*Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 24, Numbers 1 and 2, April, 1950.*

Printed in the Union of South Africa by the Government Printer, Pretoria.

# The Availability of the Calcium and Phosphorus in "Electrofos" for Bone Formation. I.—In the Rat.

By N. REINACH and J. G. LOUW, Section of Biochemistry, Onderstepoort.

On account of its high fluorine content rock phosphate is not an acceptable substitute for bone meal in the nutrition of farm animals. However, this objection has during the recent war, when a serious shortage of phosphatic supplements had to be faced, been overcome by the introduction of methods (Hill *et al,* 1945) for removing the fluorine thermally to a level which makes the resulting product safe for feeding to live stock. Unfortunately, it would appear (Bird *et al,* 1945; Ellis *et al,* 1945; Fraser *et al,* 1943; Barrentine *et al,* 1944) that some of the products thus obtained are not satisfactory sources of calcium and phosphorus for certain animals. The temperature at which defluorination is effected seems to play a role in that poorly available forms of calcium and phosphorus such as the meta- and pyrophosphates of calcium are formed under certain conditions. Tests for biological availability of all new materials of this nature are, therefore, indicated.

The technologists of a South African firm, African Metals Corporation, were not slow in appreciating the possibilities which the thermal defluorination process held out for the utilization of the phosphorus and calcium in local rock phosphate deposits. Experiments were started by them and in due course samples of two products, in finely powdered form, "electrofos I" and "electrofos II", were submitted to this laboratory for biological tests. The composition of these products is shown in Table 1.

TABLE 1.

*Analysis of Products used in Experiments.*

| Mineral. | Per Cent. Calcium. | Per Cent. Phosphorus. | Per Cent. Silica. | Per Cent. Fluorine. |
|---|---|---|---|---|
| Electrophos I..... | 30·3 | 18·3 | 11·8 | 0·024 |
| Electrophos II..... | 17·1 | 27·2 | 12·1 | 0·015 |

## EXPERIMENTAL PROCEDURE.

Using the diets described in table 2 a series of four experiments were conducted. Bone ash, total calcium and phosphorus retention, and growth were used as criteria for evaluating the test materials. In a preliminary test (experiment 1) electrophos I was compared with dicalcium phosphate at a level which furnished

Received for publication on 10th January, 1948.—Editor.

0·16 per cent. phosphorus in the diet. For this purpose a basal ration containing 0·05 per cent. P and 0·06 per cent. Ca was supplemented with the appropriate amounts of the two phosphatic products to yield diets A and B (see Table 2). Calcium carbonate was added to the diets to ensure a Ca : P ratio of more or less 2 : 1. The salt mixture used was that of Day and McCollum (1939) with the calcium carbonate omitted.

Eight trios of litter-mate albino rats of the same sex and weight were selected. At the beginning of the experiment one member of each trio was sacrificed and analysed as described below. The remaining two were pair-fed in individual cages, provided with false bottoms of $\frac{3}{8}$ inch mesh galvanized wire screen, for a period of six weeks on diets A and B, respectively.

TABLE 2.

*Composition of Diets.*

| | A. | B. | C. | D. | E. | F. | G. |
|---|---|---|---|---|---|---|---|
| Egg White | 150 | 150 | 150 | 150 | 150 | 150 | 150 |
| Gelatine | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Butterfat | 80 | 80 | 80 | 80 | 80 | 80 | 80 |
| Bacto Agar | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Harris Yeast | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Cod Liver Oil | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Sucrose | 40 | 40 | 40 | 40 | 40 | 40 | 40 |
| Dextrinized Starch | 606·9 | 606·7 | 606·0 | 607·1 | 596·9 | 606·2 | 594·7 |
| Salt Mixture | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Dicalcium Phosphate | — | 5·5 | 6·1 | — | — | — | — |
| Electrofos I | 3·7 | — | — | 5·9 | 14·6 | — | — |
| Electrofos II | — | — | — | — | — | 4·1 | 10·1 |
| Calcium Carbonate | 4·4 | 2·8 | 2·9 | 2·0 | 3·5 | 4·7 | 10·2 |
| TOTAL | 1000·0 | 1000·0 | 1000·0 | 1000·0 | 1000·0 | 1000·0 | 1000·0 |
| Per Cent. Ca | 0·33 | 0·28 | 0·30 | 0·31 | 0·62 | 0·31 | 0·64 |
| Per Cent. P | 0·15 | 0·16 | 0·18 | 0·19 | 0·36 | 0·19 | 0·35 |

The rats were fed daily and weighed weekly. Fresh distilled water was provided at all times. At the end of the experimental period they were killed with ether. The left femur of each rat was removed and its ash content determined on a dry fat-free basis. The entire rat, freed of the contents of the digestive tract, was then dried at 100° C, ashed at a low-red heat in an electric muffle, and, after the addition of the femur ash, analysed for total calcium and phosphorus.

The second experiment was essentially the same as the first one except that an extra group of rats receiving the basal ration supplemented with electrofos I at a level furnishing about 0·32 per cent. P in the diet was included. Eight quartos of albino rats were accordingly selected for a comparison of the availability of the calcium and phosphorus in diets C, D and E (see Table 2), respectively, one rat of each quarto being again sacrificed at the beginning of the experiment for the purpose of arriving at base values for the calcium and phosphorus contents of the rats. It was not possible to have litter-mates in the quartos. Rats within quartos were, however, uniform in respect of age, sex and live weight.

Experiment 3 was run concurrently with experiment 2 and was a repetition of the latter with this difference that paired feeding was dispensed with and that diets C, D and E were offered *ad lib.* to six trios of rats selected to correspond in respect of age, sex and live weight to the first 6 quartos of experiment 2. The rats sacrificed at the commencement of experiment 2 could thus also serve as controls for experiment 3.

In a final experiment electrofos II, at two levels of intake (see diets F and G in Table 2), was compared with dicalcium phosphate (see diet C in Table 2). The technique employed corresponded in every respect with that used in experiment 2.

Calcium was determined by the volumetric method of McCrudden (1911) and phosphorus by the colorimetric procedure of Fiske and Subbarow (1925).

All data were analysed statistically by Fisher's analysis of variance method.

TABLE 3.

*Weekly Feed Consumption of Rats in pair-fed Experiments (grams).*

| Rat No. | 1st Week. | 2nd Week. | 3rd Week. | 4th Week. | 5th Week. | 6th Week. | Total. |
|---|---|---|---|---|---|---|---|
| EXPERIMENT I (DIETS A. AND B.). | | | | | | | |
| 1 | 52 | 50 | 56 | 60 | 56 | 60 | 334 |
| 3 | 41 | 38 | 41 | 34 | 29 | 33 | 216 |
| 5 | 54 | 54 | 55 | 54 | 49 | 55 | 321 |
| 7 | 53 | 56 | 57 | 54 | 60 | 51 | 331 |
| 9 | 39 | 41 | 48 | 40 | 24 | 34 | 226 |
| 11 | 43 | 49 | 36 | 38 | 36 | 37 | 239 |
| 13 | 52 | 56 | 54 | 56 | 39 | 58 | 315 |
| 15 | 57 | 53 | 54 | 56 | 57 | 54 | 331 |
| AVERAGE... | 49 | 50 | 50 | 49 | 44 | 48 | 290 |
| EXPERIMENT II (DIETS C, D AND E). | | | | | | | |
| 1 | 45 | 49 | 59 | 44 | 41 | 38 | 276 |
| 4 | 39 | 42 | 52 | 47 | 48 | 44 | 272 |
| 7 | 45 | 38 | 49 | 55 | 50 | 66 | 303 |
| 10 | 44 | 49 | 48 | 61 | 56 | 56 | 314 |
| 13 | 45 | 52 | 32 | 52 | 48 | 48 | 277 |
| 16 | 35 | 46 | 50 | 51 | 62 | 67 | 311 |
| 19 | 44 | 54 | 39 | 50 | 57 | 57 | 301 |
| 22 | 52 | 48 | 61 | 66 | 64 | 70 | 361 |
| AVERAGE... | 44 | 47 | 49 | 53 | 53 | 56 | 302 |
| EXPERIMENT IV (DIETS C, F AND G). | | | | | | | |
| 1 | 32 | 41 | 45 | 40 | 36 | 45 | 239 |
| 4 | 38 | 41 | 38 | 37 | 48 | 41 | 243 |
| 7 | 38 | 41 | 47 | 46 | 53 | 55 | 280 |
| 10 | 48 | 46 | 57 | 56 | 53 | 43 | 303 |
| 13 | 40 | 46 | 52 | 52 | 54 | 57 | 301 |
| 16 | 37 | 46 | 37 | 36 | 31 | 39 | 226 |
| 19 | 38 | 44 | 42 | 48 | 54 | 60 | 286 |
| 22 | 44 | 42 | 40 | 44 | 30 | 33 | 233 |
| AVERAGE... | 39 | 43 | 45 | 45 | 45 | 47 | 264 |

## RESULTS.

*Experiment I.*

Figures for weekly feed consumption and body weights are given in Tables 3 and 4, respectively. Since the feed consumption of the respective groups of rats in experiments I, II and IV has been equalized figures for only one group in each of the experiments are tabulated. Reference to Table 4 shows that the rats on diet B (dicalcium phosphate supplemented) made greater live weight gains than those on diet A (electrofos I supplemented). This difference was found significant at $P = \cdot 05$ with a coefficient of variation of 6·19 per cent.

Data relating to the percentage retention of the ingested calcium and phosphorus are presented in Table 5. For the purpose of calculating these figures it was assumed that at the time of selection the rats of a trio or quarto contained the same amounts of calcium and phosphorus, so that the initial calcium and phosphorus contents of the experimental rats were represented by the amounts of these minerals found in the rat sacrificed at the commencement of an experiment. On an average only 23·2 per cent. of the calcium and 48·9 per cent. of the phosphorus of diet A as against 64·5 per cent. of the calcium and 76·8 per cent. of the phosphorus of diet B were retained. The differences in favour of the diet containing dicalcium phosphate were significant at $P = \cdot 001$, with coefficients of variation of 6·6 per cent. and 6·1 per cent. for the calcium and phosphorus retentions, respectively. Since the level of intake was the same for the two diets the variations in the observed retentions can be explained only on the basis of differences in availability. Electrofos I thus appears to be a poor source of calcium and phosphorus for the growing rat. Further evidence of this property of the test material is supplied by the data in Table 6. The mean weight of the ash of the femurs of the electrofos group of rats was 0·0707 gm. as against a figure of 0·1061 gm. for the dicalcium phosphate group.

TABLE 4.

*Weekly Weights of Rats in Experiment I (grams).*

| Diet. | Rat No. | Initial Weight. | 1st Week. | 2nd Week. | 3rd. Week. | 4th Week. | 5th Week. | 6th Week. (Final Weight.) | Total Gain. |
|---|---|---|---|---|---|---|---|---|---|
| A..... | 1 | 74 | 88 | 102 | 124 | 140 | 151 | 166 | 92 |
| | 3 | 54 | 65 | 75 | 91 | 98 | 100 | 100 | 46 |
| | 5 | 65 | 82 | 98 | 111 | 124 | 133 | 143 | 78 |
| | 7 | 67 | 81 | 100 | 119 | 132 | 135 | 157 | 90 |
| | 9 | 52 | 63 | 79 | 97 | 100 | 102 | 107 | 55 |
| | 11 | 67 | 74 | 87 | 94 | 104 | 111 | 114 | 47 |
| | 13 | 84 | 96 | 113 | 132 | 145 | 149 | 168 | 84 |
| | 15 | 67 | 85 | 97 | 109 | 124 | 136 | 147 | 80 |
| AVERAGE.. | — | 66·3 | 79·3 | 93·9 | 109·6 | 120·9 | 127·1 | 137·8 | 71·5 |
| B..... | 2 | 74 | 86 | 103 | 127 | 145 | 156 | 174 | 100 |
| | 4 | 54 | 69 | 80 | 90 | 103 | 104 | 111 | 57 |
| | 6 | 65 | 79 | 100 | 121 | 136 | 147 | 159 | 94 |
| | 8 | 66 | 81 | 102 | 120 | 136 | 154 | 162 | 96 |
| | 10 | 53 | 63 | 74 | 93 | 101 | 99 | 107 | 54 |
| | 12 | 67 | 76 | 92 | 100 | 110 | 116 | 122 | 55 |
| | 14 | 83 | 93 | 110 | 131 | 145 | 145 | 163 | 80 |
| | 16 | 68 | 85 | 98 | 117 | 134 | 148 | 159 | 91 |
| AVERAGE.. | — | 66·3 | 79·0 | 94·9 | 112·4 | 126·3 | 133·6 | 144·6 | 78·4 |

TABLE 5.

*Calcium and Phosphorus Retained by Rats in Experiment I.*

*(Unless otherwise stated all figures are in milligram).*

| Diet. | Rat No. | Total Ca Intake. | Ca in Rat at beginning. | Ca in Rat at End. | Ca gain. | Per Cent. Ca retention. | Total P Intake. | P in Rat at Beginning. | P in Rat at End. | P gain. | Per Cent. P retention. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | 1,095·5 | 571·1 | 794·5 | 223·4 | 20·4 | 481·0 | 395·0 | 633·0 | 238·0 | 49·5 |
| | 3 | 708·5 | 410·8 | 580·6 | 169·8 | 24·0 | 311·0 | 292·5 | 438·6 | 146·1 | 47·0 |
| | 5 | 1,052·9 | 533·0 | 672·3 | 139·3 | 13·2 | 462·3 | 370·0 | 541·6 | 171·6 | 37·1 |
| | 7 | 1,086·0 | 535·1 | 753·9 | 218·8 | 20·1 | 476·8 | 365·0 | 607·3 | 242·3 | 50·8 |
| | 9 | 741·3 | 416·8 | 613·8 | 197·0 | 26·6 | 325·4 | 285·0 | 450·5 | 165·5 | 50·8 |
| | 11 | 783·9 | 507·0 | 713·1 | 206·1 | 26·3 | 344·2 | 355·0 | 520·8 | 165·8 | 48·2 |
| | 13 | 1,033·0 | 619·2 | 898·9 | 279·7 | 27·0 | 453·7 | 430·0 | 672·6 | 242·6 | 53·4 |
| | 15 | 1,086·0 | 491·0 | 797·1 | 306·1 | 28·2 | 476·8 | 360·0 | 612·2 | 252·2 | 52·9 |
| AVERAGE | — | 948·4 | 510·5 | 728·0 | 217·5 | 23·2 | 416·4 | 356·6 | 559·6 | 203·0 | 48·9 |
| B | 2 | 921·8 | 571·1 | 1,179·0 | 607·9 | 65·9 | 537·8 | 395·0 | 842·8 | 447·8 | 83·3 |
| | 4 | 596·2 | 410·8 | 789·4 | 378·6 | 63·4 | 347·8 | 292·5 | 551·4 | 258·9 | 74·4 |
| | 6 | 886·0 | 533·0 | 1,072·0 | 539·0 | 60·8 | 516·8 | 370·0 | 753·7 | 383·7 | 74·2 |
| | 8 | 913·6 | 535·1 | 1,054·0 | 518·9 | 62·5 | 532·9 | 365·0 | 750·1 | 385·1 | 72·3 |
| | 10 | 623·8 | 416·8 | 814·9 | 398·1 | 63·8 | 363·8 | 285·0 | 555·5 | 270·5 | 74·4 |
| | 12 | 659·6 | 507·0 | 947·3 | 440·3 | 66·8 | 384·8 | 355·0 | 660·9 | 305·9 | 79·4 |
| | 14 | 869·4 | 619·2 | 1,161·0 | 541·8 | 62·3 | 507·2 | 430·0 | 824·1 | 394·1 | 77·8 |
| | 16 | 913·6 | 491·0 | 1,080·0 | 589·0 | 70·9 | 532·9 | 360·0 | 777·1 | 417·1 | 78·3 |
| AVERAGE | — | 798·0 | 510·5 | 1,012·2 | 501·7 | 64·5 | 465·5 | 356·6 | 714·5 | 357·9 | 76·8 |

TABLE 6.

*Ash in Femurs of Rats in Experiment I.*

| Diet. | Rat No. | Weight of Rat (gm.). | Weight of Fat-Free dry Femur per 100 gm. Rat. | Total Ash per Bone (gm.) | Percentage Ash of Femur. |
|---|---|---|---|---|---|
| A | 1 | 166 | 0·1028 | 0·0779 | 45·61 |
| | 3 | 100 | ·1374 | ·0544 | 39·59 |
| | 5 | 143 | ·0958 | ·0600 | 43·80 |
| | 7 | 157 | ·1067 | ·0719 | 42·89 |
| | 9 | 107 | ·1351 | ·0615 | 42·53 |
| | 11 | 114 | ·1388 | ·0705 | 44·53 |
| | 13 | 168 | ·1202 | ·0915 | 45·29 |
| | 15 | 147 | ·1138 | ·0777 | 46·41 |
| AVERAGE | — | 137·8 | 0·1188 | 0·0707 | 43·80 |
| B | 2 | 174 | 0·1293 | 0·1226 | 54·49 |
| | 4 | 111 | ·1473 | ·0779 | 47·61 |
| | 6 | 159 | ·1332 | ·1130 | 53·35 |
| | 8 | 162 | ·1313 | ·1084 | 50·94 |
| | 10 | 107 | ·1495 | ·0809 | 50·56 |
| | 12 | 122 | ·1497 | ·0960 | 52·58 |
| | 14 | 163 | ·1489 | ·1302 | 53·63 |
| | 16 | 159 | ·1440 | ·1199 | 52·36 |
| AVERAGE | — | 144·6 | 0·1417 | 0·1061 | 51·90 |

Values for percentage ash were 43·8 and 51·9 for the two groups, respectively. Both these differences were also found significant at P = ·001 with coefficients of variation of 1·5 per cent. for percentage ash and 9·3 per cent. for total ash in the femurs.

*Experiment II.*

In this trial dicalcium phosphate was compared with two levels of electrofos I. The results obtained are detailed in tables 7, 3, 8, and 9 in respect of body weights, food consumption, calcium and phosphorus retentions and femur ash, respectively.

TABLE 7.

*Weekly Weights of Rats in Experiment II (Grams).*

| Diet. | Rat No. | Initial Weight. | 1st Week. | 2nd Week. | 3rd Week. | 4th Week. | 5th Week. | 6th Week. (Final Weight). | Gain. |
|---|---|---|---|---|---|---|---|---|---|
| C | 1 | 52 | 70 | 84 | 99 | 118 | 123 | 128 | 76 |
| | 4 | 50 | 59 | 72 | 86 | 105 | 119 | 128 | 78 |
| | 7 | 52 | 57 | 77 | 92 | 115 | 129 | 150 | 98 |
| | 10 | 53 | 67 | 83 | 100 | 121 | 135 | 145 | 92 |
| | 13 | 54 | 73 | 90 | 90 | 109 | 119 | 130 | 76 |
| | 16 | 55 | 62 | 75 | 92 | 111 | 132 | 154 | 99 |
| | 19 | 61 | 74 | 94 | 99 | 115 | 131 | 147 | 86 |
| | 22 | 63 | 87 | 98 | 122 | 146 | 160 | 180 | 117 |
| AVERAGE | — | 55·0 | 68·6 | 84·1 | 97·5 | 117·5 | 131·0 | 145·3 | 90·3 |
| D | 2 | 52 | 63 | 77 | 96 | 104 | 110 | 113 | 61 |
| | 5 | 50 | 65 | 77 | 95 | 111 | 120 | 127 | 77 |
| | 8 | 52 | 61 | 75 | 89 | 110 | 124 | 150 | 98 |
| | 11 | 53 | 64 | 80 | 96 | 119 | 132 | 144 | 91 |
| | 14 | 54 | 69 | 84 | 89 | 109 | 119 | 130 | 76 |
| | 17 | 59 | 62 | 79 | 94 | 114 | 135 | 153 | 94 |
| | 20 | 60 | 70 | 90 | 95 | 114 | 130 | 146 | 86 |
| | 23 | 63 | 77 | 88 | 112 | 134 | 147 | 169 | 106 |
| AVERAGE | — | 55·4 | 66·4 | 81·3 | 95·8 | 114·4 | 127·1 | 141·5 | 86·1 |
| E | 3 | 52 | 64 | 78 | 98 | 109 | 113 | 119 | 67 |
| | 6 | 51 | 64 | 79 | 96 | 110 | 121 | 128 | 77 |
| | 9 | 52 | 66 | 77 | 91 | 110 | 123 | 141 | 89 |
| | 12 | 53 | 66 | 84 | 98 | 119 | 132 | 141 | 88 |
| | 15 | 54 | 70 | 88 | 88 | 109 | 117 | 124 | 70 |
| | 18 | 58 | 64 | 78 | 96 | 114 | 137 | 156 | 98 |
| | 21 | 62 | 72 | 81 | 99 | 114 | 129 | 140 | 78 |
| | 24 | 64 | 76 | 88 | 111 | 137 | 150 | 171 | 107 |
| AVERAGE | — | 55·8 | 67·8 | 81·6 | 97·1 | 115·3 | 127·8 | 140·0 | 84·3 |

## TABLE 8.

*Calcium and Phosphorus retained by Rats in Experiment II.*

*(Unless otherwise stated all figures are in milligram).*

| Diet. | Rat No. | Total Ca Intake. | Ca in Rat at beginning. | Ca in Rat at End. | Ca gain. | Per Cent. Ca retention. | Total P Intake. | P in Rat at Beginning. | P in Rat at End. | P gain. | Per Cent. P retention. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 1 | 828 | 391·2 | 921·5 | 530·3 | 64·0 | 491 | 287 | 664 | 377 | 76·8 |
| | 4 | 816 | 391·2 | 854·9 | 463·7 | 56·8 | 484 | 283 | 630 | 347 | 71·7 |
| | 7 | 909 | 403·3 | 972·7 | 569·4 | 62·6 | 539 | 289 | 711 | 422 | 78·4 |
| | 10 | 942 | 427·9 | 1,019·0 | 591·1 | 62·8 | 559 | 305 | 735 | 430 | 77·0 |
| | 13 | 831 | 387·1 | 941·9 | 554·8 | 66·8 | 493 | 293 | 652 | 359 | 72·8 |
| | 16 | 933 | 431·9 | 936·9 | 505·0 | 54·2 | 554 | 316 | 691 | 375 | 67·7 |
| | 19 | 903 | 464·5 | 1,054·0 | 589·5 | 65·3 | 536 | 347 | 735 | 388 | 72·4 |
| | 22 | 1,083 | 470·6 | 1,218·0 | 747·4 | 69·0 | 643 | 347 | 857 | 510 | 79·3 |
| AVERAGE | — | 905·6 | 420·9 | 989·8 | 568·9 | 62·7 | 537·4 | 308·4 | 709·4 | 401 | 74·5 |
| D | 2 | 861 | 391·2 | 706·5 | 315·3 | 36·6 | 522 | 287 | 517 | 230 | 44·1 |
| | 5 | 849 | 391·2 | 691·0 | 299·8 | 35·3 | 514 | 283 | 532 | 249 | 48·4 |
| | 8 | 945 | 403·3 | 691·0 | 287·7 | 30·4 | 573 | 289 | 574 | 285 | 49·8 |
| | 11 | 980 | 427·9 | 767·9 | 340·0 | 34·7 | 593 | 305 | 595 | 290 | 49·0 |
| | 14 | 864 | 387·1 | 742·3 | 355·2 | 41·1 | 523 | 293 | 575 | 282 | 53·9 |
| | 17 | 970 | 431·9 | 806·3 | 374·4 | 38·6 | 588 | 316 | 630 | 314 | 53·4 |
| | 20 | 939 | 464·5 | 880·4 | 415·9 | 44·3 | 569 | 347 | 652 | 305 | 53·6 |
| | 23 | 1,127 | 470·6 | 875·4 | 404·8 | 35·9 | 682 | 347 | 670 | 323 | 47·4 |
| AVERAGE | — | 941·9 | 420·9 | 770·1 | 349·1 | 37·1 | 570·5 | 308·4 | 593·1 | 284·8 | 50·0 |
| E | 3 | 1,764 | 391·2 | 990·6 | 599·4 | 34·0 | 966 | 287 | 675 | 388 | 40·2 |
| | 6 | 1,738 | 391·2 | 934·4 | 543·2 | 31·2 | 952 | 283 | 664 | 381 | 40·0 |
| | 9 | 1,936 | 403·3 | 982·8 | 579·5 | 29·9 | 1,061 | 289 | 698 | 409 | 38·6 |
| | 12 | 2,008 | 427·9 | 1141·0 | 713·1 | 35·5 | 1,099 | 305 | 785 | 480 | 48·7 |
| | 15 | 1,770 | 387·1 | 972·7 | 585·6 | 33·1 | 969 | 293 | 655 | 362 | 37·4 |
| | 18 | 1,988 | 431·9 | 1,080·0 | 648·1 | 32·6 | 1,088 | 316 | 765 | 449 | 41·3 |
| | 21 | 1,924 | 464·5 | 1,101·0 | 636·5 | 33·1 | 1,053 | 347 | 761 | 414 | 39·3 |
| | 24 | 2,308 | 470·6 | 1,239·0 | 768·4 | 33·3 | 1,264 | 347 | 867 | 520 | 41·2 |
| AVERAGE | — | 1,929·5 | 421·0 | 1,055·2 | 634·2 | 32·8 | 1,056·5 | 308·4 | 733·8 | 425·4 | 40·8 |

TABLE 9.

*Ash in Femurs of Rats in Experiment II.*

| Diet. | Rat No. | Weight of Rat (gm.). | Weight of Fat-Free dry Femur per 100 gm. Rat. | Total Ash per Bone (gm.) | Percentage Ash of Femur. |
|---|---|---|---|---|---|
| C | 1 | 128 | 0·1403 | 0·0964 | 53·7 |
| | 4 | 128 | ·1358 | ·0897 | 51·6 |
| | 7 | 150 | ·1260 | ·1000 | 52·9 |
| | 10 | 145 | ·1390 | ·1101 | 54·6 |
| | 13 | 130 | ·1425 | ·1012 | 54·7 |
| | 16 | 154 | ·1223 | ·0973 | 51·7 |
| | 19 | 147 | ·1388 | ·1127 | 55·2 |
| | 22 | 180 | ·1406 | ·1384 | 54·6 |
| AVERAGE | — | 145·3 | 0·1357 | 0·1057 | 53·6 |
| D | 2 | 113 | 0·1251 | 0·0703 | 49·8 |
| | 5 | 127 | ·1193 | ·0722 | 47·6 |
| | 8 | 150 | ·1028 | ·0702 | 45·6 |
| | 11 | 144 | ·1167 | ·0818 | 48·7 |
| | 14 | 130 | ·1196 | ·0770 | 49·5 |
| | 17 | 153 | ·1158 | ·0858 | 48·5 |
| | 20 | 146 | ·1175 | ·0883 | 51·4 |
| | 23 | 169 | ·1157 | ·0961 | 49·2 |
| AVERAGE | — | 141·5 | 0·1166 | 0·0802 | 48·8 |
| E | 3 | 119 | 0·1574 | 0·1052 | 56·2 |
| | 6 | 128 | ·1434 | ·0983 | 53·5 |
| | 9 | 141 | ·1376 | ·1073 | 55·3 |
| | 12 | 141 | ·1585 | ·1259 | 56·3 |
| | 15 | 124 | ·1501 | ·1022 | 54·9 |
| | 18 | 156 | ·1394 | ·1171 | 53·8 |
| | 21 | 140 | ·1562 | ·1208 | 55·2 |
| | 24 | 171 | ·1459 | ·1400 | 56·1 |
| AVERAGE | — | 140·0 | 0·1486 | 0·1146 | 55·2 |

Inspection of the data again leads to the conclusion that electrofos I is inferior to dicalcium phosphate as a source of calcium and phosphorus for the nutrition of the rat.

Slightly, though significantly, better growth was registered for the dicalcium phosphate group (see Table 7). Rats fed this product developed distinctly larger bones than those fed electrofos I at the same level of intake, the average values for the total ash of the femurs of the two groups being 0·1057 gram and 0·0802 gram, respectively (see Table 9). The degree of mineralization of these bones was, furthermore, higher for the animals on diet C than for those on diet D: figures for percentage ash on the dry fat-free basis were 53·6 and 48·8, respectively. These results correspond with those obtained for the retention of the relevant minerals as detailed in table 8, 62·7 per cent. of the calcium and 74·5 per cent. of the phosphorus in the dicalcium phosphate supplemented diet as against figures of only 37·1 per cent. of the calcium and 50·0 per cent. of the phosphorus in the electrofos I supplemented diet being on an average retained by the animals. The differences, in favour of the dicalcium phosphate, relating to total femur ash, percentage femur ash and retentions of calcium and phosphorus were all significant at $P = 0·001$.

With regard to the rats receiving a double dose of electrofos I (diet E) attention must be directed to the finding that whilst, as may be expected, the percentages of calcium and phosphorus retained were lower than those for the animals in the lower intake group (diet D), the higher total amount of calcium and phosphorus retained, apparently sufficed for normal bone development. Thus, the mean total weight of the femur ash of the rats on diet E was 0·1146 gram and the percentage ash of these femurs 55·1, these figures being significantly better than the corresponding values for diet D at $P = 0{\cdot}001$.

*Experiment III.*

The weekly body weights and feed consumption of the groups of rats fed diets C, D and E *ad lib.* are detailed in tables 10 and 11, respectively. Comparison of these figures with those for the rats pair-fed on the same diets (see Tables 3 and 7) reveals higher feed consumption for all the *ad lib.* groups, resulting in greater live weight increases for these animals. It is to be noted that the highest figures for feed consumption and increase in weight were recorded for the rats on diet D. This was contrary to expectations, in view of the fact that this diet seemed to be deficient in available phosphorus (c.f. tables 9 and 13), a circumstance which usually is taken to have an adverse effect on feed consumption. In this case it would

TABLE 10.

*Weekly Weights (gram) of rats in Experiment III* (Ad lib. *feeding.*)

| Diet. | Rat No. | Initial Weight. | 1st Week. | 2nd Week. | 3rd Week. | 4th Week. | 5th Week. | 6th Week. (Final Weight). | Gain. |
|---|---|---|---|---|---|---|---|---|---|
| C..... | 49 | 50 | 66 | 84 | 105 | 125 | 145 | 167 | 117 |
| | 52 | 53 | 78 | 103 | 116 | 131 | 155 | 179 | 126 |
| | 55 | 56 | 79 | 102 | 122 | 137 | 154 | 170 | 114 |
| | 58 | 59 | 80 | 100 | 117 | 138 | 149 | 163 | 104 |
| | 61 | 43 | 59 | 73 | 91 | 100 | 112 | 120 | 77 |
| | 64 | 59 | 71 | 96 | 117 | 124 | 151 | 167 | 108 |
| AVERAGE | — | 53·3 | 72·2 | 93·0 | 111·3 | 125·8 | 144·3 | 161·0 | 107·7 |
| D..... | 50 | 48 | 68 | 91 | 101 | 130 | 157 | 184 | 136 |
| | 53 | 54 | 75 | 95 | 116 | 138 | 155 | 178 | 124 |
| | 56 | 58 | 79 | 97 | 117 | 140 | 154 | 175 | 117 |
| | 59 | 58 | 82 | 101 | 111 | 130 | 141 | 156 | 98 |
| | 62 | 43 | 55 | 79 | 94 | 112 | 128 | 138 | 95 |
| | 65 | 61 | 75 | 94 | 106 | 137 | 159 | 174 | 113 |
| AVERAGE | — | 53·7 | 72·3 | 92·8 | 107·5 | 131·2 | 149·0 | 167·5 | 113·8 |
| E..... | 51 | 51 | 68 | 87 | 101 | 119 | 139 | 150 | 99 |
| | 54 | 53 | 70 | 84 | 102 | 123 | 149 | 173 | 120 |
| | 57 | 59 | 79 | 96 | 115 | 132 | 149 | 162 | 103 |
| | 60 | 60 | 78 | 97 | 121 | 143 | 165 | 182 | 122 |
| | 63 | 47 | 60 | 81 | 99 | 114 | 129 | 150 | 103 |
| | 66 | 62 | 78 | 99 | 121 | 140 | 156 | 169 | 107 |
| AVERAGE | — | 55·3 | 72·2 | 90·7 | 109·8 | 128·5 | 147·8 | 164·3 | 109·0 |

almost appear that the rats were trying to compensate for the low availability of the phosphorus with a high feed intake. It should be added here that if the 6-weekly gains in weight (see Table 10) are adjusted for the feed intake, by the application of the analysis of co-variance, they become 114·8 gm. for diet C, 108·0 gm. for diet D and 107·6 gm. for diet E, a sequence of weight increase superiority similar to that recorded for the same diets in experiment II. The difference in favour of diet C was, however, found to be insignificant.

The percentages of calcium and phosphorus retained by the rats are given by Table 12 while data relating to the total and percentage ash of the femurs occur in Table 13. The outstanding feature in these results is their essential similarity to those obtained with the same diets using the paired feeding technique: low retention of calcium and phosphorus in electrofos I resulting in poor bone formation in the case of the lower level of supplementation (diet D) but quite satisfactory in this respect at the higher level of phosphorus intake (diet E). Mean calcium retentions were 62·4, 33·5 and 36·0 and phosphorus retentions 70·2, 45·7 and 41·8 for diets C, D and E, respectively, the differences in favour of the dicalcium phosphate supplemented diet being highly signiflcant at P = 0·001. In the same order average figures for total femur ash were 0·1141 gm., 0·0825 gm. and 0·1409 gm. and those for percentage ash in these bones 53·8, 48·6 and 57·1, the odds that the differences in these results were due to chance being 1 in 1000.

TABLE 11.

*Weekly Feed Consumption (grams) of Rats in Experiment III* (Ad lib. *feeding*).

| Diet. | Rat No. | 1st Week. | 2nd Week. | 3rd Week. | 4th Week. | 5th Week. | 6th Week. | Total. |
|---|---|---|---|---|---|---|---|---|
| C | 49 | 39 | 51 | 51 | 59 | 58 | 67 | 325 |
| | 52 | 48 | 65 | 51 | 57 | 71 | 74 | 366 |
| | 55 | 51 | 65 | 65 | 56 | 63 | 64 | 364 |
| | 58 | 50 | 60 | 60 | 65 | 57 | 61 | 353 |
| | 61 | 43 | 50 | 53 | 46 | 43 | 48 | 283 |
| | 64 | 39 | 63 | 62 | 41 | 70 | 70 | 345 |
| AVERAGE | — | 45 | 59 | 57 | 54 | 60·3 | 64 | 339·3 |
| D | 50 | 44 | 58 | 58 | 68 | 83 | 90 | 401 |
| | 53 | 49 | 61 | 62 | 68 | 70 | 75 | 385 |
| | 56 | 51 | 64 | 64 | 66 | 70 | 68 | 383 |
| | 59 | 52 | 60 | 52 | 61 | 62 | 65 | 352 |
| | 62 | 39 | 56 | 52 | 54 | 60 | 60 | 321 |
| | 65 | 47 | 59 | 48 | 67 | 80 | 68 | 369 |
| AVERAGE | — | 47 | 59·7 | 56 | 64 | 70·8 | 71 | 368·5 |
| E | 51 | 37 | 51 | 52 | 58 | 64 | 64 | 326 |
| | 54 | 50 | 49 | 59 | 61 | 72 | 79 | 370 |
| | 57 | 50 | 58 | 55 | 61 | 61 | 64 | 349 |
| | 60 | 49 | 57 | 60 | 63 | 75 | 78 | 382 |
| | 63 | 44 | 58 | 56 | 52 | 57 | 65 | 332 |
| | 66 | 50 | 61 | 67 | 69 | 72 | 73 | 392 |
| AVERAGE | — | 46·7 | 55·7 | 58·2 | 60·7 | 66·8 | 70·5 | 358·5 |

TABLE 12.

*Calcium and Phosphorus Retained by Rats in Experiment III* (ad lib. *feeding*).

(*Unless otherwise stated all figures are in milligram*).

| Diet. | Rat No. | Total Ca Intake. | Ca in Rat at beginning. | Ca in Rat at End. | Ca gain. | Per Cent. Ca retention. | Total P Intake. | P in Rat at Beginning. | P in Rat at End. | P gain. | Per Cent. P retention. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 49 | 975 | 387·1 | 1,029·0 | 641·9 | 65·8 | 578 | 293 | 735 | 442 | 76·5 |
| | 52 | 1,098 | 431·9 | 1,177·0 | 745·1 | 67·8 | 651 | 316 | 802 | 486 | 74·7 |
| | 55 | 1,092 | 464·5 | 1,098·0 | 633·5 | 58·0 | 648 | 347 | 769 | 422 | 65·1 |
| | 58 | 1,059 | 470·6 | 1,195·0 | 724·4 | 68·4 | 628 | 347 | 802 | 455 | 72·5 |
| | 61 | 849 | 346·3 | 793·4 | 447·1 | 52·7 | 504 | 259 | 562 | 303 | 60·1 |
| | 64 | 1,035 | 429·8 | 1,065·0 | 635·2 | 61·4 | 614 | 323 | 769 | 446 | 72·7 |
| AVERAGE | ..... | 1,018 | 421·7 | 1,059·6 | 637·9 | 62·4 | 603·8 | 314·2 | 739·8 | 425·7 | 70·2 |
| D | 50 | 1,252 | 387·1 | 813·9 | 426·8 | 34·1 | 758 | 293 | 646 | 353 | 46·6 |
| | 53 | 1,202 | 431·9 | 834·5 | 402·6 | 33·5 | 727 | 316 | 667 | 351 | 48·3 |
| | 56 | 1,195 | 464·5 | 839·5 | 375·0 | 31·4 | 724 | 347 | 652 | 305 | 42·1 |
| | 59 | 1,098 | 470·6 | 857·4 | 386·8 | 35·2 | 665 | 347 | 635 | 288 | 43·3 |
| | 62 | 1,002 | 346·3 | 652·7 | 306·4 | 30·6 | 606 | 259 | 530 | 271 | 44·7 |
| | 65 | 1,152 | 429·8 | 829·2 | 399·4 | 34·7 | 697 | 323 | 667 | 344 | 49·4 |
| AVERAGE | ..... | 1,150·2 | 421·7 | 804·5 | 382·8 | 33·5 | 696·2 | 314·2 | 632·8 | 318·7 | 45·7 |
| E | 51 | 2,084 | 387·1 | 1,162·0 | 774·9 | 37·2 | 1,142 | 293 | 789 | 496 | 43·4 |
| | 54 | 2,365 | 431·9 | 1,200·0 | 768·1 | 32·5 | 1,295 | 316 | 820 | 504 | 38·9 |
| | 57 | 2,231 | 464·5 | 1,285·0 | 820·5 | 36·8 | 1,222 | 347 | 833 | 486 | 39·8 |
| | 60 | 2,442 | 470·6 | 1,421·0 | 950·4 | 38·9 | 1,337 | 347 | 932 | 585 | 43·8 |
| | 63 | 2,122 | 346·3 | 1,140·0 | 793·7 | 37·4 | 1,163 | 259 | 798 | 539 | 46·3 |
| | 66 | 2,506 | 429·8 | 1,267·0 | 837·2 | 33·4 | 1,373 | 323 | 852 | 529 | 38·6 |
| AVERAGE | ..... | 2,291·7 | 421·7 | 1,245·8 | 824·1 | 36·0 | 1,255·3 | 314·2 | 837·3 | 523·2 | 41·8 |

TABLE 13.

*Ash in Femurs of Rats in Experiment III.*

| Diet. | Rat No. | Weight of Rat (gm.). | Weight of Fat-Free dry Femur per 100 gm. Rat. | Total Ash per Bone (gm.) | Percentage Ash of Femur. |
|---|---|---|---|---|---|
| C | 49 | 167 | 0·1250 | 0·1108 | 53·1 |
| | 52 | 179 | ·1275 | ·1293 | 56·6 |
| | 55 | 170 | ·1289 | ·1182 | 53·9 |
| | 58 | 163 | ·1476 | ·1324 | 55·0 |
| | 61 | 120 | ·1347 | ·0817 | 50·5 |
| | 64 | 167 | ·1246 | ·1123 | 53·9 |
| AVERAGE | — | 161·0 | 0·1314 | 0·1141 | 53·8 |
| D | 50 | 184 | 0·0959 | 0·0840 | 47·6 |
| | 53 | 178 | ·1041 | ·0863 | 46·5 |
| | 56 | 175 | ·1047 | ·0864 | 47·2 |
| | 59 | 156 | ·1167 | ·0896 | 49·2 |
| | 62 | 138 | ·1008 | ·0620 | 44·6 |
| | 65 | 174 | ·1070 | ·0864 | 46·4 |
| AVERAGE | — | 167·5 | 0·1049 | 0·0825 | 48·6 |
| E | 51 | 150 | 0·1515 | 0·1296 | 57·0 |
| | 54 | 173 | ·1353 | ·1319 | 56·3 |
| | 57 | 162 | ·1605 | ·1495 | 57·5 |
| | 60 | 182 | ·1815 | ·1634 | 57·6 |
| | 63 | 150 | ·1516 | ·1268 | 55·8 |
| | 66 | 169 | ·1464 | ·1440 | 58·2 |
| AVERAGE | — | 164.3 | 0·1545 | 0·1409 | 57·1 |

To simplify comparison the average values obtained for the three diets by the pair-fed and *ad lib* methods are summarized in table 14.

TABLE 14.

*Summary of Results of Experiments II and III.*

| Diet. | Total Feed consumed (gm.) | | Total Femur Ash (gm.) | | Per Cent. Ash in Femur. | | Per Cent. Ca Retained. | | Per Cent. P Retained. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pair Fed. | *Ad lib.* | Pair Fed. | *Ad lib.* | Pair Fed. | *Ad lib.* | Pair Fed. | *Ad lib.* | Pair Fed. | *Ad lib.* |
| C... | 292·0 | 339·3 | 0·1057 | 0·1141 | 53·6 | 53·8 | 62·7 | 62·4 | 74·5 | 70·2 |
| D... | 292·0 | 368·5 | 0·0802 | 0·0825 | 48·8 | 48·6 | 37·1 | 33·5 | 50·0 | 45·7 |
| E... | 292·0 | 358·5 | 0·1146 | 0·1409 | 55·2 | 57·1 | 32·8 | 36·0 | 40·8 | 41·8 |

* Average of only first 6 rats of a group.

*Experiment IV.*

Data obtained in this final test on the relative values of electrofos II, at two levels of intake, and dicalcium phosphate as sources of calcium and phosphorus for growth and bone development in the rat are presented in Tables 15, 16 and 17.

In spite of considerable differences in composition (c.f. Table 1) electrofos I and electrofos II seem to suffer from the same defect: low availability of their calcium and phosphorus. That this is so becomes evident from a study of the results obtained with the two products.

In conformity with those of previous experiments the data on gain in body weight (Table 15) are not particularly illuminating, even though it would appear that the rats on diet C grew on an average better than those on the electrofos II supplemented diets. However, it is when the figures on calcium and phosphorus retentions (table 16) and femur ash (table 17) are considered, that more convincing evidence for the inferiority of electrofos II is revealed. At the same level of calcium and phosphorus intake the rats retained on an average only 25·8 per cent. of the calcium and 35·7 per cent. of the phosphorus in the electrofos supplemented diet F as against percentage retentions of 66·0 and 70·5 for the two respective minerals in diet C fortified with dicalcium phosphate. These results were reflected in the data on bone development described in table 17: average values for total femur ash were 0·1064 gm. and 0·0677 gm. and for percentage ash in the femur 55·4 and 46·3 for diets C and F, respectively. All differences in favour of the first-mentioned diet were found significant at the 0·1 per cent. level.

TABLE 15.

*Weekly Weights of Rats in Experiment IV (grams).*

| Diet. | Rat No. | Initial Weight. | 1st Week. | 2nd Week. | 3rd Week. | 4th Week. | 5th Week. | 6th Week. (Final Weight). | Gain. |
|---|---|---|---|---|---|---|---|---|---|
| C | 1 | 52 | 56 | 72 | 87 | 95 | 104 | 116 | 64 |
| | 4 | 56 | 64 | 75 | 85 | 90 | 102 | 114 | 58 |
| | 7 | 67 | 70 | 82 | 97 | 106 | 125 | 139 | 72 |
| | 10 | 77 | 86 | 99 | 120 | 134 | 148 | 154 | 77 |
| | 13 | 52 | 64 | 82 | 100 | 116 | 131 | 147 | 95 |
| | 16 | 54 | 62 | 80 | 84 | 91 | 95 | 107 | 53 |
| | 19 | 59 | 67 | 83 | 94 | 112 | 128 | 146 | 87 |
| | 22 | 64 | 77 | 89 | 97 | 110 | 111 | 116 | 52 |
| AVERAGE | — | 60·1 | 68·3 | 82·8 | 95·5 | 106·8 | 118·0 | 129·9 | 69·8 |
| F | 2 | 52 | 61 | 75 | 87 | 96 | 101 | 116 | 64 |
| | 5 | 57 | 64 | 74 | 82 | 90 | 102 | 108 | 51 |
| | 8 | 67 | 76 | 85 | 98 | 106 | 119 | 129 | 62 |
| | 11 | 77 | 90 | 99 | 115 | 129 | 136 | 143 | 66 |
| | 14 | 51 | 61 | 75 | 91 | 103 | 116 | 127 | 76 |
| | 17 | 53 | 63 | 77 | 81 | 88 | 92 | 104 | 51 |
| | 20 | 59 | 71 | 82 | 92 | 109 | 126 | 144 | 85 |
| | 23 | 64 | 79 | 90 | 97 | 107 | 105 | 109 | 45 |
| AVERAGE | — | 60·0 | 70·6 | 82·1 | 92·9 | 103·5 | 112·1 | 122·5 | 62·5 |
| G | 3 | 52 | 60 | 72 | 83 | 89 | 94 | 108 | 56 |
| | 6 | 57 | 64 | 74 | 86 | 95 | 108 | 117 | 60 |
| | 9 | 67 | 78 | 88 | 99 | 112 | 126 | 143 | 76 |
| | 12 | 75 | 89 | 99 | 116 | 126 | 130 | 130 | 55 |
| | 15 | 52 | 61 | 71 | 81 | 92 | 106 | 118 | 66 |
| | 18 | 56 | 64 | 72 | 79 | 83 | 84 | 88 | 32 |
| | 21 | 59 | 68 | 78 | 86 | 102 | 117 | 133 | 74 |
| | 24 | 64 | 76 | 83 | 89 | 95 | 96 | 100 | 36 |
| AVERAGE | — | 60·3 | 70·0 | 79·6 | 89·9 | 99·3 | 107·6 | 117·1 | 56·9 |

TABLE 16.

*Calcium and Phosphorus Retained by Rats in Experiment IV (Unless otherwise stated all figures are in milligram).*

| Diet. | Rat No. | Total Ca Intake. | Ca in Rat at beginning. | Ca in Rat at End. | Ca gain. | Per Cent. Ca retention. | Total P Intake. | P in Rat at Beginning. | P in Rat at End. | P gain. | Per Cent. P retention. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C. | 1 | 717 | 415·7 | 895·8 | 480·1 | 67·0 | 425 | 308 | 614 | 306 | 72·0 |
| | 4 | 729 | 431·9 | 962·2 | 530·3 | 72·7 | 433 | 324 | 636 | 312 | 72·1 |
| | 7 | 840 | 484·9 | 1,023·0 | 538·1 | 64·1 | 498 | 366 | 724 | 358 | 71·9 |
| | 10 | 909 | 601·0 | 1,200·0 | 599·0 | 65·9 | 539 | 435 | 811 | 376 | 69·7 |
| | 13 | 903 | 417·7 | 998·2 | 580·5 | 64·3 | 536 | 314 | 711 | 397 | 74·0 |
| | 16 | 678 | 433·9 | 883·1 | 449·2 | 66·3 | 402 | 306 | 586 | 280 | 69·6 |
| | 19 | 858 | 448·2 | 993·1 | 544·9 | 63·5 | 509 | 337 | 691 | 354 | 69·5 |
| | 22 | 699 | 556·2 | 1,003·0 | 446·8 | 63·9 | 415 | 392 | 664 | 272 | 65·5 |
| AVERAGE..... | — | 791·6 | 473·7 | 994·8 | 521·1 | 66·0 | 469·6 | 347·8 | 679·6 | 331·9 | 70·5 |
| F. | 2 | 729 | 415·7 | 578·5 | 162·8 | 22·3 | 447 | 308 | 467 | 159 | 35·6 |
| | 5 | 741 | 431·9 | 652·7 | 220·8 | 29·8 | 455 | 324 | 481 | 157 | 34·5 |
| | 8 | 854 | 484·9 | 796·0 | 311·1 | 36·4 | 524 | 366 | 575 | 209 | 39·9 |
| | 11 | 924 | 601·0 | 839·5 | 238·5 | 25·8 | 567 | 435 | 627 | 192 | 33·9 |
| | 14 | 918 | 417·7 | 668·7 | 251·0 | 27·3 | 563 | 314 | 642 | 328 | 35·7 |
| | 17 | 689 | 433·9 | 632·3 | 198·4 | 28·8 | 423 | 306 | 466 | 160 | 37·8 |
| | 20 | 872 | 448·2 | 652·7 | 204·5 | 23·5 | 535 | 337 | 530 | 193 | 36·1 |
| | 23 | 711 | 556·2 | 729·5 | 173·3 | 24·4 | 436 | 392 | 532 | 140 | 32·1 |
| AVERAGE..... | — | 804·8 | 473·7 | 693·7 | 220·1 | 25·8 | 493·8 | 347·8 | 540 | 192·3 | 35·7 |
| G. | 3 | 1,485 | 415·7 | 673·2 | 257·5 | 17·3 | 851 | 308 | 489 | 181 | 21·3 |
| | 6 | 1,516 | 431·9 | 788·3 | 356·4 | 23·5 | 865 | 324 | 562 | 238 | 27·5 |
| | 9 | 1,740 | 484·9 | 957·2 | 472·3 | 27·1 | 997 | 366 | 685 | 319 | 32·0 |
| | 12 | 1,883 | 601·0 | 970·3 | 369·3 | 19·6 | 1,078 | 435 | 663 | 228 | 21·2 |
| | 15 | 1,870 | 417·7 | 765·4 | 347·7 | 18·6 | 1,072 | 314 | 536 | 222 | 20·7 |
| | 18 | 1,404 | 433·9 | 696·1 | 262·2 | 18·7 | 805 | 306 | 454 | 148 | 18·4 |
| | 21 | 1,777 | 448·2 | 844·7 | 396·5 | 22·3 | 1,018 | 337 | 600 | 263 | 25·8 |
| | 24 | 1,448 | 556·2 | 849·8 | 293·6 | 20·3 | 830 | 392 | 566 | 174 | 21·0 |
| AVERAGE..... | — | 1,640·4 | 473·7 | 818·1 | 344·4 | 20·9 | 939·5 | 347·8 | 569·4 | 221·6 | 23·5 |

TABLE 17.

*Ash in Femurs of Rats in Experiment IV.*

| Diet. | Rat No. | Weight of Rat (gm.). | Weight of Fat-Free dry Femur per 100 gm. Rat. | Total Ash per Bone (gm.) | Percentage Ash of Femur. |
|---|---|---|---|---|---|
| C | 1 | 116 | 0·1533 | 0·0974 | 54·8 |
| | 4 | 114 | ·1507 | ·0973 | 56·6 |
| | 7 | 139 | ·1412 | ·1084 | 55·2 |
| | 10 | 154 | ·1521 | ·1327 | 56·6 |
| | 15 | 147 | ·1321 | ·1080 | 55·7 |
| | 16 | 107 | ·1598 | ·0956 | 55·9 |
| | 19 | 146 | ·1372 | ·1070 | 53·4 |
| | 22 | 116 | ·1637 | ·1046 | 55·0 |
| AVERAGE | — | 129·9 | 0·1488 | 0·1064 | 55·4 |
| F | 2 | 116 | 0·1198 | 0·0587 | 42·2 |
| | 5 | 108 | ·1242 | ·0624 | 46·5 |
| | 8 | 129 | ·1206 | ·0809 | 52·0 |
| | 11 | 143 | ·1282 | ·0860 | 46·9 |
| | 14 | 127 | ·0974 | ·0536 | 43·8 |
| | 17 | 104 | ·1253 | ·0615 | 47·2 |
| | 20 | 144 | ·1056 | ·0650 | 42·7 |
| | 23 | 109 | ·1385 | ·0738 | 48·9 |
| AVERAGE | — | 122·5 | 0·1199 | 0·0677 | 46·3 |
| G | 3 | 108 | 0·1289 | 0·0636 | 45·7 |
| | 6 | 117 | ·1469 | ·0818 | 49·9 |
| | 9 | 143 | ·1315 | ·0974 | 51·8 |
| | 12 | 130 | ·1443 | ·0980 | 52·2 |
| | 15 | 118 | ·1329 | ·0770 | 49·1 |
| | 18 | 88 | ·1481 | ·0622 | 47·8 |
| | 21 | 133 | ·1240 | ·0843 | 51·0 |
| | 24 | 100 | ·1586 | ·0825 | 52·0 |
| AVERAGE | — | 117·1 | 0·1394 | 0·0808 | 49·9 |

Although it somewhat depressed the percentage retention of the relevant minerals increasing the level of electrofos II intake (diet G) resulted in an improvement in bone development. Thus, femur ash rose from 0·0677 gm. to 0·0808 gm. and the percentage of this constituent in the dry fat-free bone from 46·3 to 49·9, the improvements being significant at the 1 per cent. level in the latter case and at the 0·1 per cent. level in the former case.

The mean results of all the tests are summarized in table 18. Compared with dicalcium phosphate there seems to be no doubt that the calcium and phosphorus of the electrofos products are poorly utilized. From what is known about the method of their production these products may be expected to contain large amounts of calcium metaphosphate, a circumstance which may account for their observed low availability for bone formation in the rat. Whether this low availability will also apply to our large ruminant animals will be decided by experiments now under way with sheep.

In conclusion attention may be directed to the finding that bone formation at the higher level of electrofos supplementation (diets E and G) was apparently much the same as that obtained with dicalcium phosphate at the lower level (diet C). Consequently, should it be found feasible to produce electrofos at a cost only half or less of that of dicalcium phosphate it could be expected to compete successfully with the latter product as a source of calcium and phosphorus for bone formation.

TABLE 18.

*Summary of Mean Values, Experiments I—IV.*

| Experiment No. | Level of P in Diet. Per Cent. | Supplement. | Initial Weight (gm.). | In 6 Weeks. | | Per Cent. Ca Retained. | Per Cent. P Retained. | Total Femur Ash (gm.). | Per Cent. Ash in Femur. |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Gain in Weight (gm.). | Feed Consumed (gm.). | | | | |
| I.... | 0·15 | Electrofos I | 66·3 | 71·5 | 290 | 23·2 | 48·9 | 0·0707 | 43·8 |
| II.... | 0·18 | Electrofos I | 55·4 | 86·1 | 302 | 37·1 | 50·0 | 0·0802 | 48·8 |
| III.... | 0·19 | Electrofos I | 53·7 | 113·8 | 368 | 33·5 | 45·7 | 0·0825 | 48·6 |
| IV.... | 0·19 | Electrofos II | 60·0 | 62·5 | 264 | 25·8 | 35·7 | 0·0677 | 46·3 |
| I.... | 0·16 | $CaHPO_4$ | 66·3 | 78·4 | 290 | 64·5 | 76·8 | 0·1061 | 51·9 |
| II.... | 0·18 | $CaHPO_4$ | 55·0 | 90·3 | 302 | 62·7 | 74·5 | 0·1057 | 53·6 |
| III.... | 0·18 | $CaHPO_4$ | 53·3 | 107·7 | 339 | 62·4 | 70·2 | 0·1141 | 53·8 |
| IV.... | 0·18 | $CaHPO_4$ | 60·1 | 69·8 | 264 | 66·0 | 70·5 | 0·1064 | 55·4 |
| II.... | 0·36 | Electrofos I | 55·8 | 84·3 | 302 | 32·8 | 40·8 | 0·1146 | 55·2 |
| III.... | 0·36 | Electrofos I | 55·3 | 190·0 | 358 | 36·0 | 41·8 | 0·1409 | 57·1 |
| IV.... | 0·35 | Electrofos II | 60·3 | 56·9 | 264 | 20·9 | 23·5 | 0·0808 | 49·9 |


## SUMMARY.

A basal diet, containing 0·06 per cent. calcium and 0·05 per cent. phosphorus, and albino rats, 4 to 5 weeks old and weighing approximately 60 grams, were used to ascertain the availability of electrofos I and electrofos II in comparison with dicalcium phosphate for bone formation. These phosphatic products, prepared by a thermal process, were studied at two levels giving diets containing 0·18 per cent. phosphorus and 0·36 per cent. phosphorus, the Ca : P ratio being maintained at about 2 : 1. Apart from live weight gains percentage retention of the relevant minerals as determined by slaughter tests and femur ash were used as criteria for evaluating the products.

The averages of the results obtained are summarized in Table 18.

It was found that both electrofos I and electrofos II were significantly less available for bone formation than calcium phosphate. However, more or less equal bone formation was obtained when these products were supplemented at about twice the level of calcium phosphate.



## ACKNOWLEDGMENT.

The authors wish to express their indebtedness to Dr. G. B. Laurence for carrying out the statistical analysis of the results.

## REFERENCES.

BARRENTINE B. F., MAYNARD, L. A. AND LOOSLI, J. K. (1944). The availability of the calcium and phosphorus of defluorinated rock phosphate for the rat. *J. Nutr.* Vol. 27, pp. 35-42.

BIRD, H. R., TITUS, H. W. AND CLARK, T. B. (1945). Nutritive evaluation of defluorinated phosphates and other phosphorus supplements. II. Defluorinated phosphates as phosphorus supplements for chicks. *J.A.O.A.C.* Vol. 28, pp. 118–129.

DAY, H. G. AND McCOLLUM, E. V. (1939). Mineral metabolism, growth and symptomatology of rats on a diet extremely deficient in phosphorus. *J.B.C.* Vol. 130, p. 269.

ELLIS, N. R., CABELL, C. A., ELMSLIE, W. P., FRAPS, G. S., PHILLIPS, P. H. AND WILLIAMS, D. E. (1945). Nutritive evaluation of defluorinated phosphates and other phosphorus supplements. III Utilization experiments with rats. *J.A.O.A.C.* Vol. 28, pp. 129–142.

FISKE, C. H. AND SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *J.B.C.* Vol. 66, p. 375.

FRASER, H. F., HOPPE, T. C., SULLIVAN, J. H. AND SMITH. E. R. (1943). Phosphate fertilizers as possible source of phosphorus for animals. *Ind. Eng. Chem.* Vol. 35, pp.1087–90.

HILL, W. L., REYNOLDS, D. S., HENDRICKS, S. B. AND JACOB, K. D. (1945). Nutritive evaluation of defluorinated phosphates and other phosphorus supplements. I. Preparation and properties of the samples. *J.A.O.A.C.* Vol. 28, pp. 105–118.

McCRUDDEN, F. H. (1911). The determination of calcium in the presence of magnesium and phosphates. *J.B.C.* Vol. 10, p. 187.