LXXXVIII. THE INFLUENCE OF COD-LIVER OIL, LINSEED OIL AND OLIVE OIL ON THE ASSIMILATION OF CALCIUM AND PHOSPHORUS IN THE GROWING PIG.

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THE literature of rickets contains many references to the use of fats and oils, especially cod-liver oil, as therapeutic agents. Gobley [1844] reported the successful use of ray's liver oil. Roloff [1866] advocated the use of oil-rich foods for the prevention of osteomalacia in farm animals. Kassowitz [1884, 1913] used cod-liver oil, and also other oils, as a vehicle for the administration of phosphorus. He did not however consider cod-liver oil of any special value *per se*, and notes that, in some cases, the administration of the oil alone had no beneficial action in curing rickets.

Schabad [1909], Birk [1909], Meyer [1913] and others have found that the administration of phosphorus in cod-liver oil increased the retention of calcium and phosphorus. On the other hand, Flieger [1897] showed that, in some cases at least, the phosphorised oil was ineffectual as a cure for rickets, and Schabad and Sorochowitsch [1911] and Schloss [1914] found that its use was not always followed by an increased retention of calcium and phosphorus.

Schloss, and Schabad and Sorochowitsch both found that an increased retention of calcium and phosphorus followed the administration of calcium salts in cod-liver oil.

The influence of fat without the addition of minerals has been studied by several workers. Kochmann and Petzsch [1911] found that the addition of fat to the diet reduced the absorption of calcium. On the other hand, Herter [1898], working with pigs, noted that, on the reduction of fat in the diet, the absorption of phosphorus from the intestine was decreased.

Orgler [1912] found that cod-liver oil increased the retention of calcium. Steinitz [1903], Rothberg [1907], Meyer [1908] and Orgler noted a decreased retention of calcium on increasing the amount of milk fat in the diet. Telfer [1921] investigated this apparent difference in the action of butter fat and cod-liver oil. In his experiments, no difference in calcium retention occurred with variations in the nature of the fat.

The recent literature on the therapeutic value of cod-liver oil in rickets need not be referred to. The work has been of a pathological, rather than a physiological nature. Interest has centred chiefly in bone lesions, and results have been judged chiefly by skiagraphic or histological examinations.

At this Institute during the past three years a series of investigations has been carried out on the mineral requirements of the growing pig, and on certain factors which affect the assimilation of the minerals. One of these investigations seemed to indicate that, in the pig at least, an important factor in the production of rickets is a lack of correspondence between the mineral content of the diet and the mineral requirements of the animal [Elliot, Crichton and Orr, 1922]. But it was found that the addition of cod-liver oil to a ration deficient in calcium led to an immediate increase in the amount of both calcium and phosphorus absorbed and retained [Orr and Husband, 1922].

In view of this finding and of the contradictory results of the previous workers referred to above, it seemed desirable to investigate further the influence of oils on the assimilation of calcium and phosphorus. It was intended to determine the relative influence of cod-liver oil, linseed oil and olive oil on the absorption and retention of these minerals. It was thought also that the results would show the influence of oil *per se*, and also whether the vitamin-rich cod-liver oil had a specific action.

EXPERIMENTAL METHODS.

The experiments were carried out with young hog pigs two to four months old. The animals used in each experiment were taken from the same litter and were as comparable in weight as possible. They were confined in metabolism cages in which it was possible to collect urine and faeces separately. The urine was collected in 24 hour periods. The faeces were collected as soon as possible after excretion, and placed in weighed tins with tight-fitting lids. At the end of the 24 hour period the tins were again weighed, the contents thoroughly mixed, and duplicate samples taken for immediate analysis. The cages were washed out daily with distilled water, and the washings added to the urine.

To get the pigs accustomed to the metabolic cages, to the diet, and to the routine of the experiment, they were confined in the cages for about a week before the analysis of the urine and faeces began. During this period they were fed to the limit of their appetites. The amount of food fed during the experimental period was regulated according to the amount eaten during this preliminary period, and was kept constant throughout the experimental period.

Basal Diet. The basal diet used in all experiments consisted of maize meal, middlings (wheat offal), oatmeal and blood meal in the proportions stated below. Before each experiment, samples of the food-stuffs were taken from

bulk, and the percentages of N, CaO and P_2O_5 determined. The following figures are the averages for all the samples. The exact intake of N, CaO and P_2O_5 in each experiment is stated in the tables giving the results of the experiments.

Basal diet.

	Proportions	Average composition %		
		Total N	CaO	P_2O_5
Maize	10	1.48	0.008	0.633
Middlings	10	2.72	0.082	1.615
Oatmeal	10	2.15	0.077	1.084
Blood meal	1	12.96	0.226	0.380

The calcium content of this ration is very low, containing only 0.061 % of CaO, whereas the dry matter of sow's milk contains 2.37 % CaO.

Golding, Zilva, Drummond and Coward [1922] state that they had difficulty from a technical point of view to reduce the calcium intake of pigs below 0.338 % (0.47 % CaO). From their data it would appear that their sample of wheat offal contained an abnormally high percentage of calcium. The highest figure obtained in our analyses for wheat offal was 0.12 % CaO with which the figures given by Forbes [1913] and König agree.

The food was made into a thick paste with distilled water, and the animals had distilled water *ad lib.* to drink.

As it appeared from previous experiments that the influence of the oils would be found to be greatest when the mineral content of the ration was ill-balanced, and especially when there was a deficiency of calcium, it was decided not to adjust completely the mineral matter of the ration in the first series of experiments. The results of previous work however had shown that, with such a low supply of calcium, as was present in the diet, there was a danger of pathological symptoms developing during the course of the experiment. The amount of calcium was therefore increased by the addition of 50 cc. of a 20 % solution of $CaCl_2$, which is equivalent to about 2.6 g. of CaO. This raised the CaO content of the ration to between 3 and 4 g., which is not more than half of what has been found in this Institute to be the requirement of a rapidly growing young pig three or four months old.

In Exp. 3 a further adjustment of the mineral matter was made, to determine whether the effect of the oil would still be apparent with an ample supply of calcium in the food. Details of the adjustment are given with the data for the experiment. Though the excreta of each 24 hour period in Exps. 1 and 2, and of each 48 hour period in Exp. 3, were analysed separately, the averages for longer periods are given in the tables to economise space. The lengths of the periods for which the averages are given are not identical in every case. This variation is due to the fact that there was sometimes a delay of a day in introducing or withdrawing the oil if the faecal excretion of the preceding day had not been normal in amount. Even with fairly long experimental periods, it is desirable to keep in view the danger of getting a

fallacious result owing to an abnormally large or small expulsion of faeces in the 24 hours.

The following methods of analysis were used: *total nitrogen* by the Kjeldahl method; *calcium* by precipitation as oxalate and titration with $N/10$ $KMnO_4$; *phosphorus* by the Pemberton-Neumann method.

EXPERIMENTAL DATA.

Three experiments were conducted. In each experiment one animal received cod-liver oil, one linseed oil and one olive oil.

In the first experiment the comparative effect of the three oils was tested.

This experiment was divided into three periods, the "pre-period" when the basal ration only was fed, the "oil period" when the oil was added, and the "post-period" when again only the basal ration was fed. The amount of oil added was in each case 40 cc. per day.

Experiment 1.

No. of pig	Oil	Age at beginning of experiment	Weight in kilograms	
			At beginning of experiment	At end of experiment
28	Cod-liver oil	17 weeks	26.9	43.5
24	Linseed oil	17 "	26.6	44.9
26	Olive oil	17 "	24.2	38.1

Ration for Nos. 28 and 24 was 1550 g. of basal ration plus 50 cc. of 20 % $CaCl_2$ solution.

Ration for No. 26 was 1240 g. of basal ration plus 50 cc. of 20 % $CaCl_2$ solution.

It will be seen from a study of Table I that the calcium balance of all three pigs showed a progressive increase throughout the experiment.

In Exp. 2 the pre-oil period was lengthened to ascertain what course the excretion of calcium and phosphorus would follow on the basal ration alone.

Experiment 2.

No. of pig	Oil	Age at beginning of experiment	Weight in kilograms	
			At beginning of experiment	At end of experiment
67	Cod-liver oil (40 cc.)	12 weeks	20.1	28.3
68	Linseed oil (30 cc.)	12 "	19.4	28.3
70	Olive oil (30 cc.)	12 "	17.3	29.9

The ration for Nos. 68 and 70 was 930 g. of basal ration plus 50 cc. of 20 % $CaCl_2$ solution.

The ration for No. 67 was 1240 g. of basal ration plus 50 cc. of 20 % $CaCl_2$ solution.

In this experiment it will be noted that all three pigs showed a negative calcium balance at the end of 19 days. At this period it was decided to add

Graph 1.

Average balances for Pig No. 28. Experiment 1. Table I.

Graph 2.

Average balances for Pig No. 24. Experiment 1. Table I.

Table I. *Average daily excretions and balances in grams.*

		Pig No. 28. Cod-liver oil. Duration of experiment: 31 days.									
		Nitrogen					CaO				
		Average daily weight of faeces					P ₂ O ₅				
		Intake	Urine	Faeces	Total	Balance	Intake	Urine	Faeces	Total	Balance
Pre-period	6 days	38.70	14.54	9.85	24.39	14.31	18.07	5.30	10.29	15.59	2.48
Oil period	1st 7 "	"	14.56	8.29	22.85	15.85	"	"	5.58	9.00	3.49
"	2nd 7 "	"	13.35	10.28	23.63	15.07	"	5.03	9.83	14.86	3.21
Post-period	1st 6 "	"	13.12	9.59	22.71	15.99	"	4.87	9.79	14.65	3.41
"	last 5 "	"	13.36	9.15	22.51	16.19	"	4.89	10.08	14.97	3.10
Pig No. 24. Linseed oil. Duration of experiment: 31 days.											
Pre-period	7 days	38.70	12.89	10.51	23.40	15.30	18.07	4.99	9.75	14.74	3.33
Oil period	1st 6 "	"	13.30	9.54	22.84	15.86	"	4.88	9.48	14.36	3.71
"	2nd 6 "	"	13.40	9.10	22.50	16.20	"	5.04	9.73	14.77	3.30
Post-period	1st 6 "	"	13.30	9.31	22.61	16.09	"	4.89	10.40	15.29	2.78
"	last 6 "	"	14.68	9.23	23.91	14.79	"	4.77	11.78	16.55	1.52
Pig No. 26. Olive oil. Duration of experiment: 28 days.											
Pre-period	2 days	30.96	10.41	8.10	18.51	12.45	14.46	3.96	8.04	12.00	2.46
Oil period	1st 7 "	"	11.47	7.32	18.79	12.17	"	4.21	8.10	12.31	2.15
"	2nd 7 "	"	11.00	7.68	18.68	12.28	"	3.88	7.45	11.33	3.13
Post-period	1st 6 "	"	10.92	7.68	18.60	12.36	"	4.03	7.99	12.02	2.44
"	last 6 "	"	11.16	6.98	18.14	12.82	"	4.06	8.44	12.50	1.96

NOTE:—At the commencement of the experiment pig No. 26 received the same ration as pigs Nos. 28 and 24. Unfortunately on the first day of the collection for analyses the pig refused to consume its whole ration. The ration was therefore reduced. In the analytical data for this pig only the last two days' analyses of the pre-period were taken.

Table II. *Average daily excretions and balances in grams.*

Fig No. 70. Linseed oil. Duration of experiment: 40 days.												
		Nitrogen			CaO			P ₂ O ₅				
Days	Average daily weight of faeces	Intake	Urine	Faeces	Total	Balance	Intake	Urine	Faeces	Total	Balance	
		22-66	8-63	6-67	15-30	7-36	3-30	0-19	2-07	2-26	+1-04	
1-5 inclusive	532	"	9-14	6-59	15-73	6-93	"	0-12	2-47	2-59	+0-71	
" 6-12	524	"	9-88	5-74	15-62	7-04	"	0-10	2-65	2-75	+0-55	
" 13-19	478	"	9-86	5-33	15-19	7-47	"	0-10	3-26	3-36	-0-06	
" 20-26	467	"	9-82	5-05	14-87	7-79	"	0-12	3-27	3-39	-0-09	
" 27-33	481	"	9-21	4-59	13-80	9-09	3-13	0-11	3-06	3-17	-0-04	
" 34-40	467											
Fig No. 67. Cod-liver oil. Duration of experiment: 40 days.												
Days		Intake	Urine	Faeces	Total	Balance	Intake	Urine	Faeces	Total	Balance	
		30-21	10-35	8-52	18-87	11-34	3-48	0-12	2-72	2-84	+0-64	
1-7 inclusive	638	"	11-00	7-47	18-47	11-74	"	0-13	3-18	3-31	+0-17	
" 8-13	621	"	11-48	7-89	19-37	10-84	"	0-15	3-69	3-84	-0-36	
" 14-19	716	"	11-81	8-03	19-84	10-37	"	0-19	3-77	3-96	-0-48	
" 20-22	726	"	9-49	5-34	14-83	5-31	2-32	0-12	2-03	2-15	+0-17	
" 23-28	446	20-14	8-43	4-72	13-15	6-99	"	0-07	0-71	0-78	+1-54	
" 29-34	347	"	7-35	3-25	10-60	4-65	1-70	0-05	0-49	0-54	+1-16	
" 35-40	237	15-25										
Fig No. 68. Olive oil. Duration of experiment: 38 days.												
Days		Intake	Urine	Faeces	Total	Balance	Intake	Urine	Faeces	Total	Balance	
		22-66	9-07	6-24	15-31	7-35	3-30	0-14	2-11	2-25	+1-05	
1-4 inclusive	470	"	9-30	5-55	14-85	7-81	"	0-12	2-17	2-29	+1-01	
" 5-10	429	"	9-77	5-74	15-51	7-15	"	0-12	3-10	3-22	-0-08	
" 11-16	474	"	9-80	5-29	15-09	7-57	"	0-13	3-28	3-41	-0-11	
" 17-20	432	"	10-14	5-74	15-88	6-78	"	0-15	3-29	3-44	-0-14	
" 21-26	454	"	10-17	5-12	15-29	7-37	"	0-15	2-36	2-51	+0-79	
" 27-32	401	"	22-98	10-15	5-33	15-48	7-50	3-07	0-14	2-24	+0-69	
" 33-38	437	"										

NOTE. —————> indicates the introduction of the oil.

Table III. *Average daily excretions and balances in grams.*

Fig No. 669. Cod-liver oil. Duration of experiment: 20 days.

Days	Average daily weight of faeces	Nitrogen				CaO				P ₂ O ₅			
		Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance
1-6 inclusive	490	22-77	9-56	5-91	15-47	7-30	3-54	2-56	2-67	9-77	1-77	6-17	7-94
" 7-10 "	467	"	8-87	5-78	14-65	8-12	"	2-91	3-06	"	1-84	6-19	8-03
" 11-14 "	459	22-88	10-75	5-55	16-30	6-58	3-51	3-74	3-86	9-75	2-34	7-02	9-36
" 15-18 "	517	22-98	10-12	6-07	16-19	6-79	3-49	2-50	3-58	9-73	2-30	5-87	8-17
" 19-20 "	643	"	11-02	6-50	17-52	5-46	"	1-79	1-90	9-73	2-20	5-33	7-53

Fig No. 670. Olive oil. Duration of experiment: 40 days.

Days	Average daily weight of faeces	Nitrogen				CaO				P ₂ O ₅			
		Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance
1-6 inclusive	515	22-77	10-25	6-52	16-77	6-00	14-59	0-64	11-92	9-77	0-37	7-57	7-94
" 7-12* "	512	22-98	10-93	6-33	17-26	5-72	14-55	0-61	13-03	9-72	0-38	8-38	8-76
" 13-18 "	510	"	11-19	5-96	17-15	5-83	14-52	0-50	13-27	13-29	0-59†	11-25†	11-84†
" 19-22 "	557	"	9-74	5-83	15-57	7-41	"	0-36	14-17	"	0-40	11-91	12-31
" 23-28 "	540	"	11-03	5-42	16-45	6-53	"	0-45	13-68	"	0-62	11-34	11-96
" 29-34 "	503	"	10-63	5-21	15-84	7-14	"	0-39	12-79	"	0-56	10-69	11-25
" 35-40 "	471	"	10-13	5-11	15-24	7-74	"	0-44	12-68	"	0-66	10-16	10-82

Fig No. 671. Linseed oil. Duration of experiment: 42 days.

Days	Average daily weight of faeces	Nitrogen				CaO				P ₂ O ₅			
		Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance	Intake	Urine	Faeces	Total Balance
1-4 inclusive	480	22-78	11-84	6-30	18-14	4-64	3-54	0-12	3-17	9-77	2-72	6-47	9-19
" 5-10 "	458	"	10-82	5-47	16-29	6-49	"	0-13	3-07	"	2-52	6-21	8-73
" 11-16 "	445	22-84	10-88	5-23	16-11	6-73	3-52	0-16	3-60	"	2-44	6-76	9-20
" 17-20 "	428	22-98	10-80	5-08	15-88	7-10	3-49	0-17	3-82	9-73	2-05	7-03	9-08
" 21-26 "	446	"	10-26	5-10	15-36	7-62	"	0-15	3-88	"	1-83	7-17	9-00
" 27-32 "	480	"	10-26	5-36	15-62	7-36	"	0-14	3-74	"	1-98	7-39	9-37
" 33-38 "	474	"	10-68	5-01	15-69	7-29	"	0-16	3-63	"	2-31	7-00	9-31
" 39-42 "	454	"	10-63	4-79	15-42	7-56	"	0-15	3-54	"	2-30	6-88	9-18

→ indicates the introduction of the oil.

* Ca₃(PO₄)₃ introduced on this day.† Average for four days only, figures for the first two days after the addition of Ca₃(PO₄)₃ being rejected.

40 cc. of cod-liver oil to the ration of pig No. 67 and 30 cc. of olive oil to the ration of pig No. 68. Pig. No. 70 was continued on the basal ration alone for a further period of 14 days as a control, to ascertain whether the negative calcium balance would continue.

Graph 3.
Average balances for Pig No. 26. Experiment 1. Table I.

At the end of this time 30 cc. of linseed oil were added to the ration of this pig. It will be seen from Table II that the addition of the oil to the ration resulted in a rapid conversion of the negative calcium balances to positive balances in the case of pigs Nos. 67 and 68, and in a lowering of the negative balance of pig No. 70.

Experiment 3.

No. of pig	Oil	Age at beginning of experiment	Weight in kilograms	
			At beginning of experiment	At end of experiment
669	Cod-liver oil (30 cc.)	14 weeks	19.7	25.8
670	Olive oil (30 cc.)	14 "	20.6	31.0
671	Linseed oil (30 cc.)	14 "	25.1	35.5

Ration for Nos. 669 and 671 was 930 g. of basal ration plus 50 cc. of 20 % CaCl_2 solution.

Ration for No. 670 was 930 g. of basal ration plus 26 g. of chalk and 5 g. of NaCl.

In Exps. 1 and 2 the mineral matter of the diet was ill-balanced, especially with regard to calcium, sodium and chlorine, which were markedly deficient. In this experiment those deficiencies were made good in the case of one of the pigs, No. 670, by the addition to the day's ration of 26 g. of CaCO_3 , and 5 g. of NaCl. On the 12th day of the experiment 8 g. of tricalcium phosphate

were substituted for 6 g. of the CaCO_3 , to adjust the phosphorus content of the ration. This ration thus adjusted contained the mineral ingredients in amounts approximately equal to those contained in an amount of sow's milk of equal caloric value. Of course, though these various minerals were present roughly in the same proportion as those found in sow's milk, it must not be assumed that they were present in the same utilisable form.

DISCUSSION OF RESULTS.

It will be seen from Tables II and III that prior to the addition of oil there was a progressively increasing excretion of both calcium and phosphorus. This occurred in every pig used in these two experiments. In every case where the pre-period was prolonged, the calcium balance became negative, and the phosphorus balance correspondingly reduced.

The increased excretion is in the faeces. The urinary excretion of both calcium and phosphorus in pig No. 70 decreased in the pre-period. This happened also in pigs 68 and 670.

All the pigs were in the metabolism cages for a period of not less than seven days before analysis of the excreta was begun. It is most probable that during this preliminary period also, there was a decrease in the assimilation and retention of both calcium and phosphorus. The amounts retained even at the beginning of analysis were small compared with the normal requirement for growing pigs, which has been found here to be about 4-6 g. per day for pigs of the age used in the experiments.

Correlated with the decreased retention of calcium and phosphorus, a loss of appetite was noted. As the experiments proceeded, it became difficult to get the pigs to consume the daily ration. Pig No. 67 (Exp. 2), shortly after the introduction of cod-liver oil into the ration, could not be induced to consume the full ration, and similarly pig No. 669 (Exp. 3) finally refused its food, and work with it had to be abandoned.

A notable exception to this loss of appetite was pig No. 670, which had the adjusted mineral matter. Although the calcium balances showed the same downward course as with the other pigs, it was always eager for its food. Shortly after the experiment began its appetite improved and it would have eaten much more than the ration which satisfied it in the preliminary period.

It is interesting to note that this was the only pig used in these two experiments, which at the end of the experiment was in good condition. It was still active and apparently in good health. All the other five were beginning to show signs of ill health.

There is not sufficient data available for a profitable discussion of the cause of this decreased assimilation and retention of calcium and phosphorus.

The point of importance in the present investigation is that the diet on which the oils were superimposed was a diet on which normal assimilation and retention of calcium and phosphorus did not occur. The close confinement

in the metabolism cages was very probably a contributing factor, but the fact that, under the same conditions and with the same animals, assimilation and retention markedly increased on the introduction of oil to the diet demonstrates quite clearly the beneficial effect of oil on the metabolism of these minerals.

Oil period and post-oil period. The introduction of the oil, whether cod-liver, linseed or olive oil, is followed in all cases by a definite increase in the retention of calcium and phosphorus. In Exp. 1 where the pre-period was too short for the animals to reach a negative calcium balance, the calcium retention within seven days exceeded that of the pre-period. In general, the effect on calcium is more marked than on phosphorus. There is a correlation between calcium and phosphorus retention in growing animals, as has been noted by Fraps [1918] and others. This has been observed in all the metabolic experiments with growing pigs done in this Institute.

It has been noted above that, in the pre-period, the increased excretion of calcium and phosphorus that occurred when the pigs were continued on the basal ration, was due to increased elimination in the faeces. The increased retention on administration of the oil is brought about by decreased elimination in the faeces. Compared with the faecal excretion, the effects of variations in the urinary excretion on the calcium and phosphorus balances are unimportant.

Unfortunately it is impossible to differentiate the faecal excretion into the unabsorbed portion and the excreted portion; and consequently there is no direct evidence to settle the question as to whether the oils merely cause an increased absorption of these minerals, or cause an increased retention after they are absorbed.

It has been generally believed that calcium salts are excreted chiefly by the large intestine. The fact that calcium salts, when injected intravenously, appear in the intestine, seems to support this view (a review of this work is given by Von Noorden [1907]).

The results of the recent interesting studies of Telfer [1922], however, make it appear doubtful whether, normally, calcium or phosphorus is excreted into the intestine. He regards the mineral matter of the faeces in his experiments as chiefly, if not entirely, unabsorbed residues.

If the mineral matter in the large intestine is to be regarded as unabsorbed residues, then the effect of oil is exercised in the alimentary tract. The increased absorption must however be due to something more than a mere physical or chemical change in the contents of the intestine, because in Exp. 1, where the post-period was continued for 12 days, increased retention of calcium was continued after the elimination of the oil from the diet. Indeed, retention of calcium was greater in the days 7 to 12 of the post-period than during the administration of the oil.

Comparative effect of the three oils.

A comparison of the effects of the three different oils is of interest. In Exp. 1 when the pre-period was short, and the three animals were still

retaining a considerable amount of calcium and phosphorus, the oils appear about equally potent in increasing the retention of both minerals.

In Exps. 2 and 3, when the animals had got into a pathological condition, as shown by the negative calcium balances, cod-liver oil was the most potent in increasing the assimilation and retention of the minerals. Of the other two, olive oil appears from a comparison of pigs Nos. 68 and 670 with pig. No. 671, to be greater in its action than linseed oil. On the other hand, there was a marked improvement in the appetite of the animals when given linseed oil or olive oil, but the introduction of cod-liver oil resulted in a depression of the appetite. Hart, Steenbock and Hoppert [1922] noted a similar effect of cod-liver oil on the appetite of goats.

The fact that olive oil and linseed oil, which contain little or no fat-soluble A, are almost as effective in stimulating the assimilation and retention of calcium and phosphorus as the vitamin-rich cod-liver oil, indicates that the action on the metabolism of the minerals is not due, entirely at all events, to the vitamin A.

This conclusion is supported by the work of McCollum, Simmonds, Becker and Shipley [1922] who tested a number of fats for their power to stimulate healing in rickets. They found that cod-liver oil oxidised for 12 hours to destroy fat-soluble A, still retained its power to cause the deposition of calcium in the bones of young rats suffering from rickets. A similar result was got with coconut oil, which is also deficient in vitamin A.

It is most probable that oil *per se* has an influence on the absorption of minerals from the intestine, though the above workers found that olive oil fed to rats suffering from rickets did not raise the efficiency of the tissues in utilising calcium. In the light of our results it is probable that a positive result would have been obtained by these workers if the oil had been introduced earlier, or if the experiment had continued over a longer period. Unfortunately in these experiments with rats there are no data to show the actual mineral balances.

In our experiments, when the animals had been showing a negative calcium balance for some time, the response when receiving linseed oil was not so rapid as in the case of animals still assimilating and retaining these minerals (cf. pig No. 671, Exp. 3 with pig No. 24 of Exp. 1).

The results obtained here make it clear that the effect produced on a growing animal by the introduction of cod-liver oil cannot be ascribed to the vitamin present in the oil.

NOTE. It will be seen from the tables and graphs that the nitrogen metabolism of each pig was also followed. These results seem to indicate some difference in the action of the three oils on the nitrogen metabolism. This point is being further investigated, and it is hoped to discuss the combined results in a future communication.

CONCLUSIONS.

1. Pigs confined in metabolism cages and fed on a grain ration show a progressively decreasing capability of absorbing or retaining calcium and phosphorus.

2. The introduction of cod-liver oil, linseed oil or olive oil into the grain ration leads to increased assimilation and retention of both minerals.

3. When the animal is still retaining appreciable amounts of calcium and phosphorus, the three oils are about equally potent in their action.

4. When the animal shows a negative calcium balance, both cod-liver oil and olive oil rapidly convert the negative calcium balances to positive balances. Linseed oil is much slower in its action.

5. When the animal shows a negative calcium balance the introduction of cod-liver oil into the ration causes digestive disturbances.

6. The beneficial effect of an addition of oil to a grain ration on the calcium and phosphorus metabolism appears to be largely due to the oil *per se* irrespective of its content of vitamin A.

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