixture	5	5	5	5
Manganese sulphate (anhydrous)	1 mg.	10 mg.	1 mg.	1 mg.

Cod-liver oil was incorporated in the basal diets. The diets were made up only in small quantities at a time so as to obviate any possibility of deterioration of any of the vitamins included.

The stock diet used in this laboratory consists essentially of mixed corn, wheat embryo, dried yeast, bread and fresh milk daily. The salt mixture used in the synthetic diets was a modification of that used by Osborne and Mendel; the complete composition was given in the former communication [Mapson, 1932].

Breeding on Diet E.

The general results with breeding on the original synthetic diet (Diet E) have been described fully in the former publication. Failure of lactation is common in from 50 to 70 % of the cases studied. This failure occurs at the very outset of the lactation period. The parents neglect their offspring from birth, and in many cases they kill and eat them. Out of some 60 litters bred on this diet no single case is on record in which the mother started to feed her offspring and then eventually failed to rear them. Those litters in which suckling was commenced were completely successful during all the later stages. The young weaned on this diet exhibited an exceptionally good growth rate. No pathological symptoms were seen in any of the litters in which suckling was commenced by the parent. The average weights of the offspring on this diet were: 1st week, 12–14 g.; 14 days, 23–26 g.; 21 days, 35 g.; 28 days, 45–50 g.

Substitution of "light white casein" by "Glaxo casein." Diet F.

This diet differed from Diet E only in the substitution of "Glaxo casein" (caseinogen) for "light white casein B.D.H." (Na caseinate). Breeding occurred as in Diet E and here again a large percentage of cases showed the typical immediate failure of suckling at birth. At this point, however, the resemblance between the two diets ceased, for of the mothers which succeeded in starting to feed their young only a small percentage were successful in rearing any of their offspring. With these latter litters suckling was successful up to a period varying from 14 to 28 days, when the offspring, very much undersized in weight compared to offspring bred on Diet E, showed a definite failure to grow. The onset of the symptoms in a few cases was delayed until after weaning, but in the majority of cases definite pathological symptoms were evident at approximately the 24th day. The baby animals developed sparse greasy coats, looked weak and were seen to have less than normal activity. The occurrence of these symptoms was followed rapidly by the appearance of exhaustion, and finally death ensued within a few hours. In many of the litters before this extreme condition was reached, a vitamin deficiency behaviour was shown by the more lively animals, these attaching themselves to their more moribund companions and frequently eating their entire viscera.

Post mortem examination of the young rats showed no macroscopic signs of pathological change or of infection in any of the organs, except for the extraordinary absence of fat anywhere in the body.

To all outward appearances, in those litters in which the symptoms developed prior to weaning, the mother continued suckling her young right up to the stage of exhaustion.

In the case of a small number of the litters studied some of the young offspring survived without addition of any supplement to the basal ration. That the synthetic Diet F is not completely deficient in this principle seems possible from the fact that young rats saved from death by the feeding of effective supplements were, after some 10–15 days, able to maintain themselves and grow on the basal diet alone. The explanation adopted here is that the basal ration F, though adequate for normal requirements during growth, is inadequate for the needs of the lactating female, and that this deficiency is reflected in the offspring.

Administration of active curative supplements.

To many of the offspring from these litters, effective supplements have been given; these experiments have been as yet only of a preliminary nature.

The following supplements when added to the basal diet or fed directly to the young offspring have had a curative effect.

(1) Substitution for the "Glaxo casein" in the basal diet of "light white casein" has resulted in complete recovery of 15 animals from three different litters, control animals still maintained on the basal Diet F eventually dying. The rats from these three litters had been weaned at 28 days and segregated into two groups, the two groups being placed on Diets E and F. These results are indicated in Figs. 1 and 2.

Fig. 1.

Fig. 2.

Fig. 1. Representative growth curves of control offspring maintained on Diet F. Weaned from parent 28 days old. x denotes death.

Fig. 2. Representative growth curves of offspring from the same litters, in which "light white casein" replaced "Glaxo casein" in Diet F at weaning.

(2) Extraction of the "light white casein" three times with cold 90 % acetone failed to remove the whole of the factor present in the protein. But the rate of recovery of the experimental animals when such extracted "casein" replaced the "Glaxo casein" in the diet, as indicated by gain in weight and disappearance of the sparse-looking fur, was much delayed.

(3) Administration of the 90 % acetone extract from "light white casein" resulted in two cases in the recovery of the young, control animals dying on being kept on the basal ration alone.

(4) Decidedly beneficial results have also been obtained by the feeding of a physin extract prepared from ox-liver. This extract represents material extracted from liver soluble in 90 % alcohol and 90 % acetone and was prepared as follows. Fresh ox-liver was minced and acidified to a p_H of 5.0, and the cells were broken down by means of rapid digestion by papain. After digestion an aqueous extract was made. Alcohol was added to a concentration of 90 %, the filtrate concentrated *in vacuo* at 40° and the residue dissolved in water. Acetone was then added to a concentration of 90 %. This precipitates further material. Approximately 380 mg. of acetone-soluble material are obtained from 100 g. fresh weight of liver. This material was fed in doses equivalent to 1-2 g. of original liver. A decided improvement was apparent in the animals receiving

this supplement, the outward indication of which was a rapid recovery and gain in weight, and complete disappearance of all sparse-looking fur. Death followed in control animals not receiving the supplement (Fig. 3).

Fig. 3. Representative growth curves of offspring from litters bred on Diet F. Offspring separated from mother at 28 days. ↑ Supplement; physin extract from fresh ox-liver. × denotes death.

Modifications of the synthetic diet.

(a) *Manganese.* As Orent and McCollum [1931] have demonstrated the insufficiency of a diet completely deficient in inorganic manganese for the normal requirements during lactation, the content of inorganic manganese has been varied. Diet E' represents the composition of the original synthetic diet with

Table II.

Diet of mother from weaning	E'	E	B	F
Diet of offspring	E'	E	B	F
No. of litters on experimental diet	21	30	16	25
Litters of mothers showing immediate lactation failure at birth	11	13	10	14
Litters suckled at birth	10	17	6	11
Litters successfully weaned	10	17	6	None*
Total no. of rats born	155	224	124	189
Total no. of rats born whose mothers commenced feeding at birth	68	122	41	93
Total no. of rats alive at 14 days	66	120	40	87
Total no. of rats alive at 28 days	66	118	40	43
Total no. of rats alive at 5-6 weeks	65	118	40	10

* 2 partially successful.

an increase of inorganic manganese to approximately 0.01 % of the ration. No significant differences, however, were noted using this diet.

(b) *Wheat embryo*. The composition of Diets E and F reveals their large content of wheat embryo: this had been included to provide an adequate supply of the vitamin B complex. It was thought possible that such a synthetic diet might well possess too high a standard of vitamin E, leading to a disturbance of lactation, and might thus be the explanation of the large number of mothers who failed to commence feeding their offspring. Accordingly the wheat embryo has been reduced in Diet B to 2 %, just sufficient to ensure an adequate supply of vitamin E. The results of breeding experiments on this diet have, however, still revealed the immediate failure of lactation at birth, and in general no significant difference was apparent between Diets B and E. The general results recorded in this paper are given in Table II.

DISCUSSION.

Comparison with deficiencies previously noted.

The results recorded in this paper bring out in striking contrast the nutritional differences between "Glaxo casein" (caseinogen) and "light white casein B.D.H." (Na caseinate). Certain differences have been noted before.

Coward *et al.* [1929] demonstrated the possibility of the existence of a new dietary principle necessary for the continued growth of the rat. Their basal ration was, however, deficient in vitamin E, and this objection might be raised in connection with their work. They found their growth factor to be present in "light white casein" and absent from "Glaxo casein." They also demonstrated its presence in other natural foods, *e.g.* ox-liver, milk, wheat germ. They concluded that, although possessing many properties suggestive of vitamin E, it was not vitamin E on account of its different distribution and more unstable nature. The experiments recorded here indicate quite conclusively that the deficiency studied here is not due to any lack of vitamin E.

Palmer and Kennedy [1927, 1, 2] came to the conclusion, as a result of work on synthetic diets, that a further dietary principle existed which was not identical with vitamins A, B, C, D or E. They were unable to obtain normal growth on a synthetic diet of a highly purified caseinogen, butter-fat, agar-agar and an alcoholic extract of fat-free wheat embryo. Much better growth was obtained if a commercial caseinogen was used instead of highly purified caseinogen. The rats failed in growth and developed symptoms closely allied to those noted in the present work. They found that alcoholic extracts of wheat embryo stimulated the growth rate. The main objection to their work lies in the possibility of their diet being deficient in vitamin B₂, since their wheat embryo extract which formed the sole source of the vitamin B complex was obtained by use of high alcoholic concentrations, *e.g.* 85–95 %.

The observations of Palmer and Kennedy and of Coward *et al.*, however, indicated the possibility of the existence of a further dietary principle necessary for normal growth. The results recorded in the present work have indicated the inadequacy of a synthetic diet, complete according to established criteria, for normal lactation and growth of the offspring. This deficiency is not due to vitamin E. That the deficiency is not due to a lack of the vitamin B complex is shown by the fact that many of the active supplements effecting a cure are completely deficient in vitamins B₁ and B₂, *e.g.* "light white casein," or extracts from this. Moreover, a diet such as Diet B employed here, in which the con-

centration of the vitamin B complex is smaller than that of either Diets E or F, is still adequate for supplying the vitamin B requirements both for growth and lactation.

Inadequate protein.

The possibility had to be considered that the deficiency studied here might be due to some unsuitability of the "Glaxo casein" as a source of protein. It might be argued that the death of the animals described above may have been due (1) to some inherent deficiency in the "Glaxo casein" caused by its method of manufacture; (2) to some toxic body present in this protein which upsets the maternal metabolism and lactation and which may be passed to the offspring.

Experiments to throw further light on this question are being promoted, but the following considerations would seem to negative these views. Rats taken from the stock dietary and placed on Diet F grow equally well as rats placed on Diet E. Moreover, addition of liver or fresh milk to parent animals breeding on Diet F, prevents entirely any of the deficiency symptoms described above. Further, the growth rate of offspring from such litters is equal in magnitude to that obtained when liver supplements the "light white casein" synthetic Diet E. It is difficult to see how these results could be obtained if "Glaxo casein" suffered from some inadequacy as a protein.

It is conceivable that the Glaxo protein may contain small traces of a toxic constituent, due to its method of manufacture, which may be injurious to the very young animal, having no effect on an animal some weeks older. The evidence at present in hand is antagonistic to such a viewpoint. Until more positive evidence of such toxicity is available, it seems more reasonable to adopt the deficiency hypothesis outlined in this paper.

Possible identity with the Coward factor.

The further question arises whether this deficiency is caused by an absence of the Coward factor or physin, and whether these postulated dietary principles are not identical.

Physin, as has been shown in a former publication, causes an acceleration of growth in young rats maintained on a basal diet of the nature of the diet E used in the experiments now reported. Such a diet, it was believed, should be entirely adequate in the Coward factor. Dr Coward has, however, informed me (private communication) that she believes that 20 % wheat embryo in the diet might not be sufficient for the production of maximum growth in a ration otherwise deficient in this factor.

It is thus conceivable that the stimulation of growth obtained in the previous work by the addition to the basal ration of liver may have intensified the growth rate because of a more adequate supply of this substance.

It has been shown in this paper that an active extract containing physin is effective in curing the deficiency symptoms noted above. Preliminary stages in the fractionation of physin have revealed its solubility in such solvents as 90 % alcohol, 97 % alcohol, 90 % acetone. These compare well with the reported solubilities of the substance curing the deficiency reported by Coward *et al.* These results are suggestive, but a definite answer to this question must be left for future work.

The dietary cause of the immediate lactation failure reported in this and earlier communications has still to be cleared up. That it is not due to simple inorganic manganese deficiency seems clear. Whether it may be looked upon as an acute form of physin or Coward factor deficiency remains to be determined.

In further work on this problem the ability of the rat to store the growth-promoting principle must be taken into account. The evidence at present to hand suggests that this ability may be considerable. Recent work in progress indicates that young rats weaned from the stock dietary used in this laboratory will grow well up to weights approximating to 200 g. on diets deficient in this factor.

Of equal importance in this respect is the nature of the stock dietary from which the experimental animals are derived. The inability to reproduce identical results under apparently the same external conditions may well be traced to these causes.

SUMMARY.

1. The results of breeding rats on several different synthetic diets have been recorded.

2. A deficiency disease apparent in the suckling young of rats receiving "Glaxo casein" as their source of protein has been described. Failure of growth, loss of fur and eventually death are the typical symptoms observed.

3. Nutritional differences influencing the deficiency disease have been shown to exist between "Glaxo casein" and "light white casein B.D.H."

4. Active extracts containing both physin and the Coward factor have been shown to cure this deficiency.

5. The possible identity of physin with the Coward factor is discussed.

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