# THE INFLUENCE OF CERTAIN DIETARY SUPPLEMENTS IN RELATION TO THE CALCIUM REQUIREMENTS OF GROWING AFRICAN NATIVES. II.

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(With 2 Figures.)

Results obtained in an investigation on the influence of certain dietary supplements on the nutrition of the adult African native[1] suggested to us that further work might profitably be carried out on growing subjects, particularly with a view to determining the calcium requirements.

In order that the calcium balances might be followed out in more detail than had been possible in our previous work only five subjects were selected.

## Subjects.

These five subjects were chosen from among twenty whom we considered reasonably comparable as regards age, physical development, general condition and type of prison employment. The average age of the five was 16 years. They all belonged to the Nandi tribe with one exception who was a Masai. All were employed on gardening work in Nairobi Prison where they were serving long term sentences.

## Preliminary treatment.

As a preliminary the Wassermann test was carried out on the five boys: all were negative. Further, stools were examined for parasites and blood for evidence of malaria. Where necessary the subjects were deparasitised and treated for malaria. Similar examinations were made periodically throughout the experiment. Treatment was carried out as required and did not interfere with the experimental work.

## Experimental method.

The boys were under the same strict supervision as obtained in the previous experiment, and in general the experimental method was that already described.

## Divisions of the experiment.

The experiment was divided into four periods as follows:

(1) A preliminary period of 3 weeks during which the subjects were on the usual long term prisoners' diet.

(2) A period of 5 weeks during which the prison hospital diet replaced the prison diet.

[1] *See* preceding paper in this number of *J. Hygiene*, pp. 418–428.

(3) A supplement period of 9 weeks when certain additions were made to the prison hospital diet. These additions differed in the case of each of the five subjects.

(4) A post-period of 7 weeks in which all subjects reverted to the unsupplemented prison hospital diet with the exception of a control (2640) who had received no addition hitherto but now received a supplement of cod-liver oil and mineral mixture.

## Basal diets.

The daily rations of the two diets referred to were as follows, and on analysis showed an ultimate composition as undernoted (Kelly and Henderson, 1927):

| | Prison diet (long term) | Prison hospital diet |
|---|---|---|
| Beans | 6 oz. | 6 oz. |
| Beef* | 8 ,, | 8 ,, |
| Maize | 1½ lb. | 1½ lb. |
| Potatoes | 8 oz. | 6 oz. |
| Salt | ¼ ,, | ½ ,, |
| Ghee* | 1 ,, | ½ ,, |
| Lemons | 2 per week | 2 per week |
| Protein | 116·5 grm. | 125·3 grm. |
| Carbohydrate | 626·0 ,, | 535·0 ,, |
| Fat | 53·4 ,, | 48·5 ,, |
| Energy value | 3450 C. | 3079 C. |
| Calcium (Ca) | 0·30 grm. | 0·346 grm. |
| Phosphorus (P) | 2·36 ,, | 2·65 ,, |

* In the prison (long term) diet the beef is only given on 3 days per week and the ghee on the other 4 days.

Whole maize is fed in the prison diet and maize flour in the prison hospital diet.

### *Supplements.*

The following table gives particulars of the men and shows the daily additions made to the prison hospital diet during the supplement period:

| Prisoner's no. | Tribe | Age | Initial height | Initial weight | Supplement |
|---|---|---|---|---|---|
| 2640 | Nandi | 16 | 5′ 8¼″ | 114½ lb. | None |
| 2413 | ,, | 16 | 5′ 7⅞″ | 107 | 15 c.c. cod-liver oil |
| 1217 | ,, | 17 | 5′ 8⅓″ | 125½ | Mineral mixture |
| 2415 | ,, | 16 | 5′ 9″ | 115 | 15 c.c. cod-liver oil plus mineral mixture |
| 2534 | Masai | 15 | 5′ 9¾″ | 105¾ | 1 pint cows' milk |

The mineral mixture was identical with that already noted and described in the preceding communication. It was of approximately the same composition as the ash of cows' milk, the daily dose being equivalent to the ash in 1 pint of milk. The calcium thus added daily was 0·68 grm. Ca. From analysis the calcium content of the milk fed to subject 2534 averaged 0·77 grm. Ca per day. This figure is somewhat higher than had been expected. From analysis done in Great Britain and America 1 pint of milk would contain, on average, about 0·68 grm. Ca (*i.e.* 0·12 per cent.). The analyses of milks from native cows, however, gave an average percentage of 0·1356, which is equivalent to 0·77 grm. Ca in 1 pint of the milk used in our experiment. The milk was boiled—a routine measure customary in this country.

### CALCIUM BALANCES.

The calcium balances of the five subjects were determined on nine occasions, twice in the prison diet period, three times in the prison hospital diet period and four times in the supplement-period. The urine and faeces were

Table I.

| No. | Supplement and Ca intake per 96 hours | Total calcium urine grm. | Total calcium faeces grm. | Total calcium excreted grm. | Total balance per 96 hours grm. | Average 96-hour balance in the period grm. |
|---|---|---|---|---|---|---|
| 1217 | Prison diet | 0·0582 | 1·2625 | 1·3207 | −0·1207 | −0·1417 |
| | (1·2000 grm. Ca) | 0·0495 | 1·3133 | 1·3628 | −0·1628 | |
| | Prison hospital diet | 0·0437 | 1·6924 | 1·7361 | −0·3521 | |
| | (1·3840 grm. Ca) | 0·0538 | 2·4077 | 2·4615 | −1·0775 | −0·6586 |
| | | 0·1119 | 1·8182 | 1·9301 | −0·5461 | |
| | Supplement | 0·1042 | 2·8639 | 2·9681 | +1·1415 | |
| | 125 c.c. mineral mixture | 0·0936 | 3·5329 | 3·6265 | +0·4831 | |
| | (4·1096 grm. Ca) | 0·0974 | 2·1465 | 2·2439 | +1·8657 | +1·0039 |
| | | 0·1014 | 3·4828 | 3·5842 | +0·5254 | |
| 2415 | Prison diet | 0·3690 | 1·8424 | 2·2114 | −1·0114 | −1·1878 |
| | (1·2000 grm. Ca) | 0·3666 | 2·1977 | 2·5643 | −1·3643 | |
| | Prison hospital diet | 0·1507 | 2·3073 | 2·4580 | −1·0740 | |
| | (1·3840 grm. Ca) | 0·0996 | 1·9604 | 2·0600 | −0·6760 | −0·9835 |
| | | 0·1795 | 2·4049 | 2·5844 | −1·2004 | |
| | Supplement | 0·1072 | 3·4460 | 3·5532 | +0·5564 | |
| | Minerals and cod-liver oil | 0·1527 | 4·2287 | 4·3814 | −0·2718 | |
| | (4·1096 grm. Ca) | 0·1458 | 3·0500 | 3·1958 | +0·9138 | +0·3847 |
| | | 0·1087 | 3·6406 | 3·7493 | +0·3603 | |
| 2534 | Prison diet | 0·1929 | 1·6465 | 1·8394 | −0·6394 | −0·6025 |
| | (1·2000 grm. Ca) | 0·1339 | 1·6318 | 1·7657 | −0·5657 | |
| | Prison hospital diet | 0·1409 | 1·8101 | 1·9510 | −0·5670 | |
| | (1·3840 grm. Ca) | 0·2034 | 1·4175 | 1·6209 | −0·2369 | −0·3951 |
| | | 0·1718 | 1·5937 | 1·7655 | −0·3815 | |
| | Supplement | 0·1369 | 2·5530 | 2·7199 | +1·7449 | |
| | 1 pint milk | 0·1482 | 1·9629 | 2·1111 | +2·3537 | |
| | (4·4648 grm. Ca) | 0·1724 | 2·0936 | 2·2660 | +2·1988 | +2·0234 |
| | | 0·1520 | 2·5164 | 2·6684 | +1·7964 | |
| 2413 | Prison diet | 0·0952 | 0·9990 | 1·0942 | +0·1058 | +0·0206 |
| | (1·2000 grm. Ca) | 0·0917 | 1·1729 | 1·2646 | −0·0646 | |
| | Prison hospital diet | 0·0819 | 2·1398 | 2·2217 | −0·8377 | |
| | (1·3840 grm. Ca) | 0·0587 | 2·0259 | 2·0846 | −0·7006 | −0·8003 |
| | | 0·1013 | 2·1454 | 2·2467 | −0·8627 | |
| | Supplement | 0·0890 | 2·6524 | 2·7414 | −1·3574 | |
| | 15 c.c. cod-liver oil | 0·0818 | 1·6584 | 1·7402 | −0·3562 | |
| | (1·3840 grm. Ca) | 0·0807 | 2·0686 | 2·1493 | −0·7653 | −0·5458 |
| | | 0·0836 | 1·0049 | 1·0885 | +0·2955 | |
| 2640 | Prison diet | 0·0795 | 0·9766 | 1·0561 | +0·1439 | +0·1541 |
| | (1·2000 grm. Ca) | 0·0728 | 0·9628 | 1·0357 | +0·1643 | |
| | Prison hospital diet | 0·1157 | 1·3370 | 1·4527 | −0·0687 | |
| | (1·3840 grm. Ca) | 0·0790 | 1·5142 | 1·5932 | −0·2092 | −0·0269 |
| | | 0·0871 | 1·0997 | 1·1868 | +0·1972 | |
| | Supplement | 0·1748 | 1·0746 | 1·2494 | +0·1346 | |
| | Nil. | 0·1407 | 1·0927 | 1·2334 | +0·1506 | |
| | (1·3840 grm. Ca) | 0·1158 | 1·0381 | 1·1539 | +0·2301 | +0·1738 |
| | | 0·0871 | 1·1171 | 1·2042 | +0·1798 | |

collected continuously for 96 hours on each occasion, the excreta being removed for analysis at the end of each 48 hours. These nine 96-hour collections were distributed throughout the experiment with a view to being representative of the period in which they were made. Short of making absolutely continuous collections (which we found quite impossible to undertake), this alternative appeared satisfactory.

Table I gives details of the calcium balances over 96 hours. Table II epitomises these results on the basis of a 24-hour balance.

Table II. *Average 24-hour balances.*

| Period | 1217 (Mineral mixture) | 2415 (Minerals and cod-liver oil) | 2534 (Milk) | 2413 (Cod-liver oil) | 2640 (Balance) |
|---|---|---|---|---|---|
| Prison diet | −0·0354 | −0·2969 | −0·1506 | +0·0051 | +0·0385 |
| Prison hospital diet | −0·1646 | −0·2459 | −0·0988 | −0·2001 | −0·0067 |
| Supplement | +0·2509 | +0·0962 | +0·5058 | −0·1364 | +0·0434 |

## Growth curves and blood calcium determinations.

Each subject was weighed weekly, the growth curves being represented in Fig. 1. The serum calcium, determined on two occasions, gave the following results: 2640, 10·6 and 11·0; 2413, 10·4 and 10·0; 1217, 10·4 and 10·4; 2415, 10·4 and 9·8; 2534, 10·0 mg. per 100 c.c. The second determination for subject 2534 was not made on account of an accident.

## Discussion of results.

### *Calcium requirements.*

As regards the retention of calcium during the prison diet period it will be observed from Table II that in three out of the five subjects there is a negative balance amounting to, in one case (2415), as much as 0·29 grm. Ca per day. Even the highest positive balance is only 0·0385 grm. Ca per day (2640). In the second period (prison hospital diet) all five men are on a negative calcium balance, subjects 2413 and 2415 losing 0·2 grm. Ca or more per day.

Out of fifteen 96-hour collection periods (*i.e.* three collections from each of five men in the prison hospital diet period) only one positive balance was obtained, namely + 0·1972 grm. Ca in the case of subject 2640 (Table I).

This prevalence of negative calcium balances is not what one would expect to find in growing boys. Subject 2415, for example, was increasing in weight during the first two periods at a rate of over 2 lb. per week. Sherman and Hawley (1922) found in experiments on the calcium retention of children from 3 to 13 years of age that 0·15–0·62 grm. Ca were retained per day, the amount increasing with the age and size of the child. The average daily storage in this series was 0·01 grm. calcium per kilo of body weight. For boys of the ages from 6 to 14 years Herbst (1912) also obtained similar figures (0·01–0·016 grm. Ca per kilo of body weight). Extending the application of these figures to our subjects, who, it is true, were older but were still rapidly growing, we might legitimately expect a daily calcium storage of something in the region of 0·5 grm. Ca.

So far from this being the case, we find our subjects actually losing calcium, at an average rate of 0·1 grm. per 24 hours.

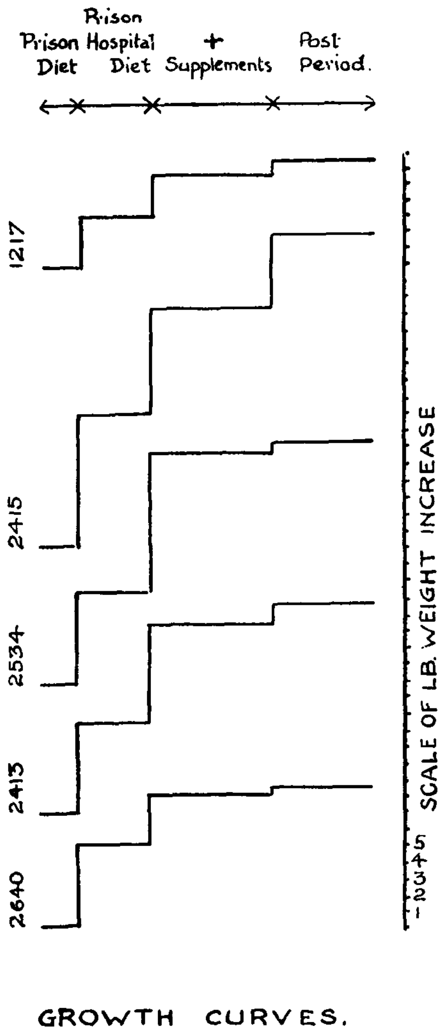


Fig. 1.

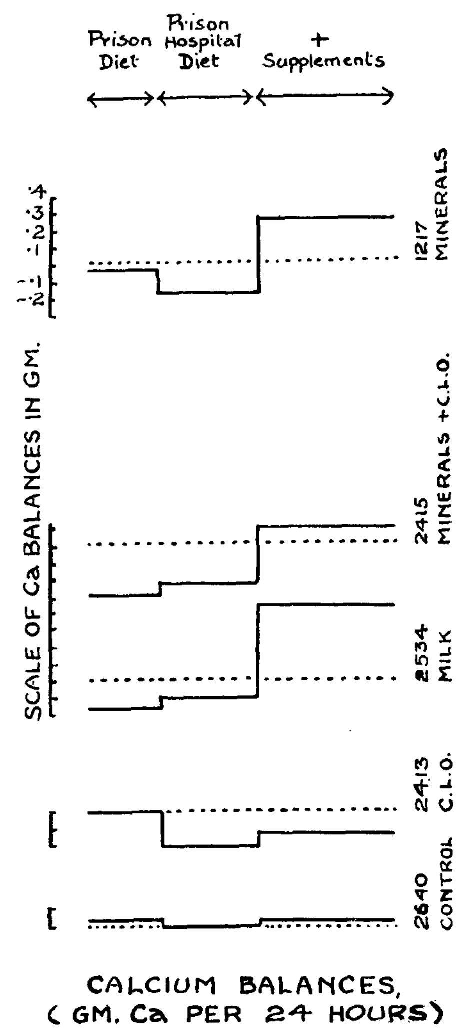


Fig. 2.

A consideration of the two prison diets and their calcium content throws some light upon the above state of affairs. The average daily intake of calcium provided by these two diets is 0·323 grm. Anyone familiar with the literature will recognise that this amount is not even sufficient for maintenance, far less for growth. Sherman (1920) in his summary of our knowledge on the calcium maintenance requirement arrives at the conclusion that the minimum of actual need is 0·45 grm. calcium per day for adults averaging 70 kilos body

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weight. This maintenance allowance of 0·45 grm. corresponds to the minimum protein maintenance allowance of 44 grm. per man per day. To bring this into line with the 100 grm. protein standard, 1·0 grm. calcium would require to be ingested. For adults this 1·0 grm. amount of calcium per day may be taken as furnishing an appropriate margin of safety over the minimum maintenance allowance of 0·45 grm. Ca. As our subjects were still growing and had an average initial weight of 50 and not 70 kilos, it is probable that their maintenance value should be nearer 0·5 grm. Ca than the 0·45 figure given by Sherman for adults. We have already assumed, however, that our subjects would require 0·5 grm. calcium per day to be retained for the purposes of growth. Thus, a calcium intake of about 1·5 grm. per day would seem to be necessary for these growing subjects. This would provide 0·5 grm. for maintenance, 0·5 grm. for storage and 0·5 grm. as a margin of safety for the inevitable loss due to imperfect absorption. In the case of the growing pig, for example (Orr and Husband, 1922), which has a capacity for calcium storage about ten times as great as the human, one finds that to ensure a retention of 5 grm. calcium per day one has to provide at least 10 grm. in the food. This shows that the 0·5 grm. moiety as a "margin of safety" in the case of humans is no mere gratuitous addition.

If, then, as it would appear, this prevalence of negative calcium balances in growing boys is largely due to the very low calcium intake in the food (0·323 grm. per day), one would expect to find some improvement on the addition of calcium. Accordingly as already stated we added, in the case of 1217, 0·6814 grm. Ca in the mineral mixture, thus making the total intake per 24 hours 1·0274 grm. Ca. This is at least an approach to the figure (1·5 grm.) which we have already deduced as desirable. In the case of 2415, 15 c.c. cod-liver oil was given in addition to the above increased calcium intake. Subject 2534 had his daily calcium intake raised to 1·1162 grm. by the addition of 1 pint of cows' milk per day. No extra calcium was given to 2640 (who acted as a control) nor to 2413; the latter, however, received daily 15 c.c. cod-liver oil. To discuss these subjects individually:

1217. (*Mineral mixture.*)

A consistently negative balance became consistently positive on the addition of the extra calcium, the subject retaining roughly 0·25 grm. Ca per day, an amount equivalent to what, on occasion, he had previously been losing per day.

2415. (*Minerals and cod-liver oil.*)

A consistently negative balance became, on the addition of the supplement, positive on three out of the four occasions. A daily loss of calcium of almost 0·3 grm. per day was converted to a retention of 0·1 grm. per day.

2534. (*Milk.*)

A consistent loss of calcium averaging over 0·1 grm. per day was converted to a consistent retention of 0·5 grm. per day.

2413. (*Cod-liver oil.*)

A negative balance of 0·2 grm. per day in the prison hospital diet period was slightly reduced on the addition of cod-liver oil to − 0·13 grm. Ca per day, but the subject did not revert to the calcium equilibrium he had shown in the prison diet period.

2640. (*No additions.*)

This subject was practically in calcium equilibrium throughout the three periods, his balance being, if anything, on the positive side and varying within very narrow limits.

These balances are graphed in Fig. 2.

It appears, from the above, therefore, that without exception those subjects who received added calcium showed a very definite improvement in their balance. Given increased calcium in the food they readily absorbed and retained it, the more readily, perhaps, since they had been on a continued low calcium intake and, at the time of analysis, at least, on a distinctly negative calcium balance. Such, on the other hand, as received no additional calcium remained on a negative balance as in the case of the subject receiving cod-liver oil alone, or more or less in calcium equilibrium as in the case of the control subject.

This would emphasise the fact that where the calcium intake is low, the most valuable addition that can be made is primarily one of calcium itself. We are aware, of course, that in giving either milk or the mineral mixture we are improving not only the calcium intake, but also the Ca : P ratio. Thus, a ratio of approximately 1 : 7 is changed to one of 1 : 3. It would further impress the futility of adding a calcium absorption promoting vitamin in the absence of an adequate calcium intake. Indeed, where there is a pressing demand for calcium, as in the case of these growing boys who have been on a diet deficient in this element, the furnishing of an adequate amount in the food, with or without an increase in the vitamin content, would seem to meet the needs of the case. Thus, subject 2415 (minerals and cod-liver oil) shows no improvement in retention over subject 1217 (minerals alone), though, it is true, 2415 was raised from a much greater negative balance. It has, however, to be pointed out that the best result of all was obtained when the calcium was added in the form of milk. This is in accordance with the work of Sherman and Hawley (1922). These findings, in so far as the experiments are comparable, are strongly confirmatory of our previous balance determinations. We put forward the results of these earlier determinations somewhat tentatively on account of the shortness of the collection periods and the small number of subjects examined. The two series of results are now seen, however, to be mutually supporting.

To return to a consideration of the maintenance requirement we have already assumed that about 0·5 grm. calcium per day is necessary. In actual fact we find that four of the five subjects lose per day, on the low average intake of 0·32 grm. Ca, the following amounts respectively: (1217), 0·10 grm. Ca;

(2413), 0·09 grm. Ca; (2415), 0·27 grm. Ca; (2534), 0·12 grm. Ca. Adding these small negative balances to the intake we obtain as maintenance requirements the following values: (1217), 0·42 grm. Ca; (2413), 0·41 grm. Ca; (2415), 0·59 grm. Ca; (2534), 0·44 grm. Ca. In the case of 2640 since he is in effect in calcium equilibrium, we may conclude that his maintenance value is equal to his intake, *i.e.* 0·32 grm. Ca per day. The average of these five values gives a figure for maintenance of 0·43 grm. Ca per day which is in fair agreement with the 0·50 grm. postulated.

Accepting then this maintenance requirement of 0·43 grm. Ca per day, it is obvious that if the intake was only 0·32 grm. originally, then, in those cases in which we increased the intake by 0·68 grm. an amount of 0·11 grm. Ca (0·43–0·32) is actually needed to bring the subject into equilibrium. Therefore 0·57 grm. Ca is left for storage purposes. Of this we find an average of almost 0·20 grm. retained which is an approximation to the 50 per cent. retention of any additional calcium over and above the maintenance allowance. In the case of the subject receiving milk, 2534, whose intake was raised by 0·77 grm. Ca daily, this value amounts to 74 per cent.

One will observe that these marked improvements in the calcium balances were brought about by an addition of calcium which, though approaching to, did not equal the postulated optimum daily intake of 1·5 grm. Ca for growing subjects. That an even more marked retention might have resulted from a 1·5 grm. daily intake seems not improbable.

*Blood calcium.*

The serum calcium values were initially normal and remained so throughout the experiment.

*Growth.*

In the previous experiment our subjects were adult men whose weight had been practically stationary. Any increase, therefore, due to the addition of a supplement was uncomplicated and easily observed. Here, however, the fact that the subjects were growing fairly rapidly probably masks any improvement in weight due to the addition of supplements. Further, no criteria of growth in native tribes are available, though we understand data for such are being collected. Admissions to hospital for minor ailments, which were not infrequent during the experiment, constitute a further complication. We have, however, graphed the averaged growth curves for each period omitting those weights recorded when a subject was in hospital.

It is perhaps worthy of note that the best growth curves during the supplement period are those of the subjects receiving cod-liver oil (2415 and 2413) or milk (2534). Further, growth after withdrawal of the supplement is best maintained by subjects who had received cod-liver oil. These effects may be satisfactorily explained by the growth stimulating influence and possible storage of vitamin A.

The growth curve for the basal subject (2640) is, it is true, somewhat anomalous in so far as it shows no rise in the post-period on the addition of minerals and cod-liver oil. To what extent this is related to the fact that he was the only subject initially in calcium equilibrium and with the highest serum calcium is uncertain.

A consideration of the balance graphs and growth curves shows conclusively that growth may take place in the absence of adequate calcium storage. Indeed, on supplying cod-liver oil increased growth may be brought about (2413) while the calcium balance still remains negative. It is to be noted, however, that, apart from the subject receiving milk, the one who had both minerals and cod-liver oil shows the best growth and passes from what was the most markedly negative to a definite positive calcium balance. It is evident, of course, that continued growth along with a negative calcium balance must necessarily be unsatisfactory in that while body weight increases, skeletal growth must suffer. McCollum and Simmonds (1917) demonstrated that growth in rats on a diet low in calcium was of a stunted type. A similar observation has been made by Telfer and Crichton (1924) working with young goats on a mineral-deficient diet. While in this country skeletal deformities are not patent they are by no means unknown. In this connection tropical sunlight probably plays a beneficial part.

Should further research show that the diet of the native in his natural environment is as deficient in calcium as we have shown these institution diets to be, this deficiency may in some part account for the lack of resistance which is a recognised feature of many East African tribes. It is generally conceded that the above institution diets are markedly superior to the usual native diet. Since, however, our knowledge of native dietary habits is scanty, research is being actively prosecuted in this direction.

## SUMMARY AND CONCLUSIONS.

1. Of five experimental subjects—growing East African native boys—four proved to have definitely negative calcium balances on the diet in use at the Nairobi Prison, while the fifth was merely in calcium equilibrium.

2. The diet in question was found to be so low in calcium (0·32 grm. Ca per day) that the occurrence of negative balances is not altogether surprising.

3. These negative calcium balances became positive on the addition of calcium as milk or in a mineral mixture with or without cod-liver oil.

4. Cod-liver oil alone was not effective in bringing about any appreciable reduction of a negative balance.

5. Our findings showed that 0·43 grm. Ca daily was the maintenance requirement for the subjects under experiment. This figure is practically the same as that found by Sherman in a summary of work on white races.

6. On giving additional calcium in a mineral mixture it was found that after the maintenance requirement had been satisfied, as much as 50 per cent.

of any excess might be retained. The calcium of milk, on the other hand, was found to be utilised more efficiently; the corresponding retention figure being over 70 per cent.

7. The influence of the various supplements on growth is briefly discussed.

REFERENCES.

HERBST (1912). *Jahrb. Kinderheilk.* **76**. Ergänzungsheft, 40.
KELLY and HENDERSON (1927). *Kenya Med. J.* **4**, 232.
—— —— *See* pp. 418–428 of this number of *J. Hygiene.*
McCOLLUM and SIMMONDS (1917). *J. Biol. Chem.* **32**, 29.
ORR and HUSBAND (1922). *Scottish J. of Agricult.* **5**, No. 3.
SHERMAN (1920). *J. Biol. Chem.* **44**, 21.
SHERMAN and HAWLEY (1922). *Ibid.* **53**, 375.
TELFER and CRICHTON (1924). *Brit. J. Exp. Path.* **5**, 84.

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