The Retention of Calcium and Phosphorus by the Rat from Wholemeal Bread, with and without added Calcium, and from White Bread fortified with Calcium and Vitamin B₁

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McCance & Widdowson (1942a) have shown in extensive experiments that human adults retained Ca more satisfactorily from diets in which 40–50% of the calories were derived from white bread made from 69% extraction flour than from diets similar but containing bread made from 92% extraction flour. The difference was attributed to the much higher phytic acid content of the brown bread. McCance & Widdowson showed that, in order to maintain the subjects in positive Ca balance, it was necessary to add 65 mg. Ca/100 g. white flour, and 200 mg. Ca/100 g. flour of 92% extraction. The carbonate or the mono-hydrogen phosphate were equally effective as sources of Ca.

Towards the end of 1940, when these experiments had been completed but were not yet published, we had the opportunity of discussing their results with the authors. It was felt that somewhat similar experiments with young rats would be of additional interest. It was realized that the bearing of such experiments with growing rats on problems of human adult nutrition would have to be cautiously interpreted. The rat is not only specially well equipped to deal with phytate P, probably because of the possession of an intestinal phytase (Lowe & Steenbock, 1936; Patwardhan, 1937; Laskowski, 1937; Krieger, Bunkfeldt & Steenbock, 1940a; Giri, 1940), but its Ca and P metabolism differs from that of man in several respects, of which the refractoriness of the rat to rickets when the Ca and P intakes are normal may be quoted as an example.

EXPERIMENTAL

Plan of the experiments. The experiments were designed to compare bread made from the lowest extraction of flour available, 72%, with 100% wholemeal bread when both formed the almost exclusive diet of the experimental animals. At the time when these experiments were being planned the Ministry of Food were considering the production of white bread of 72% extraction fortified with the addition of ½ lb. of CaCO₃ and of 1 oz. of vitamin B₁ concentrate (equivalent to approximately 0.2 g. aneurin)/280 lb. sack (Moran & Drummond, 1940). The possibility of using purified bone meal as a source of additional Ca was also contemplated.

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Two sets of experiments were therefore planned. In one the Ca content of wholemeal bread was taken as a basis and white bread was suitably supplemented with CaCO₃ or bone meal so that all three breads contained the same amount of Ca. In the second the comparison was with the fortified white bread of Moran & Drummond (1940). Here CaCO₃ or bone meal were added to wholemeal bread again to equalize the Ca content.

A third experiment was done to measure the availability of Ca from the two Ca supplements, Creta praeparata and bone meal. For comparison dried skim milk was also studied.

Preparation of breads. The breads were baked at the Laboratories of the Research Association of British Flour Millers (now Cereals Research Station of the Ministry of Food). Flour of 72% extraction and the wheat mixture from which it was milled were obtained from one of the members of the Association. The wheat was then ground to wholemeal and the two flours were analyzed for Ca. For baking, flour mixtures were made by addition of the requisite quantities of vitamin B₁, CaCO₃ or bone meal. The CaCO₃ was in the form of Creta praeparata, and the bone meal was a proprietary purified brand. After baking, the breads were sliced, crumbled and allowed to dry overnight at room temperature; they were then ground in a hammer mill.

The composition of the flour mixtures and analysis of the breads are given in Table 1.

Feeding tests. It was not possible for technical reasons to study the seven breads simultaneously. Two experiments were carried out; in the first, breads 1–5 inclusive were fed, while in the second, breads 4–7 were used. In this way breads 4 and 5 acted as a link between the two experiments. Full details of most of the experimental technique and methods of analysis will be found elsewhere (Henry & Kon, 1937). The phytate P content of the breads was determined by the method of McCance & Widdowson (1933).

Exp. 1. The retention of Ca and P from breads 1–5. Six litters of five male rats 23–25 days old were allocated by lot to the five breads. The intakes of breads having the same Ca content, i.e. 1, 2 and 3, and 4 and 5, were equalized, as far as possible, between litter-mates. The breads were fed mixed with 24 times their weight of distilled water to minimize scattering. Where an animal consistently refused to eat or scattered its food badly, equalization was discontinued. Each animal received daily, separately from the bread, the following additions: 10μg. vitamin B₁, 40μg. riboflavin, 100μg. calcium pantothenate and 4 i.u. carotene. Vitamin D was not given. The animals were given distilled
Kon & phytic acid underwent hydrolysis. Widdowson (1941) noticed that, in the baking of bread from flour of 99% extraction, 31% of the phytic acid was hydrolyzed.

water to drink. Excreta were discarded for the first 3 days and were subsequently collected for a period of 5 weeks. The animals were then killed, the femora and humeri removed and their ash content determined as described by Kon & Henry (1935).

Exp. 2. The retention of Ca and P from breads 4–7. Six litters of four male rats 23–25 days old were used; the experimental procedure was exactly the same as in Exp. 1. The intakes of the two white breads 4 and 5 and of the two wholemeal breads 6 and 7 were equalized between pairs of litter-mates.

Exp. 3. The retention of Ca and P from dried skim milk, Creta praeparata and bone meal. The diets used in this experiment were similar to diets used previously in this laboratory for comparisons between the availability of Ca from milk and from an inorganic source (Henry & Kon, 1939a). They were planned to supply suboptimal amounts of Ca but adequate P. The composition and analysis of the three diets used are given in Table 2. Six litters of three male rats 27–30 days old were used. The experimental procedure was essentially the same as in Exps. 1 and 2, but each rat received daily separately from the diet 10 μg. vitamin B₂, 40 μg. riboflavin, 10 μg. vitamin B₆, 300 μg. calcium pantothenate, 3 mg. choline chloride, 1 mg. inositol, 1 mg. nicotinic acid, 3 mg. p-aminobenzoic acid and 4 i.u. carotene. No vitamin D was given. The diet intake was equalized between litter-mates.

RESULTS

The results of the three experiments are shown in Tables 3 and 4. The equalization of intakes proved difficult in the comparison of breads 1, 2 and 3, as the wholemeal bread was consumed much more readily than the white which the rats persistently scattered, and as a result slightly more of it was eaten. The Ca intake from this source was therefore some 10% more than from the white breads. The faecal loss was, however, also definitely higher, and, though the absolute retentions were the same, the percentage retention was significantly lower for the wholemeal bread. With the white breads the Ca was equally available from the carbonate and from the phosphate. The retention of Ca from breads 1 and 2 was lower than that observed in a previous experiment (Henry, Houston, Kon, Powell, Carter & Halton, 1941) with breads containing very much the same percentage of Ca derived from skim milk. In that experiment the Ca of added milk was available to the extent of 95% and the over-all retention from the 2% milk bread was almost 80%, that is as much as could be expected allowing for unavoidable losses of Ca in urine and faeces. The lower figure of 70% now observed is due to decidedly higher faecal losses, some 0.7 mg. daily as against 0.4 mg. for milk bread or unsupplemented white bread found by Henry et al. (1941). In the present experiment unsupplemented bread was not examined, and it is impossible to say whether the relatively low availability of Ca from breads 1 and 2 is due to a peculiarity of the experimental animals or to a lowering

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**Table 1. The composition of the flour mixtures and analysis of the experimental breads**

<table>
<thead>
<tr>
<th>Bread no.</th>
<th>Composition of flour mixture from which bread was made</th>
<th>Analysis of breads (% of air-dried matter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 9 lb. white flour + 1.965 g. Creta praeparata + 0.85 g. vitamin B₆ concentrate</td>
<td>Ca</td>
<td>Total P</td>
</tr>
<tr>
<td>2 9 lb. white flour + 2.36 g. bone meal + 0.85 g. vitamin B₆ concentrate</td>
<td>0.0470</td>
<td>0.1115</td>
</tr>
<tr>
<td>3 Wholemeal flour, no addition</td>
<td>0.0468</td>
<td>0.1168</td>
</tr>
<tr>
<td>4 9 lb. white flour + 7.29 g. Creta praeparata + 0.85 g. vitamin B₆ concentrate</td>
<td>0.0484</td>
<td>0.3465</td>
</tr>
<tr>
<td>5 9 lb. white flour + 8.75 g. bone meal + 0.85 g. vitamin B₆ concentrate</td>
<td>0.0504</td>
<td>0.1174</td>
</tr>
<tr>
<td>6 9 lb. wholemeal flour + 5.31 g. Creta praeparata</td>
<td>0.0615</td>
<td>0.1461</td>
</tr>
<tr>
<td>7 9 lb. wholemeal flour + 6.37 g. bone meal</td>
<td>0.0683</td>
<td>0.3523</td>
</tr>
<tr>
<td></td>
<td>0.0711</td>
<td>0.3656</td>
</tr>
</tbody>
</table>

* As the wholemeal flour contained 0.2747% phytic acid P on the dry basis it can be calculated that 29% of the phytic acid underwent hydrolysis during baking.

**Table 2. Composition and analysis of diets used in Exp. 3**

<table>
<thead>
<tr>
<th>Component</th>
<th>Creta praeparata</th>
<th>Bone meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk diet</td>
<td>11.63</td>
<td>15.92</td>
</tr>
<tr>
<td>P-free salt mixture</td>
<td>2.33</td>
<td>2.38</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.000</td>
<td>0.061</td>
</tr>
<tr>
<td>KCl</td>
<td>0.051</td>
<td>0.052</td>
</tr>
<tr>
<td>K citrate</td>
<td>0.291</td>
<td>0.293</td>
</tr>
<tr>
<td>Mg citrate</td>
<td>0.103</td>
<td>0.104</td>
</tr>
<tr>
<td>Na₂HPO₄.12H₂O</td>
<td>1.22</td>
<td>2.54</td>
</tr>
<tr>
<td>Spray-dried skim milk</td>
<td>12.93</td>
<td>0.397</td>
</tr>
<tr>
<td>Creta praeparata</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca, P and moisture content of diets (%)</th>
<th>0.1666</th>
<th>0.1677*</th>
<th>0.1624*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>0.2445</td>
<td>0.2398</td>
<td>0.2372</td>
</tr>
<tr>
<td>Moisture</td>
<td>12.03</td>
<td>11.71</td>
<td>11.54</td>
</tr>
<tr>
<td>Ca/P</td>
<td>0.68</td>
<td>0.70</td>
<td>0.68</td>
</tr>
</tbody>
</table>

* Approximately 90% derived from Creta praeparata and bone meal.
of the availability of the Ca of *Creta praeparata* and bone meal when added to the bread.

When the Ca intake was increased (breads 4 and 5) the retention also rose markedly. As a matter of fact it almost kept pace with the increased supply. The growth of the rats was slow, of the same order as that observed by us before with rats receiving bread supplemented only with CaCO₃ (Henry *et al.* 1941), and their intake of Ca/g. gain in weight outstripped the 'normal' requirement over this weight range (7.5 mg./g.) as defined by Sherman & Macleod (1925). Hence these animals were probably ingesting more Ca than they were able to utilize, and there was thus a rise in their faecal excretion of Ca and a slight lowering of the percentage retention of this element.

The findings with breads 4 and 5 in the second experiment are very similar to those reported in the first experiment. Ca was again equally well retained from both breads; the absolute and percentage retention was somewhat higher than in Exp. 1. This is accounted for by a lower excretion of Ca in the faeces.

All five diets supplied relatively more P than Ca and the amounts of P consumed were well above the 'norm', 6 mg./g. gain in weight, of Sherman & Quinn (1926). The retentions from breads 1 and 2 were substantially the same, about 80–90 mg., but some 130 mg. were retained from the wholemeal bread. Rats receiving this bread made slightly

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**Table 4. Statistical treatment* of differences in percentage Ca retentions in the three experiments**

| Exp. 1 | Breads 1, 2 | -0.1 | ±2.8 | 1:1 | N.S. |
| Exp. 1 | Breads 1, 3 | +7.0 | ±2.1 | 1:47 | S.  |
| Exp. 1 | Breads 2, 3 | +7.2 | ±2.2 | 1:47 | S.  |
| Exp. 1 | Breads 4, 5 | -0.1 | ±2.4 | 1:1 | N.S. |

**Exp. 2**

| Breads 4, 5 | +0.5 | ±3.8 | 1:1 | N.S. |
| Breads 6, 7 | +3.6 | ±2.1 | 1:7 | N.S. |
| Breads 4, 6 | +3.1 | ±2.5 | 1:3 | N.S. |
| Breads 4, 7 | +6.7 | ±3.0 | 1:13 | N.S. |
| Breads 5, 6 | +2.6 | ±3.5 | 1:2 | N.S. |
| Breads 5, 7 | +6.2 | ±2.2 | 1:27 | S.  |

**Exp. 3**

| Milk, | +5.1 | ±0.7 | 1:1086 | S.  |
| *Creta praeparata,* | | | |
| Milk, bone meal | +9.4 | ±0.8 | 1:14286 | S.  |
| *Creta praeparata,* bone meal | +4.3 | ±0.8 | 1:333 | S.  |

N.S. = not significant; S. = significant.

* All comparisons were made between litter-mates using the paired *t*-test of 'Student' (1908, 1925).

† *P* = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

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better growth than those on the supplemented white breads, and part of this excess P was certainly used to supply the needs of the greater weight of their soft tissues. The depositions of Ca were sensibly the same in all three groups, hence the amounts of P used for bone must have varied but little, and all the extra P must have been retained in the soft tissues. There is no doubt that the higher P retention with wholemeal bread represented a greater saturation of tissues than with breads 1 and 2.

Thus it can be calculated from the data of Table 3, on the assumption that 99% of the Ca is stored in the bones and that the Ca/P ratio of the normal rat bone is 2.31 (Brooke, Smith & Smith, 1934; Kramer & Shear, 1928), that rats receiving bread 1 retained 2-6 mg. of extra-skeletal P/g. gain in weight, whereas wholemeal bread gave a retention of 3.2 mg./g. gain in weight. The reason for this increased retention of P from wholemeal bread is not clear; though the amount of P supplied was very much greater than with the white breads, there is no doubt that even the latter contained more than enough P, especially as much more was absorbed than was retained.

The animals receiving the supplemented wholemeal breads consumed some 30% more diet and Ca than their litter-mates eating the supplemented white breads; they also grew almost twice as fast, and consequently their Ca intake judged by the standard described on p. 119 was suboptimal while that of the white-bread animals was again rather above the normal. Hence a comparison of the percentage retentions of Ca from the two types of bread would be biased in favour of the wholemeal groups. Nevertheless, relatively more Ca was retained from the white breads, the difference between breads 5 and 7 being statistically significant, and it is highly probable that Ca was not quite as available from wholemeal bread as from white bread. There is also an indication that the phosphate was less satisfactory than the carbonate as an addition to wholemeal bread, though statistical confirmation is lacking.

The results of the direct comparison of the carbonate with the phosphate carried out in Exp. 3 (Tables 3, 4) point more forcibly in the same direction. The rats in this experiment grew faster than any animals in the previous two experiments and reached a higher final weight. The 'normal' Ca requirement of rats of their size is 8-8 mg./g. gain in weight (Sherman & Macleod, 1925), and their actual intakes may be taken as suboptimal. The retention of Ca from Ostra praeferata and bone meal was 92 and 97% respectively; Table 4 shows that this difference was statistically significant. Of the milk Ca 97% was retained. This figure was statistically superior to those obtained for the mineral additions and confirms our earlier finding (Henry & Kon, 1939a) of the very high availability of milk Ca. The value of 87% now obtained for bone meal may be compared with the values, 96 and 92%, previously reported by us for CaHPO₄.2H₂O (Henry & Kon, 1939a, b).

It should be recorded that in a recent paper Yudkin (1943) found the carbonate and the phosphate of Ca equally effective in increasing the bone-ash content of rats, when added to white flour, wheatmeal flour, or maize gluten mixture, in quantities similar to those used by us.

A comparison of the P retention from breads 1 and 4 is of interest as the diet and P intakes from both were very similar, though the Ca intakes naturally differed. The increased retention of Ca from bread 4 was, as would be expected, accompanied by additional deposition of P. However, the P retained for this purpose accounts for only 70% of the difference in retention from the two diets, and the extra-skeletal stores must have been rather better supplied from bread 4. The better retention of P was chiefly due to a diminished urinary excretion.

From the figures for the metabolism of P derived from bread 3 it is possible to gain an insight into the utilization of phytate P by the rats. Of the 0.8414 g. P supplied by this bread, 0.4075 g. was phytate P and 0.4339 g. non-phytate P (cf. Tables 1, 3). As the absorbed P amounted to 0.6257 g., it must have contained at least 0.1918 g. of phytate P, or 47% of that present in the bread. This calculation underestimates the availability of phytate P and sets a minimum figure for its utilization.

It is obvious that non-phytate P is not 100% available, and to obtain a more satisfactory estimate a correction might be introduced for the non-phytate P lost through the gut. For example, it might be assumed that the non-phytate P of brown bread is as available as that of white bread. For this calculation the very small amount of phytate P in white bread may be neglected. The intake of P from bread 1 was 0.2606 g., of which 0.0676 g. was excreted in the faeces. Taking the minimum unavoidable faecal excretion of P as 1.1 mg. daily (Henry & Kon, 1939 a), one finds that 0.0291 g. of the bread P was not available; pro rata the unavailable non-phytate P of the brown bread would amount to 0.0485 g., and the absorbed phytate P would be not 0.1918 g. but 0.2403 g. or 59%.

The higher limit for the availability of phytate P may be calculated in the following way: it may be assumed that the excess/100 g. diet of faecal P derived from bread 3 over that obtained from bread 1 represents unabsorbed phytate P. The excess works out at 59.9 mg., and as the phytate P intake in 100 g. of bread 3 was 167.8 mg., 36% of the intake was excreted. The 64% hydrolyzed in the intestine would represent the maximum phytate P potentially available. Similar calculations with breads 4 and 6 yield figures for the minimum estimate of
46%, for the corrected estimate of 62% and for the amount hydrolyzed in the intestine of 66%.

In this connexion it is of interest to point out that though the excess faecal P derived from bread 3 as compared with bread 1, or from bread 6 as compared with bread 4, may well be phytate P, it cannot possibly be in the form of Ca phytate. Ca phytate contains almost equal weights of Ca and P (Krieger et al. 1940b), yet the excess faecal P/100 g. bread was 59-9 mg. for bread 3 and 57-8 mg. for bread 6, while the excesses of Ca were only 3-5 and 4-1 mg. respectively. The similarity of the values obtained for Ca and P in the two independent experiments is noteworthy.

A clearer indication of the effects of wholemeal bread on the assimilation of Ca may be gained from a consideration of the faecal excretions. It will be seen that 90–100 mg. of Ca were lost in this way from the supplemented wholemeal breads, whereas only 34 mg. were correspondingly lost from the unsupplemented wholemeal bread in Exp. 1. Assuming that in both experiments the Ca of wholemeal bread was equally available, and making allowance for unavoidable faecal losses of Ca of 0.1 mg. daily (Henry & Kon, 1939a), it can be calculated that with breads 6 and 7 there should be a faecal loss of 39 mg. of Ca originally present in wholemeal bread. Breads 6 and 7 supplied, however, in addition 155 and 151 mg. Ca respectively from Creta praeparata and bone meal. From the availability of Ca from these sources found in Exp. 3, their contribution to faecal Ca may be calculated as 11 and 18 mg. respectively. The total calculated amount of unavailable Ca derived from wholemeal bread and mineral addition amounts, therefore, to 50 mg. for bread 6 and 57 mg. for bread 7; allowing for unavoidable losses one arrives at over-all figures of 54 and 61 mg. respectively. However, the actual excretions were 90 and 100 mg. respectively and the difference represents the Ca of Creta praeparata or bone meal rendered unavailable by the wholemeal bread.

The superior growth of the animals receiving the wholemeal breads at both levels of Ca intake is of interest. In Exp. 1 the intake was very similar for breads 1, 2 and 3 and the calorie intake, calculated from data in the tables of McCance & Widdowson (1940), was, if anything, lower from the wholemeal bread, yet all the animals receiving the wholemeal bread grew faster than their litter-mates on the white breads. In Exp. 2 the consumption of wholemeal bread was some 28% higher and growth some 100% better than with white bread.

Extensive studies by Chick (1940, 1942) and Copping (1943) have shown the superior nutritive value, as measured on rats, of higher extraction flours. The present results may be taken to confirm and support their findings. It should be noted that in our experiments ample quantities of vitamin B1, riboflavin, and pantothenic acid were added to all breads. The superiority of the wholemeal bread which manifested itself under these circumstances was, therefore, probably due to differences in other factors.

**DISCUSSION**

Viewed as a whole our experiments certainly support the findings of McCance & Widdowson (1942a) that breads made from higher extraction flour have a depressing effect on the assimilation of Ca. Our experiments were of a more drastic nature in that all the calories and Ca were derived from bread, while the human diet was more varied and with it only about 50% of calories was obtained from bread. Yet the effects observed by us were much milder than those noted by McCance & Widdowson. The explanation probably lies in the choice of our experimental animal. A young growing rat will, under suitable conditions, absorb nearly 100% and retain over 98% of ingested Ca (Ellis & Mitchell, 1933; Henry & Kon, 1939a), while children and adults utilize only some 20% (cf. Kon, 1943). Apart from that, it seems possible that the possession of an intestinal phytase enables the rat to break down phytate P more efficiently than man. Though the destruction of phytic acid in the human gut proceeds apparently to a marked extent (Rogozinski, 1910; McCance & Widdowson, 1935, 1942a), a large part of it may take place at a level too low for absorption to occur. In the rat the enzymic breakdown higher up in the gut may well favour absorption. In fact, our figures indicate an absorption by the rat of some 60% of the ingested phytate P as against the estimate for man of 50% by McCance & Widdowson (1942a). There is ample evidence in the literature, already quoted on p. 117 of this paper, that phytate P is less well utilized by the rat than other forms of this element, and that in the presence of large quantities of phytic acid the metabolism of Ca and P as measured by the deposition of bone may be impaired through shortage of available P. The evidence, however, for any marked direct depressing effect of phytic acid on the utilization of Ca by the rat, as found by Harrison & Mellanby (1939) for the dog and by McCance & Widdowson (1942a, b) for man, is almost entirely lacking. In fact Krieger et al. (1940b) report that the Ca of Ca phytate is as available to the rat as that of CaCO₃. We believe, however, that our observations prove that, in the rat, phytic acid exerts effects similar to those in man and the dog, though probably to a lesser degree. The depressing effect of phytate P on the utilization of Ca by the rat has also very recently been reported by Marcy (1944). He used diets containing 0.12–0.25% P and with a Ca/P ratio of 2:1.
SUMMARY

1. The retention of Ca by rats receiving diets mainly consisting of wholemeal bread or of bread made from 72% extraction flour has been studied in metabolic experiments.

2. In one experiment Creta praeparata or bone meal were added to the 72% extraction flour to bring the Ca content of the diet to that of the wholemeal bread diet. In another, the two substances were added to the 72% extraction flour at a rate equivalent to 1 lb. of CaCO₃/280 lb. sack of flour, and the wholemeal flour was similarly supplemented to bring its Ca content to the same level.

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The Formation of Mono- and Di-glycerides during the Hydrolysis of Triglyceride by Pancreatic Lipase

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It has been generally assumed that triglyceride is rapidly broken down by pancreatic lipase to fatty acid and glycerol. This is a necessary assumption if it is believed that triglyceride must be completely hydrolyzed to fatty acid and glycerol before it can be absorbed from the intestinal lumen (Verzar & McDougall, 1936; Bloor, 1943). It can be shown, however, that triglyceride and fatty acid behave differently both during and after absorption. Triglyceride absorption is associated with globular dispersion of fat in the intestinal cell, with milkiness of the lacteals, with an increase of the particulate fat in the systemic blood and with subsequent deposition in the fat depots, whereas fatty acid absorption gives rise to a granular appearance in the intestinal cell, an increase of fatty material in the portal blood and deposition in the liver (Frazer, 1938, 1943a). It is generally agreed that trigly-