

**The food value of mangolds and the effects of deficiency of vitamin A on guinea-pigs / by Ellen Boock and John Trevan.**

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ON GUINEA-PIGS

BY

ELLEN BOOCK

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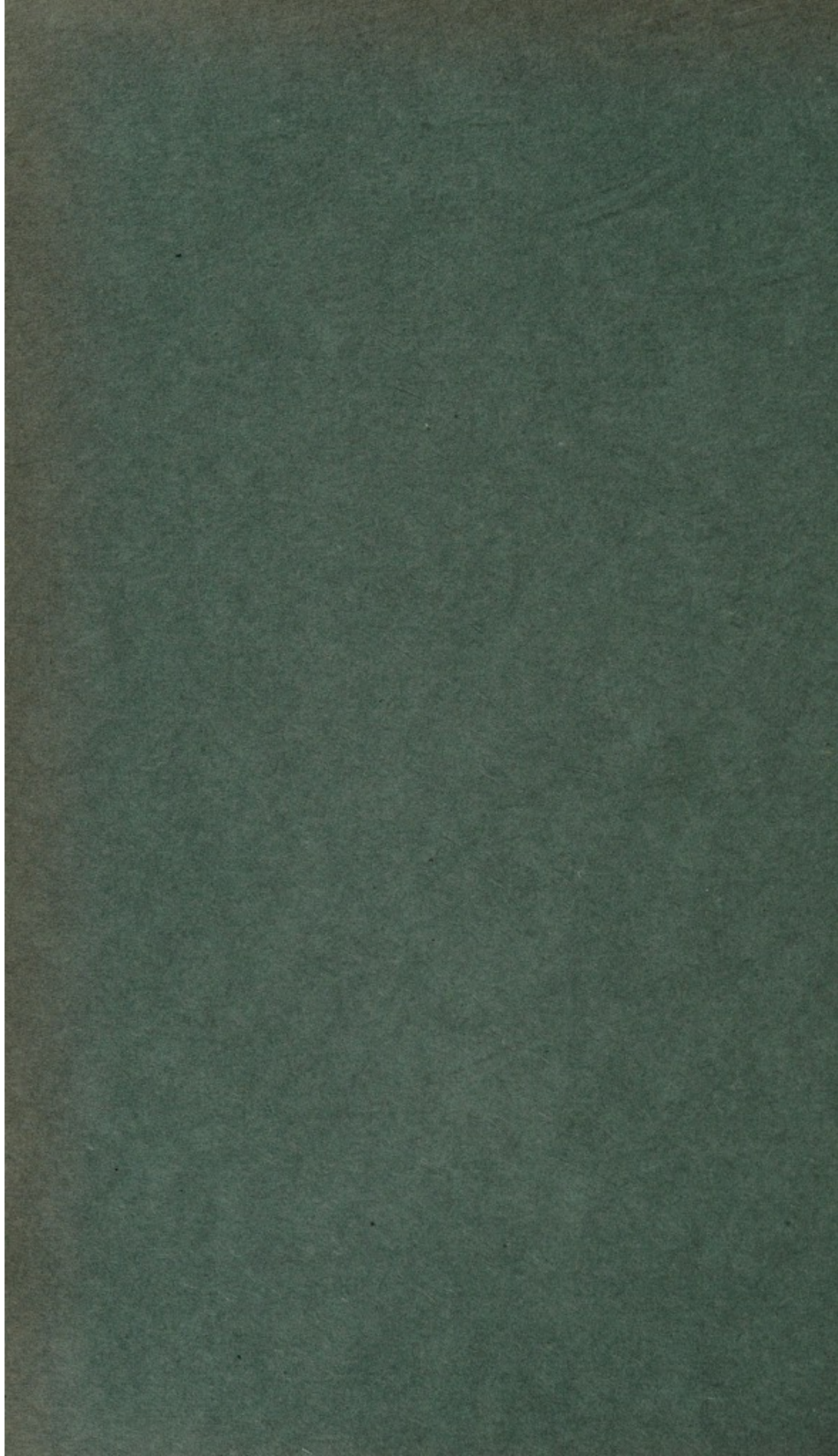
JOHN TREVAN


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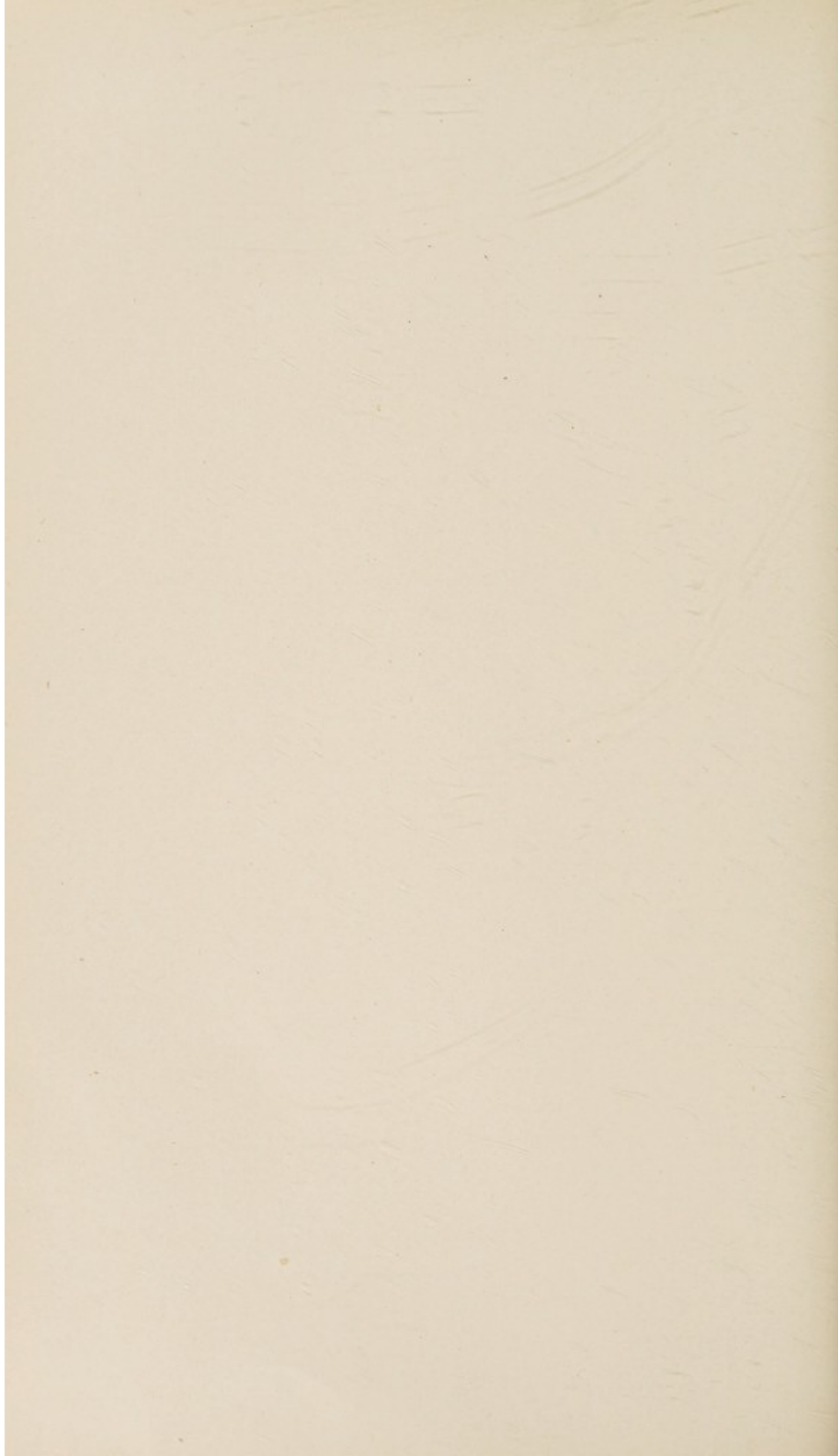






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## LXXXII. THE FOOD VALUE OF MANGOLDS AND THE EFFECTS OF DEFICIENCY OF VITAMIN A ON GUINEA-PIGS.

BY ELLEN BOOCK AND JOHN TREVAN.

*From the Wellcome Physiological Research Laboratories.*

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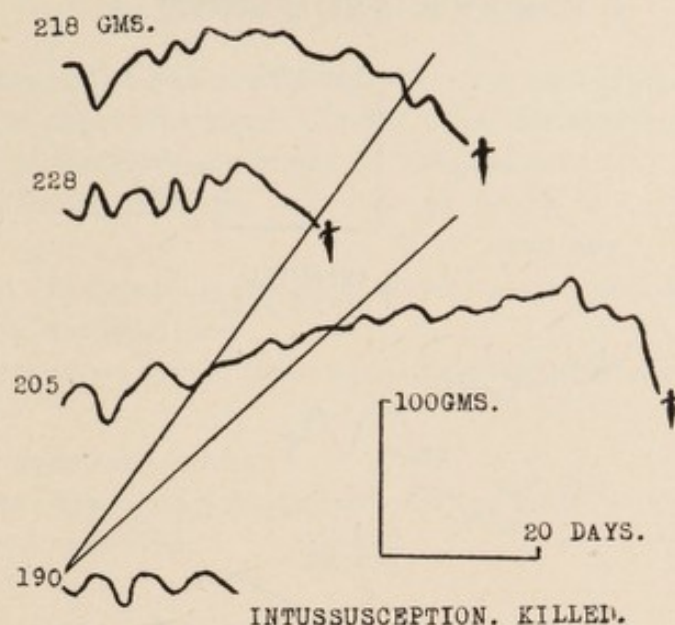
OUR colleagues Glenny and Allen [1921] presented to the Pathological Section of the Royal Society of Medicine an account of an investigation into an epizootic amongst a stock of guinea-pigs. They presented conclusive evidence that the epizootic could be entirely controlled by alteration of the diet. The diet which resulted in the outbreak consisted of bran, oats, water and mangolds. Substitution of the mangolds by cabbage, grass or lucerne stopped the epizootic. When guinea-pigs in adjacent runs were fed on the two diets, those on mangolds were attacked, whilst those with grass remained healthy, although no precautions whatever were taken to prevent infection of the healthy animals. Isolation was not carried out, the runs were not disinfected, and the attendants handled both groups of animals indiscriminately. These results are of such importance that the following attempt was made to determine the factors or deficiencies in the mangold which rendered the guinea-pigs sensitive to the epizootic. A few experiments were done by Glenny in which an alcoholic extract of carrot was given to the guinea-pigs with a view to supplying a possible deficiency of vitamin A. These indicated that the supply of vitamin A, although it reduced the incidence of the disease, did not stop it, and the guinea-pigs did not put on weight.

The following experiments were begun after the epizootic had been controlled by dietary means, and the effect of the diet was re-investigated.

Chart I shows the weight curves of four guinea-pigs fed on unlimited bran, oats, mangolds and water, the animals eating about 40 g. of mangold each per diem, and Chart II those of guinea-pigs kept on a control diet of bran, oats, autoclaved milk and greenstuff. These latter gained weight rather faster than Miss Hume's [1921] "standard" guinea-pigs (43.7 % in 25 days, as against 34.2 % in Miss Hume's experiments).

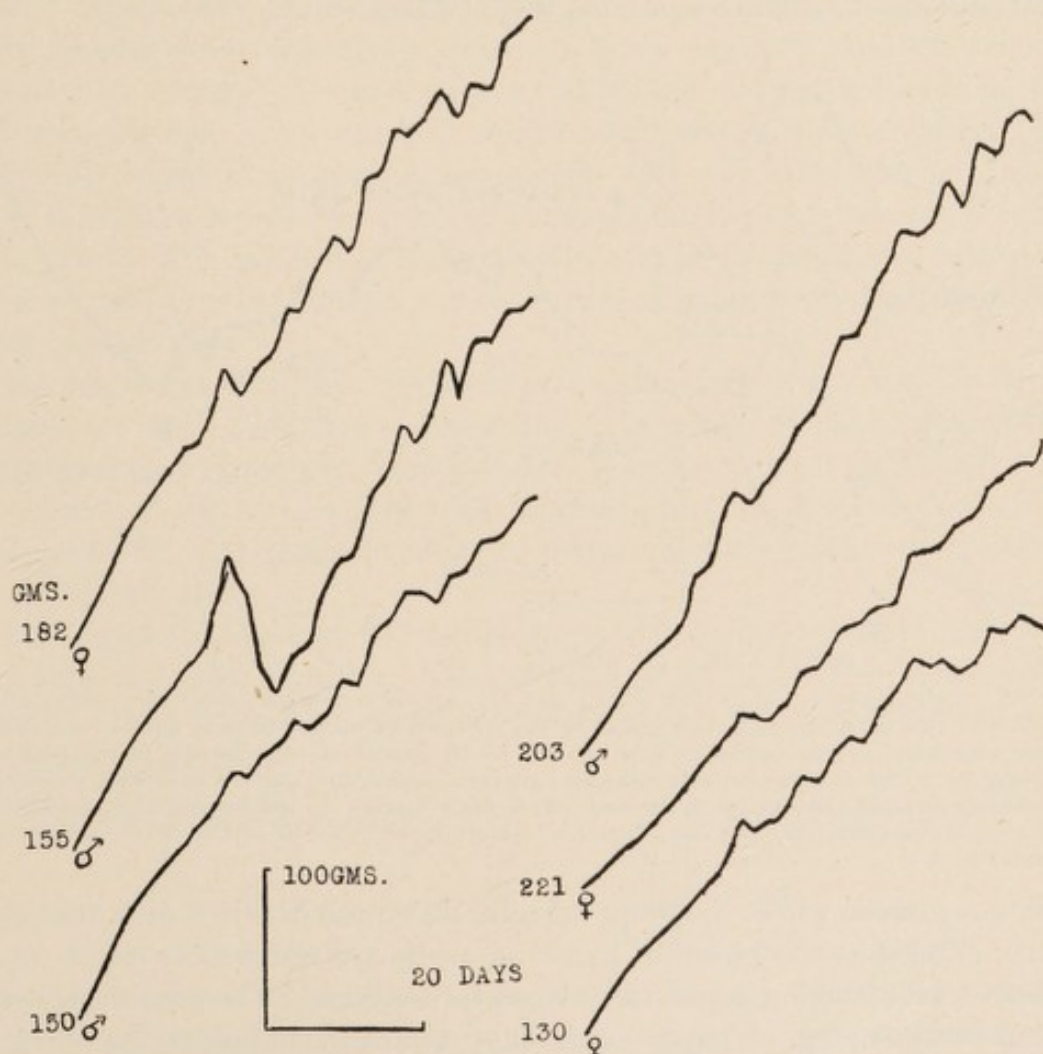
It was found that all the guinea-pigs placed on the mangold diet eventually succumbed within a period of about two months. In Chart I, the guinea-pigs were about 200 g. weight when started. In Chart III, they were younger—about 150 g.—and the younger guinea-pigs succumbed earlier than the older ones.





The charts are drawn to different scales indicated in each case. The oblique straight lines on each chart represent the greatest and least rate of growth shown in the experiment with a normal diet represented in Chart II. The abscissa for each curve is at a different level; the figure at the beginning of each curve shows the initial weight of the guinea-pig in grams.

*Chart I.* Effect of basal diet of bran, oats, and mangolds. One of the animals had an intussusception and was killed. We have met this condition fairly frequently in animals under 200 g. in weight which have been deprived of greenstuff.



*Chart II.* "Normal" diet consisting of bran, oats, lucerne, grass and autoclaved milk. This diet was used as a control for some experiments on scurvy. We have obtained since writing this paper even better results than these, with the "synthetic" diet described later.



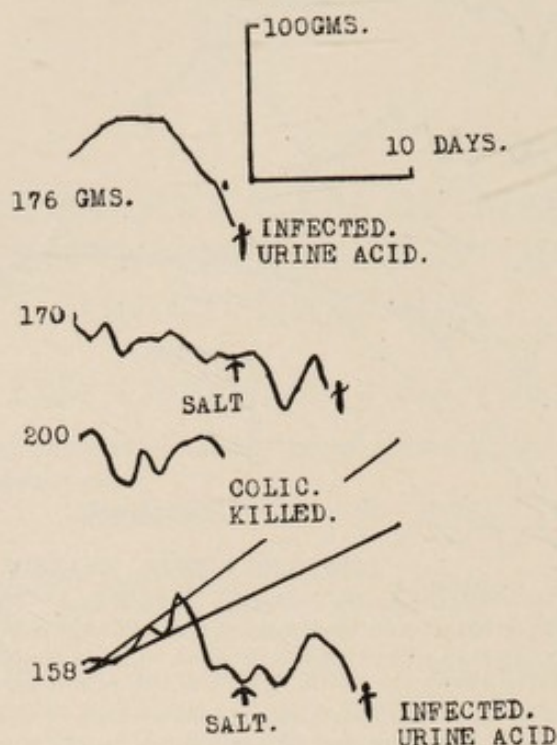


Chart III. Effect of basal diet of bran, oats, and mangolds on younger guinea-pigs. It will be seen that these animals died sooner than those in experiment I.

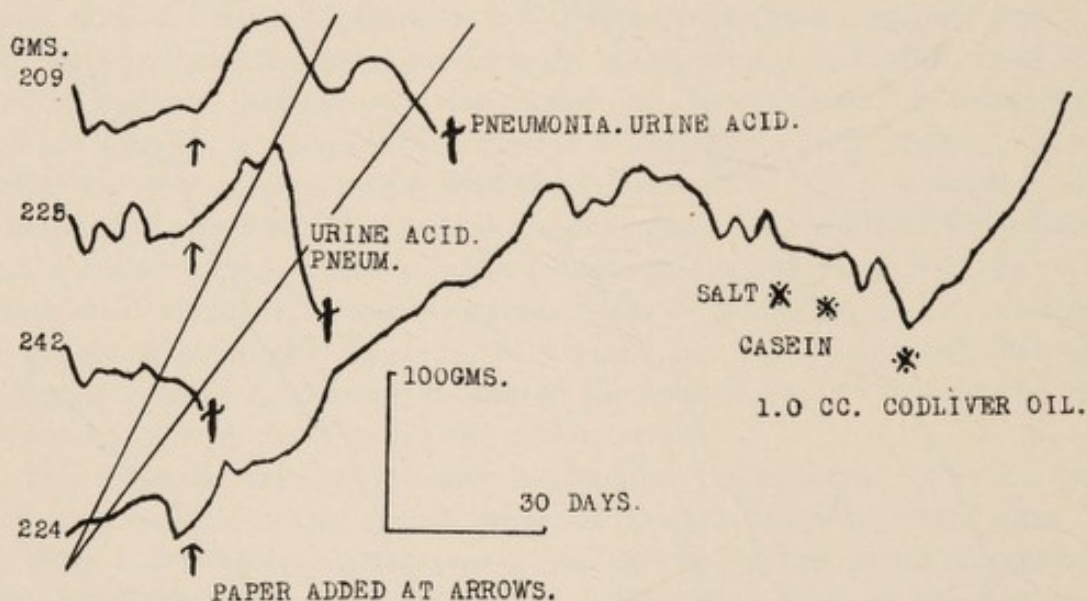


Chart IV. Guinea-pigs on basal diet as in I and III, to which was added packing paper at the arrows. One of the animals died the same day, the others started to grow again, one growing for over two months before decline set in. As the result of experiments which had been going on in the meanwhile, salt mixture (calcium lactate and sodium chloride), caseinogen and cod-liver oil were added to the diet (see further charts). It will be seen that the limiting factor in this guinea-pig was the absence of something supplied by cod-liver oil—presumably vitamin A.

It was noticed whilst weighing the animals on the deficient diet, that they eagerly nibbled at any pieces of paper or cardboard which they could reach; so clean white packing paper was placed in the cages. The guinea-pigs each consumed about 5 g. of paper every day. Chart IV indicates the effect on



the growth, and it will be seen that out of four guinea-pigs, one died on the first day that the paper was given, but the other three gained in weight as a result, and one of the three continued to gain for over two months before a decline set in. Suggestions are put forward below as to the explanation of this.

Post-mortem examinations revealed several abnormalities in the guinea-pigs whilst on the mangold diet.

As in Glenny's experiments, there was constantly some sign of an infection present, either

- (a) Alkaline stomach contents.
- (b) Pneumonic consolidation or abscess of the lung.
- (c) Purulent pericarditis.
- (d) Enteritis.

When the urine was tested during life and post-mortem, it was always found to be strongly acid, and therefore quite free from the normal precipitate of phosphates which renders the urine of the healthy guinea-pig turbid. This observation led to the suggestion that the mangold, bran and oats diet is deficient in certain basic constituents, and following the indication of McCollum's feeding experiments on rats [1920] (from which he concludes that roots and seeds, besides other deficiencies, lack the three inorganic elements calcium, sodium and chlorine), it was decided to add a salt mixture to the bran, oats and mangold diet. A mixture of calcium lactate and sodium chloride was made and mixed as thoroughly as possible with the bran and oats, about 1.5 g. of calcium lactate and 1.0 g. of sodium chloride per guinea-pig per diem being given. This resulted in a return of the urine to the normal alkalinity, when examined during life and post-mortem, and improved the growth curves. (First part of Chart V.)

In connection with this, we made an examination of the ash, by drying mangold at 100°, and then incinerating in a muffle furnace. Magnesium, sodium, potassium and iron were found to be present, but no qualitative test for calcium could be obtained, and in attempting to estimate calcium by Cahen and Hurtley's method [1916], no potassium permanganate was used up in the final titration.

The beneficial effect of the salt mixture is therefore probably due to its supplying the extra calcium. The packing paper also helps in the same direction, for analyses of the ashed paper showed that there is a certain amount of calcium present:

Percentage of ash in paper ...	...	1.7 %	1st determination.
"      "      "      "      " ...	...	1.6 %	2nd      "
"      " calcium in paper ...	...	0.126 %	1st      "
"      "      "      "      " ...	...	0.190 %	2nd      "

Using Sherman and Gettler's tables [1912], we have calculated that the calcium supplied by the 5 g. of paper eaten per guinea-pig per diem would



be about equal to that supplied by 6 cc. of cow's milk per diem, and is about equal to half the calcium in 40 g. of cabbage (Miss Hume's standard amount).

Besides supplying this small amount of calcium, it is possible that the paper may act as "roughage" in the diet, the alimentary tract of the guinea-pig being only suited to an extremely bulky diet, and requiring a comparatively large amount of ballast in order to secure normal intestinal movements. It is, however, very difficult to believe that a guinea-pig devouring 40 g. of mangold per diem is in need of any further cellulose, and we are inclined to the opinion that the paper acts chiefly by partially supplying the calcium deficiency. Mangold, though deficient in calcium, contains about the same percentage of potassium as cabbage.

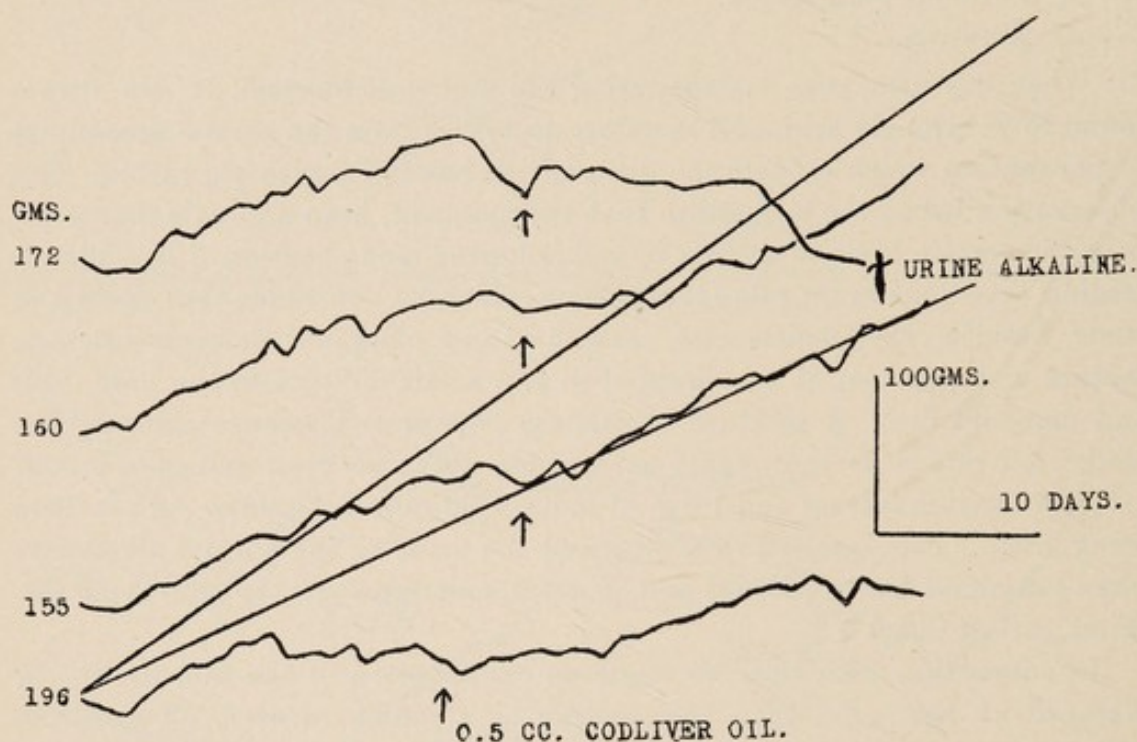


Chart V. Guinea-pigs on basal diet as in I and III, plus salt mixture and paper. 0.5 cc. cod-liver oil per guinea-pig per diem administered at the point marked by the arrows. One guinea-pig only then approached a normal rate of growth, the deficiency of protein probably being the limiting factor in the case of the other three guinea-pigs, one of which ultimately succumbed.

An important point in connection with the first part of Chart V arises in consideration of the changes in the acidity of the urine.

On the mangold diet, the urine is acid. On a greenstuff diet, the urine is alkaline. 40 g. of cabbage, according to Miss Hume's results, is sufficient for normal growth in guinea-pigs. If, however, a comparison is made between the ash of mangold and cabbage, it will be found that whereas 40 g. of ashed mangold give sufficient base to neutralise 35.2 cc. *N*/10 acid (this is obtained from a direct titration of the ash—is probably therefore too high because of the loss of sulphur, but the error is only of the order of 2 or 3 cc.), 40 g. of ashed cabbage will neutralise 42.6 cc. *N*/10 acid (Sherman and Gettler, calcu-



lated), not a significant difference. There are two explanations that offer themselves:

(1) The potassium salts of the mangold are not absorbed by the intestinal mucous membrane in the absence of calcium salts to balance their toxic effects. It is a commonplace that potassium poisoning is very difficult or impossible to produce by oral administration of potassium salts. As a result, the acid-base balance is disturbed—the animal's only source of salts is the bran and oats, the ash of which is acid.

(2) The deficiency of calcium may lead to a disturbance of the metabolism, possibly secondarily to an infective process, which leads to excessive breakdown of body protein, and the consequent production of abnormal amounts of sulphuric and phosphoric acids in excess of the neutralising value of the food salts absorbed.

The experiments so far done are in favour of the first explanation, for animals that died as a result of other deficiencies in diet, but with a good supply of calcium lactate, mostly had an alkaline urine, whereas animals fed on the diet without mangold, and with no other source of vitamin C, although supplied with calcium lactate, developed an acid urine.

This is only one of many indications of the need for further investigation of the physiological nature of the disturbances of the animal economy by a deficient diet, and is one which we are further examining.

In Chart V, four guinea-pigs were started on an initial diet of mangolds, bran and oats, salt mixture and paper, and it will be seen that the salt mixture and paper did not sufficiently supplement the diet to give continued normal growth curves, and after about a month, all the guinea-pigs started to decline in weight. The similarity of the post-mortem lesions to those found in rats fed on diets deficient in vitamin A suggested the addition of some source of vitamin A. This had been attempted before with carrot extract prepared by Zilva's method, but without much success, for other deficiencies of the mangold were not made up. We now tried cod-liver oil instead of the carrot extract and 0.5 cc. per guinea-pig per diem was administered by hand. This was found to cause a great improvement, and the guinea-pigs, with only one exception, resumed a fair rate of growth.

It follows, therefore, that the mangold, bran and oats diet, besides being deficient in calcium and perhaps roughage, is also lacking in the fat-soluble vitamin A. Our impression is that the dose of 0.5 cc. of the oil used in this experiment for our guinea-pigs was marginal, which may account for the failure of one of the four guinea-pigs to survive on the oil-supplemented diet. We have frequently noted that rats kept for a prolonged period on a diet deficient in vitamin A seem to undergo some permanent change, which renders them unresponsive to any treatment with cod-liver oil. It is possible that guinea-pigs also develop a similar condition of unresponsiveness, when deprived of the vitamin for prolonged periods of time.

But further feeding experiments with another set of guinea-pigs on a diet



of bran and oats, mangolds, salt mixture, paper and oil, revealed a further deficiency in the diet, viz. protein.

Chart VI shows the weights of two guinea-pigs which were started with a diet of bran, oats, mangolds, paper and salt mixture, and then when a decline in weight, due to deficiency of fat-soluble A, occurred, oil was given, with a result that growth was again resumed. After about a month of this supplemented diet, however, a fresh drop in the weights of both guinea-pigs occurred, and one of them succumbed, but the other guinea-pig quickly picked up weight again on adding caseinogen to the diet. This guinea-pig continued a normal rate of growth, and was still continuing to grow at the time of writing, so that it is concluded that a fully supplemented diet has been attained. (See also the latter part of Chart IV.) The question arises whether the caseinogen acts beneficially by:

(a) Supplementing the phosphorus intake.

(b) Supplementing deficient protein, either a deficiency in certain amino-groupings, or a deficiency in total amount.

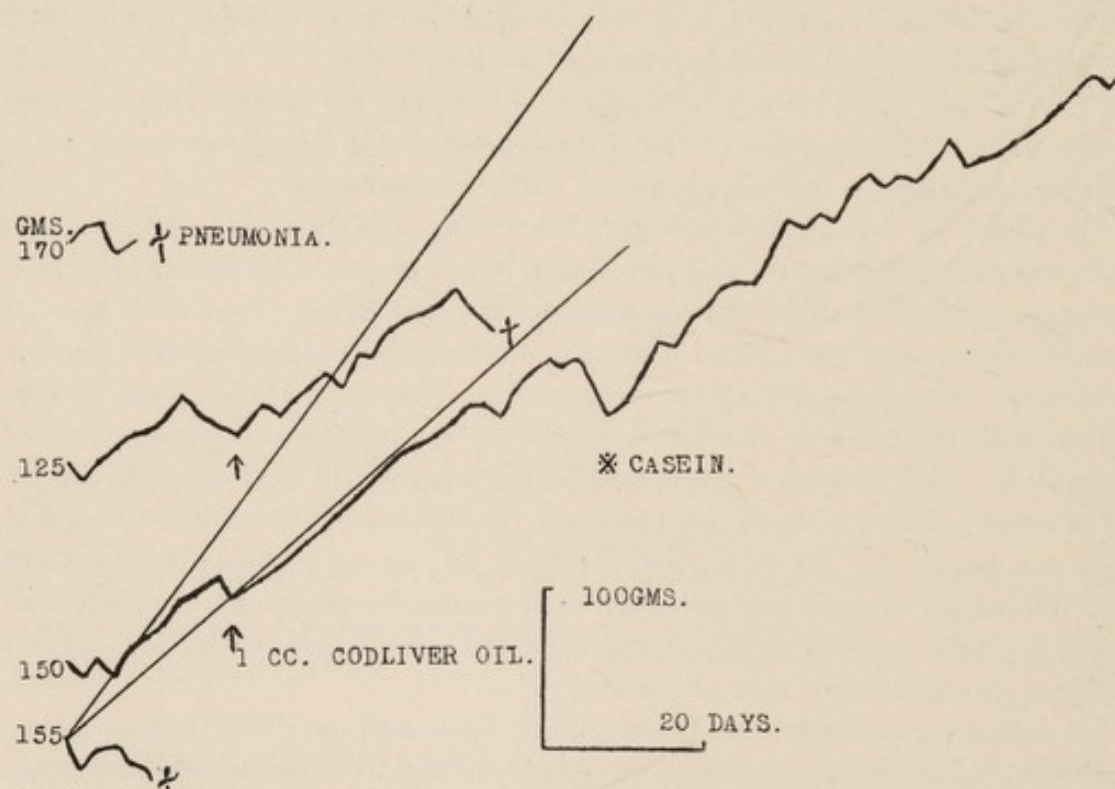


Chart VI. Guinea-pigs on basal diet as in I and II, plus salt mixture and paper. Two succumbed before administration of oil was commenced (at arrows). Of the two survivors, one eventually succumbed to protein deficiency, but the other quickly resumed a normal rate of growth on the addition of caseinogen to the diet (at star).

Osborne and Mendel [1918] have shown with rats, that a shortage of phosphorus leads to a considerable slowing in growth followed by a fall in weight, but on adding caseinogen to the phosphorus-free diet, an improvement was made, and still greater improvement when inorganic phosphorus was added. The addition of edestin (a phosphorus-free protein), however, only led to complete cessation of growth and a decline.



The 12.5 g. of bran and oats eaten in a day contains 0.05 g. of phosphorus [Sherman and Gettler, 1912], whereas 40 g. of cabbage only adds 0.013 g. of phosphorus, an amount which could be easily covered by a small extra consumption of bran and oats, such as often takes place. We are led to conclude that, since cabbage can fully supplement a bran and oats diet, the addition of phosphorus is probably not the influencing factor, but that both caseinogen and cabbage supply a certain amino-acid or acids not present in bran and oats or in mangolds. This is a rather remarkable conclusion, considering the small amount of nitrogen in greenstuff. It is paralleled by the observations of Thomas that the N of potato is especially effectual in supplying the N requirements of the body.

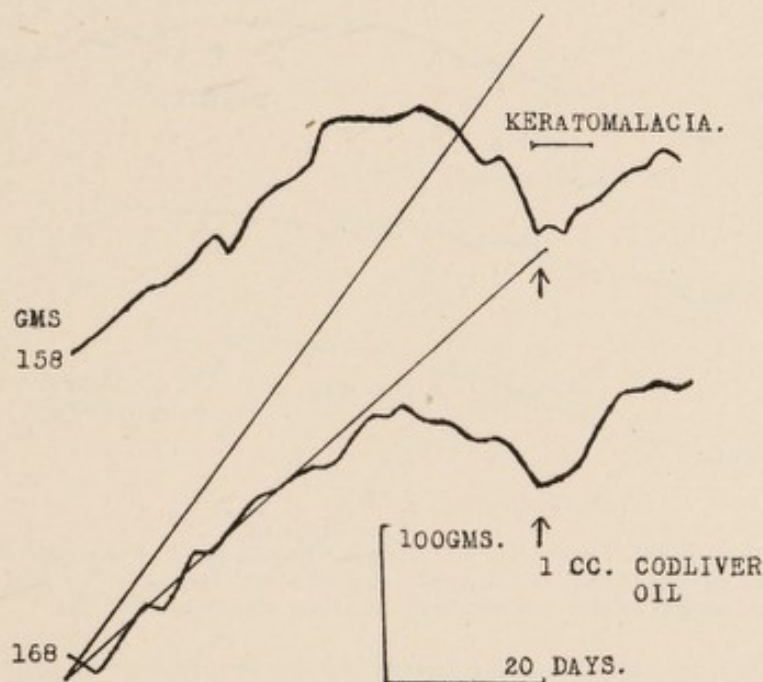


Chart VII. Guinea-pigs on diet of bran, oats, salt mixture, caseinogen and mangolds. After definite symptoms of vitamin A deficiency had become apparent, cod-liver oil was administered, at the arrows. Duration of the keratomalacia marked by straight line.

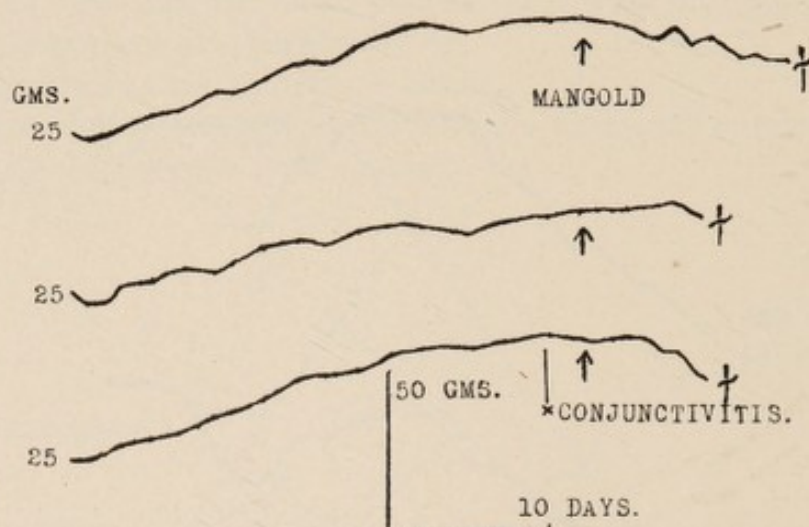
Two guinea-pigs, Chart VII, were started on a diet of bran and oats, caseinogen, salt mixture, paper and mangolds, and it will be seen that this diet was complete with one exception, viz. fat-soluble A. After a good initial growth for over a month, both guinea-pigs dropped in weight considerably, and one of them developed keratomalacia. There was a clouding of the cornea of one eye, which gradually became opaque. No haemorrhagic discharge was seen, and no conjunctivitis. In the Report of the Medical Research Committee [1919], it is stated that in rats it is the swelling of the eyelids and conjunctivitis which appears first, and this, if untreated, leads to thickening and clouding of the cornea, and ultimate blindness. With guinea-pigs, however, we have found that the corneal clouding always appears first, and several times we have received guinea-pigs from stock with completely opaque corneas, which have gone on to panophthalmia. We have never observed any haemor-



rhagic or purulent discharge preceding the keratomalacia in guinea-pigs. In rats, we have sometimes observed the uncomplicated corneal change first, but more frequently conjunctivitis precedes the corneal cloudiness.

When the guinea-pigs had each lost about 50–100 g., and the keratomalacia had developed unmistakably in the one, 1 cc. of cod-liver oil was given to each guinea-pig every day, which resulted in an almost immediate increase in weight, and a resumption of a normal rate of growth. There was a complete disappearance of the eye trouble after about one week's feeding with the oil.

We have confirmed the absence of fat-soluble A from mangold by feeding experiments with rats (Chart VIII).



*Chart VIII.* Rats on purified synthetic diet deficient in vitamin A. Conjunctivitis developed at point marked by cross, and here mangold was added to the diet. No improvement in the condition and weight of the rats resulted, and they all eventually died.

The fact that our guinea-pigs developed no symptoms of scurvy whilst on the mangold diet supplemented as above, for a period extending over more than two months, indicates that the mangold supplies an adequate amount of the anti-scorbutic vitamin C. Vitamin B is supplied in sufficient quantity by the bran and oats mixture, and is also present in the mangold.

Our experiments therefore lead to the conclusion that mangold is deficient in the following: (1) calcium, (2) fat-soluble A, (3) protein, (4) ? roughage; but contains a sufficiency of the anti-scorbutic vitamin to keep guinea-pigs free from scurvy. This confirms the rule laid down by McCollum for seeds, roots and tubers generally.

The only essential dietary substance added by the mangold to bran and oats is vitamin C, although there is of course some energy value in the various other constituents, and by feeding experiments on rats we have shown that there is a fair quantity of vitamin B present.

Once again, as other observers have noted, vitamin A deficiency accompanies a low calcium content, so that it can almost be laid down as a general rule that where there is vitamin A deficiency, calcium deficiency probably



runs parallel with it; and conversely, where there is a good supply of the fat-soluble vitamin, there is often a high percentage of calcium, as the following figures taken from Sherman and Gettler's tables show:

			Vitamin A	Calcium content %
I.	Egg yolk	...	+	0.143
	Cow's milk	...	+	0.124
	Beans (dried)	...	+	0.165
	Nuts	...	+	0.270 (almonds)
II.	Egg white	...	-	0.011
	Fish (white)	...	-	0.022
	Potatoes	...	-	0.006
	Rice	...	-	0.006-0.01

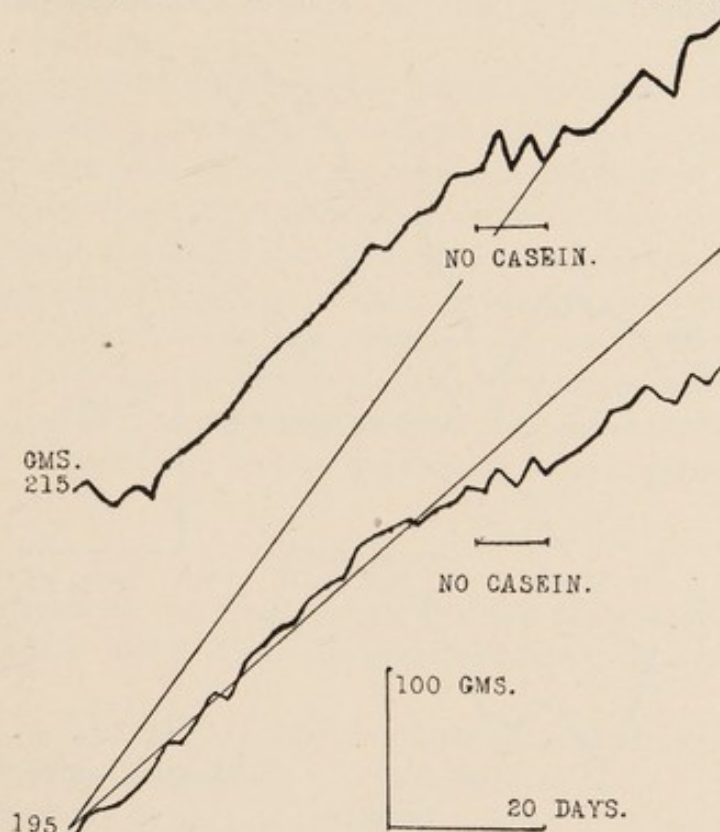


Chart IX. Guinea-pigs on complete diet of bran and oats, salt mixture, orange juice, cod-liver oil and caseinogen. Flattening of the growth curves when caseinogen was withdrawn from the diet for a period marked by the horizontal line.

We have made various efforts to feed guinea-pigs on a basal artificial diet similar to that used in feeding experiments on rats, but made up according to calculations based on analyses of bran and oats, milk and grass, with the idea of supplementing this basal diet with the three vitamins A, B and C in turn. All our experiments, however, have been brought to an untimely conclusion by the fact that the guinea-pigs refuse to eat any of the artificial diet.

Starting with bran and oats as a basal diet, and supplementing this with heated caseinogen, salt mixture as above, and paper, one can, however, study the effects of the vitamins A and C in the guinea-pig in a more or less uncomplicated way.

Two guinea-pigs were placed on this "synthetic" diet, as shown in Chart IX, bran and oats, heated caseinogen, salt mixture and paper being given, together



with 3 cc. lemon juice and 0.3 cc. cod-liver oil per guinea-pig per diem. After a time, however, 5 cc. orange juice was substituted for the lemon, as it was found that the guinea-pigs were averse to the sour taste of the lemon, whereas they drank the orange juice eagerly. It will be seen from the curves that a very satisfactory rate of growth can be maintained on this diet. A flattening of the growth curves occurred when caseinogen was withdrawn from the diet for a short period, thus emphasising the need of supplementing the protein of the bran and oats. It is, of course, of great importance that the temperature of the animal house in which the guinea-pigs are kept should be kept as constant as possible.

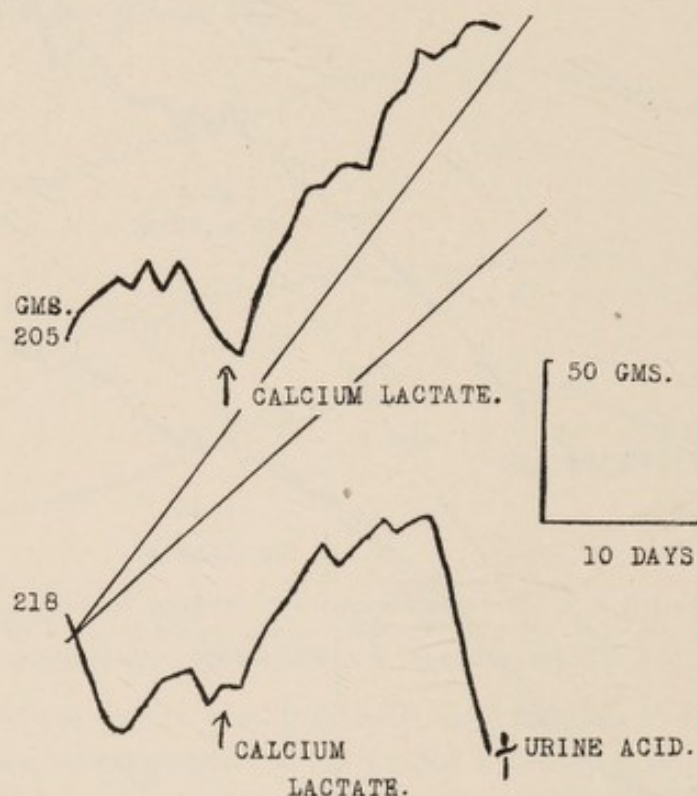


Chart X. Showing effect of calcium deficiency. Guinea-pigs on bran and oats, mangold and cod-liver oil. (Paper and caseinogen were omitted because of the possibility of their containing small quantities of calcium.) A rapid decline set in, from which both animals quickly recovered on the addition of calcium lactate to the diet. One guinea-pig later succumbed to protein deficiency.

We are unable to confirm Miss Hume's statement [1921] that guinea-pigs have an intolerance for unemulsified fat, for we have performed a large number of experiments in which we have fed guinea-pigs with cod-liver oil, and in every case the oil has had a beneficial effect provided other essential food factors are not missing. We would suggest that Miss Hume's failure to obtain growth on oil was probably due to the basal diet of bran and oats and orange juice being inadequate, so that when the test substance, *e.g.* butter-fat or oil, was added to the diet, the beneficial effect of the addition of vitamin A was masked by the protein and inorganic deficiencies. For example, in Chart X, guinea-pigs were put on a diet of mangolds, bran and oats and cod-liver oil, and very soon a decline in weight occurred, which might have been ascribed



to an intolerance for oil on the part of the guinea-pig. That this was not the case was shown by supplying the deficient calcium of the dietary, when a quick recovery of the weight of the guinea-pigs was obtained, and they continued growing satisfactorily for some time, the oil still being continued, until finally, the deficiency of protein became apparent.

It is obvious that the need of the guinea-pig for an adequate supply of calcium is an urgent one, and the effects of its absence show themselves earlier than those of the vitamins. Vitamin A deficiency takes about 40-50 days to become apparent, and scurvy takes about three weeks to develop. On a diet containing inadequate calcium, however, guinea-pigs lose weight in a few days (see Chart X).

These experiments fully confirm Miss Hume's conclusion that guinea-pigs require a large amount of vitamin A, and they have the advantage that the addition of vitamin A to the diet was made in the form of cod-liver oil—much less admixed with other essential substances than in the case of the greenstuff and milk studied by her. The effect of deprivation of vitamin A is much more regularly obtained in guinea-pigs than in our own stock of rats, several of which grow for long periods on a deficient diet and even continue to grow whilst developing obvious eye changes. The larger dose of oil necessary for the guinea-pigs also suggests that they may be more suitable than rats for the estimation of vitamin A, and we are making some experiments with that in view.

#### CONCLUSIONS.

(1) The deficiencies of a diet of mangold, bran and oats and water which was the controlling factor in an epidemic amongst guinea-pigs have been investigated.

(2) Vitamin A and calcium salts have been shown to be deficient, and the protein to be deficient in quantity or composition or both.

(3) Keratomalacia in the guinea-pig as a result of vitamin A deficiency has been observed and cured by the administration of cod-liver oil.

(4) The administration of cod-liver oil to guinea-pigs has been shown to be an adequate means of administering vitamin A, and to be well tolerated by guinea-pigs if the diet is otherwise satisfactory.

(5) Direct confirmation of the necessity for vitamin A in the diet of guinea-pigs, inferred by Miss Hume, has been obtained.

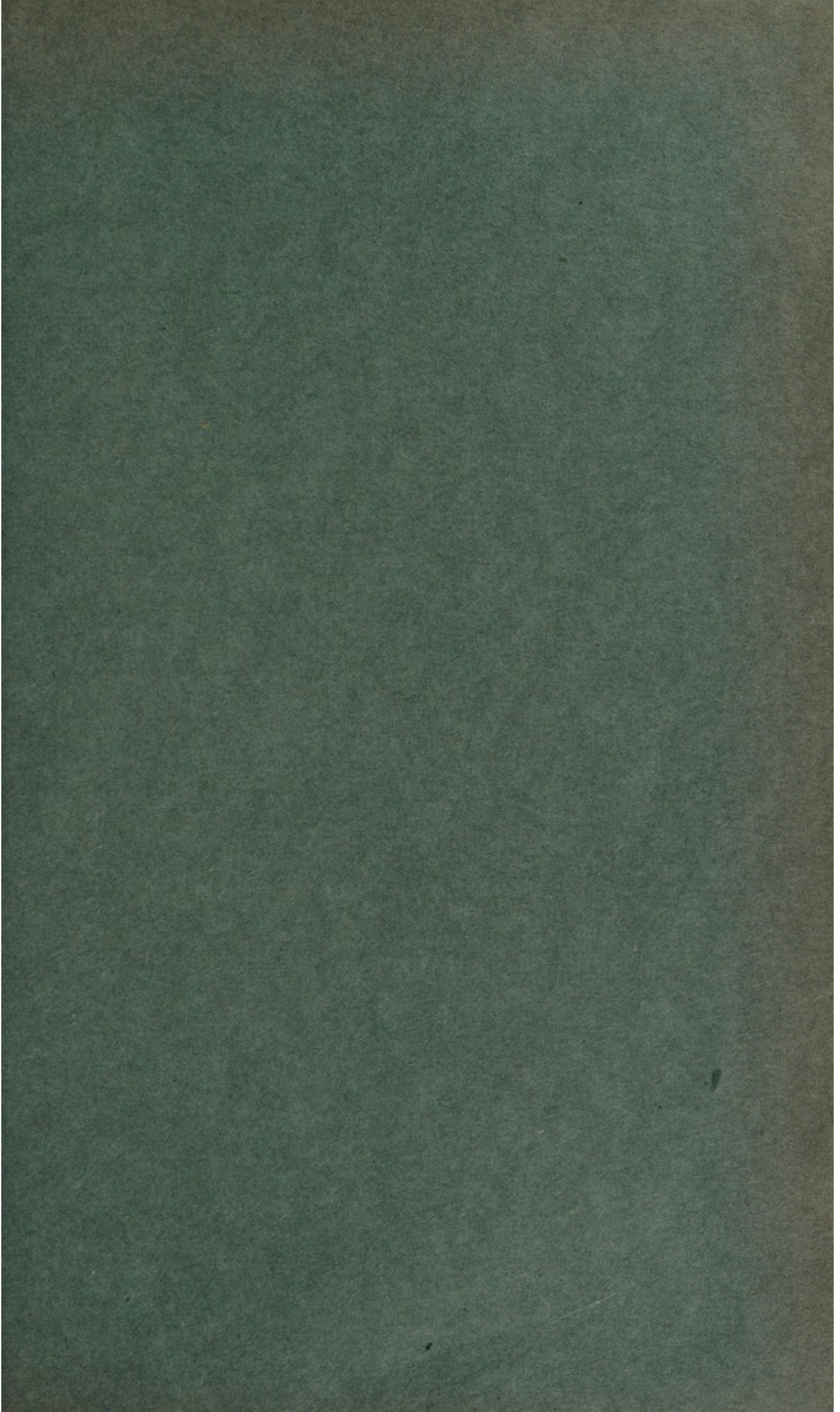
(6) Another instance is provided of a foodstuff in which a deficiency of vitamin A is accompanied by a deficiency of calcium.

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