II. INVESTIGATIONS INTO VITAMIN A-FREE BASAL DIETS. I.

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Two of the main difficulties associated with the use of diets for work on vitamin A have been the occasional failure of experimental animals to reach a "plateau" of inhibited growth—to "run out" of vitamin A, in the phrase of certain investigators—and rather more frequent failure of the animals, after they have ceased to grow, to respond to even relatively large doses of the vitamin with anything approaching normal growth performance. It is generally held that the first difficulty is due to high vitamin reserves, the result of a too liberal pre-experimental (including pre-natal) diet and that the second is due to some inadequacy in the basal diet, since, if this is deficient in other constituents besides vitamin A, the mere provision of the latter will not bring about continued normal growth.

Most of the published work that bears on these problems has appeared incidentally in papers dealing with the detection or estimation of vitamin A, and there have been relatively few investigations into the experimental technique as such. Notable exceptions, however, are the papers by Coward and her colleagues [1929, 1, 2], who have studied amongst other things especially the effect of different sources of protein (caseinogen) on the ability of the animals to resume growth when vitamin A is restored to their diet. Mention must also be made of the very interesting critical study recently published by de Lourero [1931], who has examined and discussed all the chief constituents generally present in vitamin A-free diets. A recent communication by Culhane [1932] was concerned with the regularity of the incidence of xerophthalmia in relation to the source of protein in the basal diet, but the details of her work have not yet been published. Moore [1932] has also made passing reference to similar problems.

These problems are of considerable importance, quite apart from their theoretical interest, since a multiple deficiency in the basal diet might quite conceivably cause an over-assessment of the amount of vitamin A in a substance that was being compared with a simple solution of carotene in vegetable oil. Further, the question of securing uniform and rapid response to a diet deficient only in vitamin A is of as immediate concern to all workers in the field as is that of securing quick and normal response to curative treatment.

Although our interest in this problem was partly stimulated by Coward's findings, we have for years been attempting to approximate more closely to a theoretically perfect basal diet without complete success, and we are presenting here in a condensed form such of our results as seem either to throw light on the unsolved problems or to call for some explanation that we ourselves have been unable to find.

Various preliminary experiments, undertaken as time and the necessary stock of animals were available, indicated an inadequacy of commercial yeast extract (marmite) as a source of the vitamin B complex, since better growth was obtained either by increasing the amount of the extract or by supplementing the diet with wheat germ; in this we have confirmed the findings of several other workers. The preliminary experiments also suggested that the weight of the animals when they begin to receive the basal diet is a less important factor in determining their running-out age or weight than is their age. In this we find ourselves in agreement with Nelson [1928], who has advocated transferring the nursing mother and sucklings to the basal diet some 16 to 18 days after the birth of the litter. Further, our experiments indicated that, if this is done, the replacement in the stock diet of a New Zealand dried milk, with its relatively high vitamin A content, by an English winter-dried milk has little, if any, effect on the running-out time. These observations tally well with some work recently published by Dann [1932]. Again, the time of survival of animals on a vitamin A-free diet, their initial growth rate and the time of reaching a growth plateau do not appear to be significantly influenced by the presence or absence of fat in that diet.

The animals employed in this work were exclusively of the albino variety of the Norwegian rat (*Mus norvegicus*); all were descended by the closest inbreeding from three bucks and six does obtained in the winter of 1923 from the Wistar Institute of Anatomy and Biology, Philadelphia, U.S.A. All animals were removed by at most one generation from animals that were themselves the product of continuous brother-sister mating of descendants from the original nine animals. We have never followed the practice advocated by some workers of reducing the numbers of all litters after birth to 6 or 7. The mothers are allowed to bring up as many of their young as they are able; this has once been as high as 17, and the average size of litters at weaning has been 6.7.

In all the work discussed in this paper we have, unless otherwise stated, compared only isogenic animals (animals of the same sex and from the same litter).

For the last three years we have fed our general breeding stock (those, that is, whose F_1 litters alone have been used in these investigations) on a diet constituted as follows:

Whole-meal flour	...	...	...	...	...	70 parts
Full-cream New Zealand dried milk...	...	...	...	...	...	30 "
Commercial yeast extract (marmite)	...	...	...	...	...	5 "
Water	...	...	...	...	...	45 "

Lettuce (about 2 g.) or other green stuff is fed daily, liver or lean meat (about 2 g.) twice weekly, and fish (a few g.) once a week.

This diet, which is a modification of Sherman's stock diet B [Sherman and Burtis, 1928], was introduced to replace a diet of mixed kitchen scraps, on which our whole stock had previously been fed, since this was not always available in sufficient amount and must in any event have varied considerably in its content of fat-soluble vitamins; the scrap diet, supplemented with various sources of vitamins and minerals, is now used only for a selected number of special pairs bred as a "genealogical" stock to produce the general breeders; its replacement does not appear in any way either to have reduced the numbers of young animals born or to have affected their health.

In spite, however, of the uniform diet and the intensely inbred nature of the animals, there is still a large, unexplained, and uncontrollable variation in

the response of individual animals to basal diets. Many animals receiving a diet on which some animals cease growing at about 120 g. weight (males) in 8 to 10 weeks will continue to grow for months with no evident signs of any kind of hypovitaminosis (see negative control animal, Fig. 1). Others cease growing at a relatively high weight, 180 g. or more; their growth response to any supplement is then limited by the normal growth rate for that weight. Some animals appear to stop growing for as long as 14 days and then spontaneously continue to grow again for 4 or more weeks, behaving exactly as if a vitamin supplement had been administered, though actually no known change whatever has been made in their diet. The literature abounds in graphs showing animals that have been

Fig. 1. Anomalous growth curves of animals on vitamin A-free diet; no cessation of growth in negative control animal and secondary growth plateau in animal receiving curative treatment.

Fig. 2. Anomalous growth curve of negative control animal on vitamin A-free diet; cessation of growth with long survival (and no xerophthalmia).

considered "run out" when their growth has only slackened, without even actually ceasing, for a few days, and one may be permitted to wonder whether their "response" to vitamin supplements was wholly due to the supplement.

The onset of xerophthalmia in our stock is so uncertain that it has been found useless as a criterion of avitaminosis, though when it does occur it is invariably relieved or cured by administration of sufficient vitamin A, whether with or without resumption of growth. It is not uncommon, again, for "negative control" animals to reach a growth plateau, at 120, 150 or even 200 g. weight or more, and then to continue to live in apparently perfect health for weeks, or even months, with no change in diet or weight (see negative control animal,

Fig. 2). In so far as our experiments cover the same ground as those of Culhane [1932], we agree with her that the incidence of xerophthalmia does not appear to be markedly different with soluble caseinogenate and heat-treated caseinogen, and is both low and erratic with either. We have no experience of the alkaline-heated caseinogen that she found efficacious in the regular production of the eye-trouble.

It can, however, be said that the basal diet at present in use, combined with the technique of Nelson mentioned above, allows quite a large number of litters to respond satisfactorily to the diet, but the unexplained vagaries just described make it essential to discard whole litters that do not respond reasonably well to the diet, to compare as far as possible only isogenic animals and always to have a certain number of "negative controls" in each experiment.

A complete confirmation of our views both on the need for the use of isogenic animals and of the fundamental importance of an inbred stock is to be found in the fact that the variations of response to diet within a litter are far less than the variations between litters (see Series i below). This experience has been reported by workers in other branches of vitamin research [*cf.*, for example, Bourdillon *et al.*, 1931].

EXPERIMENTAL.

Preliminary series. These experiments will not be described in detail. Forty-five animals were distributed into seven groups receiving various diets of two main types, corresponding with diets A and B in Table II (except that no wheat germ was included in the diets of type B). The main source of protein was varied for the sub-types of both main types, heated caseinogen (Coward's "Glaxo casein"), the same caseinogen before heating, both fat-extracted and unextracted, a "soluble" sodium caseinogenate (Coward's "light white casein"), and the same after extraction of fat, being all examined. The results were inconclusive, but some reliance can be placed on the animals given the four diets:

- (a) Type A with caseinogen.
- (b) Type A with caseinogenate.
- (c) Type B with caseinogen.
- (d) Type B with caseinogenate.

The isogenic groups used each contained 3 males and 4 females (3 males and 3 females only in group (a)), and the animals were given the unsupplemented basal diets until they died or were moribund. The average survival periods were as follows:

- (a) 131 days (2.5).
- (b) 211 days (1.6).
- (c) 156 days (1.7).
- (d) 129 days (0.8).

The animals' condition at death varied from complete protection to acute xerophthalmia. On an arbitrary score of 3 = complete protection; 2 = slight xerophthalmia; 1 = severe xerophthalmia; 0 = acute xerophthalmia with blindness, the average condition of the animals can be calculated and is shown by the figures in brackets after the average survival period. The indication is that the caseinogenate protects rather less completely from xerophthalmia than does the heated caseinogen.

The other averages to be obtained from these figures are as follows:

Diet type A (13 animals)	...	...	...	174 days
Diet type B (14 animals)	...	...	...	143 „
Soluble caseinogenate (14 animals)	...	...	...	170 „
Heated caseinogen (13 animals)	...	...	...	144 „

There is thus an indication, though nothing more, first that diet B gives a more rapid "running-out" period than diet A, and subsequent experiments make it well-nigh certain that this is at any rate partly due to the lack in diet B of something present in fat-free wheat germ, and, secondly, that the soluble caseinogenate allows of longer survival than the heated caseinogen.

Series i. This had for its object the comparison of the three different types of diet shown in Table II. As it was wished to use 18 males and 18 females on each diet, the principle of isogenic control was abandoned in this case, and the animals were arranged as shown in Table I.

Table I. *Distribution of animals and diets used in Series i (see Figs. 3 and 4).*

Diets	Litter-groups of 6	
	Males	Females
A	i. ex 6 ♂ and 1 ♀ (30)	iv. ex 4 ♂ and 6 ♀ (36)
	ii. ex 6 ♂ and 3 ♀ (27)	v. ex 6 ♂ and 7 ♀ (30)
	iii. ex 6 ♂ and 7 ♀ (30)	vi. ex 2 ♂ and 7 ♀ (28)
B	i. ex 7 ♂ and 1 ♀ (28)	iv. ex 2 ♂ and 7 ♀ (28)
	ii. ex 7 ♂ and 1 ♀ (27)	v. ex 1 ♂ and 6 ♀ (26)
	iii. ex 6 ♂ and 1 ♀ (28)	vi. ex 3 ♂ and 6 ♀ (28)
C	i. ex 6 ♂ and 1 ♀ (30)	iv. ex 5 ♂ and 6 ♀ (31)
	ii. ex 6 ♂ and 2 ♀ (31)	v. ex 2 ♂ and 7 ♀ (32)
	iii. ex 7 ♂ and 3 ♀ (33)	vi. ex 3 ♂ and 7 ♀ (25)

Table II. *Percentage composition of diets.*

Diet		A	B	C
Carbohydrate	Dextrinised rice starch	74	—	—
	Rice starch	—	55	50
Fat	Aerated cottonseed oil	—	10	—
	Arachis oil	—	—	10
Protein	Soluble caseinogenate	15	—	—
	Heated caseinogen	—	20	20
Salt mixture	Steenbock 40	4	—	—
	McCullum	—	5	5
Roughage	Agar-agar	2	—	—
Vitamin B	Marmite	—	5	5
	Dried yeast	5	—	—
	Wheat germ (fat-free)	—	1 g. fed separately to each animal per day	5
Vitamin C	Lemon juice	—	5	5
Vitamin D	—	Diet irradiated	20 units in 10 g. diet; irradiated ergosterol dissolved in oil of diet	100 units twice a week; irradiated ergosterol fed separately to each animal

Diet A is similar to that used by Coward, and the source of protein, described as "soluble caseinogenate," is identical with her "light white casein." Our diet, however, which was identical with an earlier diet described by Drummond,

Coward and Handy [1925] contained 3 % less yeast than Coward's. Diet B, without the supplement of wheat germ, had been used by us for some years; some preliminary experiments had shown that marked improvement in growth on this diet followed the daily administration of 1 g. of wheat germ that had been extracted with acetone to remove oil and fat-soluble vitamins. The addition may have brought about the improvement by virtue of its content of vitamin B, of extra protein, or of roughage, or of a combination of these. The replacement of the aerated cottonseed oil by refined arachis oil did not appear to introduce any traces of vitamin A, so that arachis oil was included in diet C, which is now the only vitamin A-free diet used regularly in our laboratories. In diets B and C the caseinogen used was the acid-precipitated heat-treated caseinogen described by Coward as "vitamin-free casein (Glaxo)." Diet A was fed dry and diets B and C as pastes. It will be seen that diets A and B differ in a number of respects, whereas diet C is simply an improved form of diet B, itself a modification of the diet at first advocated by Drummond and Watson [1923].

Fig. 3.

Fig. 3. Typical growth curves for two different (isogenic) groups on two different basal diets (see Table II).

Fig. 4.

Fig. 4. Average growth curves for groups of six animals each, on three different basal diets (see Table II) during "running-out" period.

In Table I we have included data as to the size of the original litter and the age (figures in brackets) of the litter groups at the beginning of the experiment; examination of all the average growth curves of the 18 groups showed that neither of these factors influenced significantly the animals' response to the diets. In Figs. 3 and 4 are given respectively the growth curves for all the animals of two typical groups (A i and C vi), and the average curves for all the males and all the females on each diet. The behaviour of the animals in this experiment showed

- (1) a very reasonable regularity of response of the individual animals in the same litter groups;
- (2) a fair regularity of average response of the groups of one sex on the same diet;
- (3) the usual superiority of male growth rate over female;
- (4) a considerable difference of average response to the three different diets.

How far the difference noted under (4) is a true expression of different dietary conditions, and not of differences between the litters, selected at random, for the three diets, is uncertain, but in so far as it is so, diets A and C would seem to approximate more closely to a theoretically sound diet than does diet B. The experiment was, however, concluded after 70 days, and it became necessary to probe rather more deeply into the possible causes of the difference in average response to the three diets. The difference between response to diets B and C is, as we have mentioned, not attributable to the difference in the oils used, unless the peroxide in the aerated cottonseed oil was causing destruction of the vitamin D dissolved in it. The evidence of other preliminary tests, however, suggests that this was not the case, but that the wheat germ supplement was almost solely responsible for the animals' improved growth.

Direct comparison between the fat-free diet A and diet C is less simple, since two different sources of protein were used and, according to Coward, this affects the response of the animals to curative treatment rather than to the deprivation of vitamin A. We have therefore investigated the effect on response to cure of animals on diets of both types A and C, using each source of protein with each diet.

Series ii. Forty-eight animals were used in this test, six groups, each of four isogenic males, and six groups, each of four isogenic females. One animal of each group received a diet of type A containing one of four sources of protein, three being different samples of heated caseinogen and one a sample of soluble caseinogenate. The distribution of animals and diets is shown in Table III. Two groups of the males and two groups of the females were given no curative treatment; all the other animals received after the "running-out" period a daily supplement of one drop (20–25 mg.) of cod-liver oil known to be a good source of vitamin A (Carr-Price blue value about 12). All the latter animals remained alive, and in two cases (females) in which xerophthalmia had developed

Table III. *Distribution of animals and diets used in Series ii (see Figs. 5 and 6).*

Diet type A.					
		Caseinogen			
Litter-group		1	2	3	Caseinogenate
♀ I, II, III, IV		+	+	+	+
V, VI		0	0	0	0
♂ VII, VIII, IX, VI		+	+	+	+
X, XI		0	0	0	0

+ =Received cod-liver oil supplement.   0=Received no supplement.

this was cured. All the unsupplemented males died or were moribund after 150 days or less on the diet; five of the unsupplemented females died in 120 days or less, while the other three, though still alive after 175 days, had made no growth for some weeks. Of these 16 negative controls one male and one female developed slight and one female definite xerophthalmia.

In Figs. 5 and 6 are shown the average growths on each diet of the eight animals (four males and four females) which received the supplementary dose of cod-liver oil; the 16 negative control animals are omitted from these averages, since three died before the period of supplementary feeding began, and since clearly none of the other 13 can be included with the animals whose response to cure is being discussed. Examination of the individual groups showed that response to cure followed litter-grouping and not the nature of the source of

protein used, and the latter point is made clear by the average curves of response. Thus the animals in male groups VIII and IX made good response; those in male group VI and in female groups III and IV made fair response; those in male group VII and in female groups I and II made very poor response; male groups X and XI and female groups V and VI were negative control groups. It is perhaps noteworthy that three of the four negative control females in

Fig. 5. Average growth curves for groups of four animals each, on basal diet A (see Table II), using two different types (3 and 1 samples) of protein, during "running-out" period.

Fig. 6. Average growth curves for recovery period, corresponding with preparatory period illustrated in Fig. 5.

group VI survived the experimental period, and that the males in group VI, which were part of the same litter, made only fair growth response to curative treatment, so that animals having, theoretically, excessive "reserves" nevertheless failed to make good response to curative treatment.

Each average curve for the "running-out" period is for four animals up to 70 days and for two animals for the last 10-day periods, since 2 male and 2 female groups were put on to supplementary doses between the 70th and 80th days of experiment. The average curves for the curative period are, however, all constructed with the first day of supplementary feeding as day 0, and represent a curative period of 100 days.

Inspection of the curves reveals the following characteristics.

1. A slightly poorer growth performance during the preparatory period by the males receiving caseinogenate than by those receiving caseinogen.
2. No significant difference in the effects of the two proteins in the "running-out" period of the females.
3. A slightly more vigorous growth by both sexes on the first of the three heated caseinogens than on the other two.
4. No significant difference in response to cure associated with the two different caseinogens. The slightly greater initial growth of the males receiving the caseinogenate is most probably associated with their lower average weight at the beginning of the curative period.
5. A very poor total response to curative treatment, compared with that reported by many other investigators, including especially Coward. All the growth-curves show a tendency to "flattening" at about the same weight, 200 g. for males, 150 g. for females.

Series iii. In this experiment only two litters were used, one of 8 males and one of 6 males and 4 females. All these animals received diet C, but 4 males from the first litter and 3 males and 2 females from the second had only heated caseinogen (sample No. 3) as a source of protein, while the rest had the soluble caseinogenate only. Two males on each source of protein in the first litter and 1 male and 1 female on each source of protein in the second litter received no supplement and acted as negative controls, while all the other 10 animals received from the 94th to the 158th day daily doses of cod-liver oil (20–25 mg. of an oil with a Carr-Price blue value of about 10). Owing to the relatively small number of animals, the averages have been calculated for males and females together, and the results are plotted in Fig. 7.

Fig. 7. Average growth curves for nine animals (preparatory period) and five animals (recovery period) on basal diet C (see Table II), using two different types of protein.

Owing to the number of animals used it is undesirable to stress even the negative results of this experiment, which shows no significant difference in effect on running-out weight, running-out time or extent of recovery between the two sources of protein. The similarity of these average growth-rates is closely reflected in the individual curves for those animals receiving curative treatment; two of the males receiving no supplement, however, grew slowly but steadily throughout the experimental period, at the end of which they were comparable in weight with isogenic animals which had run-out and then been given cod-liver oil. Both of these animals came from the group fed on caseinogenate and are responsible for the slightly steeper average curve for the running-out period of these animals; but for them the two sets of curves would have been well-nigh

indistinguishable. The average growth increase of the animals receiving heated caseinogen for the first 37 days of the recovery period was 54 g.; for the animals receiving soluble caseinogenate the corresponding figure was 60 g. Here again we have no significant difference in response and a markedly sub-normal growth rate for both groups of animals during the recovery period.

These results raised at once the question whether diet C, though it seemed to possess no theoretical disadvantages, was indeed the ideal vitamin A-free diet for which we were seeking. This could be tested only in one way, and the test was made in the next experiment.

Series iv. Here six isogenic groups of males were used; the five males of each group were distributed over the five diets described below.

Group G. Kitchen-scrap supplemented diet, as used for "genealogical" stock.

Group S. Stock diet as used for breeding stock.

Group P. Diet C, containing heated caseinogen and supplemented with adequate vitamin A¹.

Group K. Diet C containing heated caseinogen, unsupplemented for 90 days, and then supplemented as for Group P.

Group C. Diet C containing heated caseinogen, unsupplemented.

The experiment was continued for one year, with the result summarised in Fig. 8. The curve for group G has been omitted since it ran continuously parallel

Fig. 8. Average growth curves for four groups of six isogenic animals on stock, supplemented basal, and unsupplemented basal vitamin A-free diets, for whole experimental period of 370 days.

with that for group S; at the end of 370 days the average weight of the animals in group G was 344 g., and of those in group S 323 g., and the spread of the individual weights suggests that the difference is not significant. The conclusions to be drawn from the four curves shown do not allow of any doubt.

(1) Diet C, supplemented with adequate vitamin A, is not capable of giving normal growth.

(2) Diet C, unsupplemented, allows animals to "run out" in such a manner that, when adequate vitamin A supplement is added to it, the animals will make as much recovery as the limitations of the diet permit. The average final weights of the animals in groups S, P and K were: S, 323 g.; P, 254 g.; K, 252 g.

(3) Diet C, unsupplemented, is incapable of maintaining growth beyond a point definitely below that reached if it is supplemented with vitamin A, either prophylactically or curatively.

¹ Unsaponifiable matter from 50 mg. of cod-liver oil having a Carr-Price value of 10, determined on the oil, and 16 determined on the unsaponifiable matter.

DISCUSSION.

The results, in their bearing on the source of protein in vitamin A-free diets, do not confirm those reported by Coward, and no explanation of the discrepancy is put forward. Her standard vitamin A-free diet, it is true, contains 8 % of an active dried yeast, and it is just possible, though in my opinion very improbable, that this is a more complete source of the B-vitamins than the 5 % of marmite and 5 % of wheat germ present in diet C. This point is being tested in further experiments now in hand¹. It is also a fact that several of our batches of wheat germ were extracted with acetone; the first runnings of extract contain considerable amounts of water, since wheat germ has a moisture content of 12–15 %, and there is the possibility that the aqueous acetone may be extracting some of the vitamin B from the germ. This point is also under investigation.

I have had the benefit of Dr Coward's own views on these results, and we are unable even jointly to offer any explanation. The beneficial effect of wheat germ on my diet B is consistent with Dr Coward's finding that wheat germ is a source of the unknown "factor" when fed at a level of at least 20 %. Diet C, however, only contains 5 % of wheat germ; it should therefore be inadequate as a supplement of her factor to the heated caseinogen, while the soluble caseinogenate should need no such supplement. Yet the experiments in Series iii detected no difference whatever between the two sources of protein in a diet containing 5 % of wheat germ. Again, there can be little question as to the adequacy of the vitamin D supplies to animals receiving diet C. They were given about 200 units per week; Coward (private communication) gives her animals 8 units, certainly more than sufficient. The poor recovery of the animals on so high a dose as the equivalent of 50 mg. of good cod-liver oil is doubtless a summation of two (or more) causes—the relatively high weight at which the animals "run out" and the unknown limiting factors of the basal diet itself.

A possible explanation of some of our results might be given in terms of vitamin A storage by the animals during their pre-experimental (including pre-natal) lives. This should result in an unduly long period of running out, with the attainment of an unduly high growth plateau, so that the animals would have a potentially lower recovery rate than animals which stopped growing sooner. There are, it seems to me, two unanswerable objections to this suggested explanation. First it can in no way account for the failure of the animals in group *P*, Series iv, to make growth equal to that of the animals in groups *G* and *S*, since the three groups were absolutely comparable in pre-experimental history. High initial vitamin A reserves can hardly explain failure to reach normal weight with adequate vitamin A administration. Secondly, we have experimental evidence which is extremely difficult to reconcile with the current conception of vitamin A storage and its effect on resistance to the ill-effects of deficient diets. (See the following paper, p. 17.)

We are therefore compelled to accept the simple explanation that the failure of animals receiving vitamin A and diet C to make growth approximating to that of stock-diet fed animals must be associated with some unrecognised deficiency of diet C. Possible sources of such deficiency are:

- i. Shortage of one or more substances in a diet qualitatively correct, *e.g.* vitamins of the B group, amino-acids.
- ii. Absence of some constituent or factor already known, *e.g.* vitamin E, roughage.

¹ So far they quite refute the idea that diet C is in any way deficient in vitamin B.

iii. Absence of some new or unrecognised factor, *e.g.* "physin" [*cf.* Mapson, 1932], a caseinogen factor [*cf.* Coward *et al.*, 1929, 1, 2].

iv. A combination of two or more of these causes.

Experiments are in hand, and more are planned, to investigate some of these possibilities, but they must of necessity be of fairly long duration, since they involve observing the weight and condition of adult animals reared from youth on experimental diets.

Meanwhile the apparently inherent limitations of our basal diets lead us to emphasise the desirability of assessing the growth-producing potentialities of it or any similar diet, wherever possible, by carrying out positive control tests of the prophylactic as well as of the curative type.

SUMMARY.

1. Attention is drawn to various types of anomaly shown by rats fed upon vitamin A-free diets, both during the preparatory "running-out" period and under curative treatment.

2. With two different vitamin A-free diets, one containing no fat, running-out time, weight and degree of recovery under curative treatment seemed to be independent of the sources of protein examined—a heat-treated "vitamin-free" acid caseinogen, and a "soluble" sodium caseinogenate ("light white casein").

3. In no case was growth under curative treatment resumed to an extent equal to that of normal animals receiving stock diets.

4. Evidence is submitted that the vitamin A-free diets used must have been deficient in more than vitamin A.

5. The bearing of these results on the technique of vitamin A testing is discussed.

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