VIII. THE EFFECT OF IRRADIATION AND DIET ON CALCIUM AND PHOSPHORUS METABOLISM.

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THE metabolism of calcium and phosphorus is intimately involved in the action of ultra-violet irradiation. Hess and Lundagen [1922] have shown that in infants there is a seasonal tide of blood phosphate depending on the seasonal variation in sunlight. The increased deposition of calcium and phosphorus in bone as a result of irradiation, whether natural or artificial, has been amply proved by such work as that of McCollum and others [Powers, Park, Shipley, McCollum and Simmonds, 1921]; and numerous experiments have been carried out in investigating the effect of ultra-violet light on growth and the prevention of xerophthalmia.

In most of the above instances, however, the changes in calcium and phosphorus metabolism have been deduced from a final chemical analysis of the tissues, or from histological examination. In only very few cases has a complete calcium and phosphorus balance been followed out.

The present work was accordingly undertaken with the object of obtaining a quantitative estimate of the retention of these minerals under the influence of light over a fairly long period. Previous work, for the greater part, where it has not been on the human subject, has been carried out on small animals. For the present investigation the pig was selected for the following reasons:

(1) The growing pig of about three months old retains large amounts of calcium and phosphorus—roughly about 5 g. of CaO and of P_2O_5 per diem [Orr, 1923].

(2) A considerable amount of data on the retention of these minerals by the pig has already been accumulated at this Institute and serves as a guide.

(3) In the pig there is no thick coat to interfere with irradiation of the skin.

The importance of the proportions in which inorganic constituents are present in the diet has been shown by Elliot, Crichton and Orr in their work on rickets in pigs [1922]. In the case of the rat, Hess, Unger and Pappenheimer [1922] find that the rickets-producing effect of a diet adequate in calcium but low in phosphorus may be prevented by short exposures to direct sunlight. In the present investigation the calcium and phosphorus retention was accordingly determined in irradiated pigs which were on diets ill-balanced and

well-balanced respectively, particularly as regards their calcium, phosphorus and magnesium content. This aspect of the problem is of interest since the interrelation between ultra-violet light and vitamin A has already been carefully worked out by Hume [1922], Goldblatt and Soames [1923], Steenbock and Nelson [1923]. The problem, too, is not without its clinical significance in the treatment of rickets [Mellanby, 1924].

Two separate experiments were carried out. In the first, two pigs were used. One was irradiated and one kept in the dark. Both pigs were on a diet badly balanced as regards several of the mineral constituents. In the second experiment three pigs were used; one irradiated, one in darkness and one in diffuse light. All three were on a diet well-balanced as regards its mineral content.

EXPERIMENTAL.

Experiment 1. Animals. Two hog pigs of ten weeks old were selected from the same litter. They were closely comparable as regards condition, and at the beginning of the experiment each weighed 21.5 kg.

Housing. The pigs were kept in large metabolism cages from which urine and faeces could be collected separately. All sunlight entering the room traversed glass; further, the cages were rendered practically dark by means of green cloth canopies at a height of 9 inches above each cage and draped down the sides. There was no interference with ventilation. The animals were weighed every morning and were allowed to run about the animal room for half an hour for exercise.

Diet. The ration was as follows:

	g. per day	CaO %	P ₂ O ₅ %	N %
Oatmeal	180	0.0819	1.048	2.101
Maize-meal	270	0.0188	0.526	1.511
Middlings	450	0.1245	1.528	2.261
CaCO ₃	6	3.8898	—	—
Distilled water	<i>ad lib.</i>	—	—	—
Intake per 48 hours	—	8.2300 g.	20.366 g.	36.072 g.

A comparison of the mineral ratios of this diet and of sow's milk shows the diet to be ill-balanced with regard to several of its inorganic constituents. Thus:

	Sow's milk	Diet
CaO : P ₂ O ₅	10 : 9	10 : 25
CaO : MgO	20 : 1	20 : 19

The content of the ration in fat-soluble vitamin is low.

Analysis. The metabolism cages were washed out with distilled water every morning. The excreta were collected over 48-hour periods and the analyses then carried out. Calcium was precipitated as oxalate and estimated volumetrically. A slightly modified Pemberton-Neumann method was adopted for phosphorus.

Irradiation. The source of light was a carbon arc lamp which took .36 ampere at 45 volts. The pig was irradiated for 1 hour daily at a distance of 3 feet from the lamp.

The time during which the animals were in the cages may be divided into three periods. (1) A preliminary period of 10 days to accustom them to their environment and to determine their appetites. (2) A pre-period of 10 days during which analyses of the excreta were carried out. (3) An experimental period of 24 days during which the analyses were continued and the irradiation of the irradiated pig was in progress. The condition of the non-irradiated pig later in the experiment became so unsatisfactory that for the last few days an attempt was made to improve it by irradiation.

Forty-four days after the pigs had been put in the metabolism cages they were killed. Autopsies were done and also analyses of the blood and bones.

Table I. Exp. 1. *Average daily excretions and balances in grams.*

		Calcium (intake: 4.11 unless indicated thus *)				Phosphorus (intake: 10.18 unless indicated thus *)					
Days		Wt. of faeces	Wt. of pig (kg.)	Urine	Faeces	Total	Balance	Urine	Faeces	Total	Balance
Irradiated pig 1193.											
Light	1-10	522	21.9	0.09	3.56	3.65	0.46	1.51	6.79	8.30	1.88
	10-18	735	22.4	0.14	3.42	3.56	0.55	2.23	6.56	8.79	1.39
	18-24	513	21.7	0.23	1.73	1.96	1.78	2.48	4.68	7.16	2.54
	24-32	614	22.1	0.14	1.63	1.77	2.10*	2.76	4.10	6.86	2.65*
Non-irradiated pig 1192.											
Light	1-10	488	22.0	0.08	4.02	4.10	0.01	1.31	7.08	8.39	1.79
	10-18	373	22.2	0.10	2.64	2.74	0.26*	1.86	4.98	6.84	0.58*
	18-24	290	22.5	0.10	2.53	2.63	0.35*	1.51	4.38	5.89	1.45*
	24-32	457	22.7	0.10	3.02	3.12	0.10*	2.34	5.49	7.83	0.15*

* Owing to pig 1192 latterly refusing to consume the whole ration the average daily intake for the last three periods was 3.00, 2.98 and 3.22 g. CaO; and 7.42, 7.34 and 7.98 g. P₂O₅.

Table II. Exp. 2. *Average daily excretion and balances in grams.*

		Calcium (intake: 11.94 CaO)						Phosphorus (intake: 12.77 P ₂ O ₅)				
Days		Wt. of faeces	Wt. of pig (kg.)	Urine	Faeces	Total	Balance	Urine	Faeces	Total	Balance	
Irradiated pig 1322.												
Light	{ C.L.O.	1- 8	447	37.5	0.41	6.50	6.91	5.03	0.85	6.69	7.54	5.23
		8-16	510	40.2	0.46	7.06	7.52	4.42	0.87	7.12	7.99	4.78
		16-24	397	43.1	0.42	6.12	6.54	5.40	0.60	6.36	6.96	5.81
		24-32	463	45.2	0.31	6.81	7.12	4.82	0.69	7.14	7.83	4.94
		32-40	391	48.1	0.51	6.32	6.83	5.11	0.88	6.61	7.49	5.28
		40-50	398	51.6	0.56	6.52	7.08	4.86	0.87	6.73	7.60	5.17
Non-irradiated pig—in darkness 1321.												
C.L.O. last 6 days	{	1- 8	489	37.1	0.37	7.10	7.47	4.47	1.21	6.90	8.11	4.66
		8-16	459	38.4	0.30	7.77	8.07	3.87	1.40	7.84	9.24	3.53
		16-24	458	42.6	0.26	6.52	6.78	5.16	1.33	7.42	8.75	4.02
		24-32	378	45.1	0.27	6.24	6.51	5.43	1.04	6.53	7.57	5.20
		32-40	420	48.1	0.31	6.47	6.78	5.16	1.00	7.51	8.51	4.26
		40-50	401	51.7	0.21	6.57	6.96	4.98	1.14	6.71	7.85	4.92
Non-irradiated pig—in diffuse light 1323.												
	{	1- 8	445	33.8	0.51	7.75	8.26	3.68	0.58	7.82	8.40	4.37
		8-16	479	36.2	0.45	7.85	8.30	3.64	0.60	7.63	8.23	4.54
		16-24	423	38.9	0.28	7.10	7.38	4.56	0.78	7.11	7.89	4.88
		24-34	388	42.2	0.29	6.32	6.61	5.33	0.65	6.55	7.20	5.57

Results. Fig. 1 shows the calcium and phosphorus balances for each pig. The abscissae mark days, while the ordinates represent retention of CaO and P_2O_5 in g. per diem. Before irradiation was begun the CaO and P_2O_5 balances were fairly steady, the CaO having shown a slight decrease. Comparing the experimental with the pre-period in the irradiated animal the following points may be noted:

Fig. 1. Retention of calcium and phosphorus in irradiated and non-irradiated pigs.

Fig. 2. Showing rise in urinary and fall in faecal excretion of P_2O_5 in irradiated pig.

(1) Both curves show a dip immediately after irradiation was begun, the pig actually reaching a negative calcium balance on the fourth day of irradiation. Thereafter there is a definite and steady rise. The rise is most marked in the case of the calcium retention. The average retention of CaO per diem during the pre-period was .46 g.; during the irradiation period it rose to an average of 1.45 g. The corresponding figures for P_2O_5 retention were 1.88 g. and 2.07 g. respectively.

(2) The P_2O_5 and CaO balance curves approach each other in the experimental period and run a parallel course.

(3) The ratio of the urinary to the faecal excretion of calcium and phosphorus rises markedly during the experimental period. As regards the calcium, of the total CaO excreted in the pre-period 2.51 % was excreted in the urine; in the experimental period this figure rose to 6.94 %. An average of 18.21 % of the total P_2O_5 was excreted in the urine during the pre-period, and of 32.59 % during the experimental period. Fig. 2 shows the rise in urinary and fall in faecal excretion of P_2O_5 which occurred during the experimental period. It will be seen that, towards the end, the excretion in faeces and in urine was almost equal.

Non-irradiated Pig. After the tenth day of the experiment the non-irradiated pig suffered from gastro-intestinal disturbances, periods of diarrhoea and constipation alternating. As a result of these conditions balances became very irregular and it was impossible to graph 48-hour results. By averaging varying periods, however, it was obvious that the balances were gradually falling. This is not surprising in view of previous results [Husband, Godden and Richards, 1923] in which "pigs kept in metabolic cages and fed on a grain ration showed a progressively decreasing capability of absorbing and retaining CaO and P_2O_5 ." In the present case the average balances will be seen from Table I. Fig. 1 also shows the marked contrast between the rising retention of the irradiated pig and the falling retention of the non-irradiated pig. In the case of the latter, average periods are graphed: in the former, however, the balance from day to day is set out. The clinical picture corresponded with these findings, for the non-irradiated pig became less active than the irradiated, showed some loss of appetite and, on one occasion at least, vomited its food. In this condition irradiation, when applied for 10 days before the animal was killed, failed to have a beneficial effect; indeed, it possibly accelerated the downward trend. The balances at least became more irregular still. There was a rise in the ratio of urinary to faecal excretion with respect to both CaO and P_2O_5 . Thus before irradiation 2.08 % of the CaO excreted appeared in the urine; after irradiation 3.36 % was thus excreted. As regards the P_2O_5 , before irradiation 21.3 % was excreted in the urine; after irradiation this proportion rose to 29.8 %.

Table I shows the weight increments for the irradiated and for the non-irradiated pig. The weight gained over six weeks is remarkably small. When the pigs were put in the metabolism cages each weighed 21.0 kg. Forty days later, *i.e.* at the end of the experiment, the non-irradiated pig weighed 22.2 kg., and the irradiated pig 22.4. The latter, however, showed no signs of malnutrition.

Post-mortem Findings. The following are the results of the analyses of the blood and bones. In each case the right radius and ulna and the right tibia and fibula were removed and analysed separately.

	Irradiated Pig 1193		Non-irradiated Pig 1192		Pig 1168		Pig 1112	
	Tibia and fibula	Ulna and radius	Tibia and fibula	Ulna and radius	Tibia and fibula	Ulna and radius	Tibia and fibula	Ulna and radius
Wet weight of bones ...	140.99 g.		108.93 g.					
% ash in dry fat-ex- tracted bone ...	45.86	43.39	39.77	41.23	43.00	43.92	39.48	39.77
% CaO in dry fat-ex- tracted bone ...	24.25	23.35	20.84	22.17	21.28	22.86	20.73	21.02
% P ₂ O ₅ in dry fat-ex- tracted bone ...	20.32	18.39	18.12	17.39	18.71	19.51	15.36	16.92
Blood: CaO ...	12.9 mg. per 100 cc.		16.3 mg. per 100 cc.					
P ₂ O ₅ ...	138.4	100	143.1	100				

(Figures for two pigs, 1168 and 1112, from another experiment are placed here for comparison. These pigs showed definite clinical symptoms of rickets and on post-mortem examination there was "marked beading of the costochondral junctions" and "very marked beading" respectively, as also definite histological appearances of rickets.)¹

In neither animal was any pathological condition found. Neither gross nor histological signs of rickets were observed in the bones. What appeared to be the only abnormality was an enlarged thyroid in the case of the non-irradiated pig.

Experiment 2. Object. Having obtained a definite effect of irradiation on the calcium and phosphorus balances when a badly balanced diet was being fed, it was determined to investigate the effects of irradiation, darkness and diffuse light when the animals were on a well-balanced ration.

Animals. Three comparable hog pigs, eleven weeks old were selected from the same litter.

Housing. The general conditions were identical with those in Experiment 1. As regards lighting, there were the following differences between the experimental conditions of the three animals.

(1) Pig 1321 was kept in a darkened metabolism cage and received no irradiation.

(2) Pig 1322 was kept under the same conditions, but had one hour's irradiation daily.

(3) Pig 1323 was kept in a metabolism cage without a canopy. That is, it was in the ordinary diffuse light of the animal room; it received no irradiation.

The pig in the dark and the irradiated pig occupied the cages for 66 days; the irradiation period of the latter was 42 days. The pig in diffuse light occupied the cage for 45 days.

Diet. The diet fed daily consisted of oatmeal, maize-meal, middlings—400 g. each. To this was added 50 cc. of a 10 % solution of NaCl; 20 g. CaCO₃, 40 g. blood-meal and 10 cc. olive oil. In the case of the irradiated pig and of the pig kept in the dark, 30 cc. of cod-liver oil was added to their daily ration from the 32nd and the 44th day respectively of the experiment.

¹ I am indebted to Dr J. P. McGowan for these specimens.

The intake per diem on the above ration was 11.94 g. CaO, 12.77 g. P_2O_5 and 28.83 g. N. The mineral ratios of this diet are roughly as follows:

	Diet	Sow's milk
CaO : P_2O_5	12 : 13	10 : 9
CaO : MgO	12 : 5	20 : 1

It is thus a better balanced diet than that employed in Experiment 1, particularly with respect to its calcium, phosphorus and magnesium content. NaCl and olive oil were added on account of their beneficial effect as indicated in some experiments at this Institute. The fat-soluble vitamin content of the ration is low. All experimental conditions were similar to those of Experiment 1.

In the case of the irradiated pig the analyses were carried out for 50 days. This was made up of an 8 days pre-period, and 42 days irradiation: during the last 18 days it was receiving in addition 30 cc. of cod-liver oil daily. Analyses of the excreta of the pig kept in darkness were conducted for 50 days, during the last six of which it received daily 30 cc. cod-liver oil. The analysis period of the pig in diffuse light lasted 34 days. Sixty-six days after being first put in the cages the irradiated pig and the pig in darkness were killed. Autopsies were done and analyses of the blood and bones.

Results. Table II (p. 54) shows the balance in the case of the irradiated pig. It will be seen that following irradiation there is a slight dip, but this is later compensated for. Despite oscillations, the balances during the irradiation period show no very definite tendency up or down. Since it was thought that the animal might be on a maximum retention of CaO and P_2O_5 , 30 cc. of cod-liver oil was added to the daily diet on the 32nd day. There followed a depression and later an elevation of the balance: the average, however, remained practically the same as in the preceding period. In this connection it must be remembered that the animal had from the beginning of the experiment been receiving olive oil [Husband, Godden and Richards, 1923].

Table II gives also the average balances of the pig kept in darkness. On the whole they show no marked tendency to rise or fall. The cod-liver oil period towards the end is admittedly short, but sufficient to have shown a rise had such been striking. A characteristic feature of the table is the tendency of the P_2O_5 balance to fall below that of CaO. Further the P_2O_5 and CaO curves when graphed in detail show a decided departure from their general parallelism on the 20th and on the 42nd day. On these dates the CaO balance is raised and the P_2O_5 markedly depressed.

The parallelism of the calcium and phosphorus curves in this animal is not so striking as in the irradiated pigs of either Experiment 1 or 2, or as in the "diffuse light" pig of the latter experiment.

It will be noticed from Table II that the balances of the pig in diffuse light start at a slightly lower level than in the other two pigs. In this case, however, the tendency is undoubtedly upwards during the whole metabolic period with the P_2O_5 in general above the CaO.

The following table gives a rough comparative idea of the tendencies of

the balances in the three cases. It will be seen that while the intervals represent the pre-period, the period of irradiation alone, and the period of irradiation + cod-liver oil in the irradiated pig these merely correspond to arbitrary periods in the other animals. The table, however, serves the purpose of a broad summary.

Average retention of CaO and P₂O₅ per day.

Period	Irradiated pig		Pig in darkness		Pig in diffuse light		Remarks
	CaO	P ₂ O ₅	CaO	P ₂ O ₅	CaO	P ₂ O ₅	
First 8 days	5.0	5.2	4.4	4.7	3.7	4.4	Pre-period for irradiated pig
Next 24 days	4.9	5.2	4.8	4.2	4.7	5.0	Light period for irradiated pig
Next 18 days	4.9	5.2	4.9	4.6	—	—	{ Irradiated pig, light: cod-liver oil. { Darkness pig: 6 days cod-liver oil.

As regards the ratio of urinary to faecal excretion of CaO and P₂O₅ there is little to note. It varies within fairly narrow limits and shows no special tendencies.

The weight increments of all three pigs were comparable and show a steady rise (Table II). The irradiated pig put on, on the average, .34 kg. per day, the pig in darkness .35 kg. and that in diffuse light .36 kg. The last, however, had been about 1.5 kg. lighter than the others at the beginning of the experiment.

Post-mortem Findings. The following are the results of the analysis of the blood and bones of the irradiated pig and that kept in the dark. In each case the left ulna and radius were used for analysis.

	Irradiated pig. Ulna and radius		Pig in darkness. Ulna and radius	
Wet weight of bones	99.2 g.		101.9 g.	
% ash in dry fat-extracted bone	55.65		52.48	
% CaO in dry fat-extracted bone	29.57		28.06	
% P ₂ O ₅ in dry fat-extracted bone	21.44		19.90	
Blood: CaO	9.43 mg. per 100 cc.		9.24 mg. per 100 cc.	
P ₂ O ₅	121.2 " 100 "		98.5 " 100 "	

No abnormal condition was observed in either pig. Sections of the ribs showed normal calcification in each case.

DISCUSSION OF RESULTS.

In considering the results of Experiments 1 and 2 the main difference is the marked contrast between the irradiated pig and its control in Experiment 1, and, on the other hand, the comparatively small differences in the second experiment between the irradiated pig, the pig in diffuse light and that kept in darkness. We are thrown back upon the conception that the balance of the diet is of fundamental importance [Orr, 1924]. When this is well adjusted the influence of light would seem to be at a minimum; for all three pigs in Experiment 2 grew normally, remained in good condition and, on the showing of their balance curves, retained adequate and fairly consistent amounts of calcium and phosphorus. On the other hand, in Experiment 1 neither pig grew, and the irradiated pig, which retained more calcium and phosphorus, alone remained in fair condition.

In the first experiment irradiation caused a marked rise in the retention of calcium and phosphorus but it is noteworthy that in each pig there was a slight dip in the balance curves following immediately upon irradiation; in the experimental pig a negative calcium balance was actually reached. Even in the irradiated pig of Experiment 2 there is a suggestion of a depression immediately after irradiation. This may be a specific effect of the light representing a negative phase or at least a period of re-adjustment. On the other hand the depressions of the curves in Experiment 1 were contributed to by an increased faecal excretion, for both pigs after irradiation suffered from diarrhoea for some days. Grant and Gates [1924] report cases of diarrhoea occurring among rabbits after irradiation.

Apart from stimulating the retention of calcium and phosphorus it appears that irradiation has a stabilising effect. Under its influence these minerals are probably retained in an optimum ratio. Both in Experiments 1 and 2 there is a well defined parallelism of the calcium and phosphorus retention curves during irradiation.

The relation of urinary to faecal excretion demands some notice. In the irradiated pig in Experiment 1 one of the most marked results of irradiation was the increase in urinary phosphorus and calcium. It will be seen from Fig. 2 that, while the total excretion was diminishing, the urinary excretion was steadily increasing. The same result, though in lesser degree, was obtained when in turn the non-irradiated pig was irradiated (Table II). There was, however, no such result following upon irradiation in Experiment 2. The increase in urinary excretion is a feature, then, of irradiation of the pig on a badly balanced diet; it is at least not marked when the diet is well balanced. In the treatment of rachitic children a similar result may be observed [Orr, *et al.*, 1923]. This increased excretion *via* the kidney would indicate that there has been on the part of the intestine a greater absorption, a diminished excretion, or both.

In view of recent work [Telfer, 1923] it is probable that the calcium and phosphorus of the faeces consist mainly of unabsorbed residues. If this be so, the effect of the irradiation is to cause an increased absorption by the bowel wall and thus present a greater amount of calcium and phosphorus to the organism. From this reasoning it is obvious that the increase in CaO and P_2O_5 absorbed by the bowel under the influence of light is even more than what is indicated by a graph of the total retention.

The increase in urinary excretion, particularly of phosphorus, also suggests that it is possible as the result of irradiation to induce absorption by the gut at a rate greater than the utilising mechanism can cope with. In the case of the non-irradiated pig in Experiment 1 there was, judging by the criterion of increased urinary excretion, increased absorption but little evidence of utilisation.

It might have been expected that even on a well-balanced diet and a maximum retention of calcium and phosphorus there would have been an

increase in the amount of these minerals in the urine following irradiation. Table II indicates that there was no increased absorption with rejection of the superfluous calcium and phosphorus. It may be that not only was the animal retaining a maximum but that in the circumstances a maximum was being absorbed by the wall of the gut. Thus neither irradiation nor cod-liver oil could cause any increase in absorption.

The failure of growth in the irradiated pig in Experiment 1 is striking. By many it is assumed that growth is essential to rickets [Parks, 1923] and it seems at first sight an attractive theory that irradiation may in part exercise its anti-rachitic effect through inhibiting undue general growth; that failure to grow is really a protective mechanism. There are, however, too many results showing growth under the stimulus of light to make this idea tenable. At the other extreme there are workers who, as a result of experiment, hold that the "growth factor" and the "anti-rachitic factor" are identical [Steenbock and Nelson, 1923]. In the present instance of the irradiated pig in Experiment 1 it is obvious that light may stimulate the retention of calcium and phosphorus without causing any concomitant increase in general body growth. Webster and Hill [1924] have recently pointed out an interesting example which, in their opinion, suggests that "the radiant energy necessary to promote growth appears to be other than that required to cure rickets." In the case of a rat which on irradiation showed no increase in growth over the controls, the P_2O_5 content of the carcass was nevertheless appreciably higher.

The analyses of the bones as detailed above reveals a difference in the direction one would have been led to expect. Thus, averaging the two sets of bones in Experiment 1, it is found that those of the irradiated pig contain 4.6 % more CaO and 3.2 % more P_2O_5 than those of the pig in darkness. As regards the corresponding pigs in Experiment 2 the figures are respectively 1.15 % and 1.14 %. That a terminal analysis of bones, however—unless it be the whole skeleton—gives only a rough indication of the amounts of CaO and P_2O_5 absorbed and deposited, is seen from the fact that had the ulna and radius alone been compared in corresponding pigs no marked difference would have been observable.

It will be noticed that in Experiment 2 the irradiated pig has a slightly higher blood calcium and a markedly higher blood phosphorus. The phosphorus retention in this pig had been greater than in the pig kept in darkness. But in Experiment 1 the control pig has a higher blood content of both minerals. It must be remembered, however, that this control had been irradiated for the previous 10 days and that while it was not retaining and depositing calcium and phosphorus it was absorbing considerable quantities as is shown by the urinary excretion of CaO and P_2O_5 . It is not surprising that the blood calcium and phosphorus are high after irradiation, particularly in an animal which is not depositing these minerals. It is doubtful, however, if much can be deduced from the figures for the blood analysis. There are possibly in pigs marked individual differences in the CaO and P_2O_5 content

of the blood. Had it been possible the variation under light treatment would have been followed out in each individual pig but this was found incompatible with the success of the metabolic experiment since it was feared that the disturbance occasioned the pig by taking a blood sample might upset the balances.

SUMMARY.

(1) In a pig on a diet badly balanced particularly with respect to phosphorus, calcium and magnesium ($P_2O_5 : CaO : MgO—3 : 1 : 1$) 1 hour's irradiation daily for 24 days with a carbon arc lamp definitely increased the calcium and phosphorus retention as compared with a control animal kept in the dark. The balance curves for these minerals tended to approach each other in the light period and run a parallel course.

(2) Following upon irradiation the calcium and phosphorus in the urine rose, both actually and relatively to their excretion in the faeces, suggesting an increased absorption from the gut or a decreased excretion.

(3) The above results took place in the irradiated pig without any concomitant increase in body weight. This would indicate that a stimulus to calcium and phosphorus retention does not necessarily involve a stimulus to growth in general.

(4) In pigs on a satisfactory diet, well balanced particularly with respect to calcium and phosphorus ($P_2O_5 : CaO—1 : 1$), darkness, diffuse light (through glass) and irradiation of 1 hour per day for 52 days caused little difference in the calcium and phosphorus retention, though such difference as there was, was in favour of the irradiated pig.

(5) In the irradiated pig on a well-balanced diet no tendency was observed for the urinary calcium and phosphorus to increase after irradiation.

(6) Analyses of the bones were in general agreement with the findings of the metabolic analyses.

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