# University of Glasgow

# LIST OF DEGREES

## Conferred on 16th October, 1922

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### DOCTORS OF MEDICINE (M.D.).

I. With Honours.

Alexander Scott, M.B., C.M.—Thesis: "The Dermatoses of the Paraffin Workers of the Scottish Shale Oil Industry."

II. With High Commendation.

William M'Kendrick, M.B., Ch.B.—Thesis: "An Inquiry into the Possible Diagnosis of Enteric-group Carriers by means of a Cutaneous Reaction."

III. Ordinary Degree.

Duncan Cameron, M.B., Ch.B., B.C.(Cantab.), F.R.C.S.(Eng.)—Thesis: "Causes and Treatment of Primary Inertia Uteri."

### MASTER OF SURGERY (Ch.M.).

Spencer Mort, M.D., Ch.B., F.R.C.S.Ed., F.R.S.Ed.—Thesis: "Surgical Work at North Middlesex Hospital, Edmonton; A record of operative and clinical work."

### DOCTOR OF SCIENCE (D.Sc.).

Walter Elliot Elliot, B.Sc., M.B., Ch.B., M.P.—Thesis: "Study in Mineral Metabolism with special reference to Rickets in the Pig and similar bone lesions in other animals and in the Human."

### BACHELORS OF MEDICINE AND BACHELORS OF SURGERY (M.B., Ch.B.).

I. With Honours.

Robert Campbell Garry.

II. With Commendation.

Catherine Harrower.
Robert Crawford.
James Grant.
Alexander Thomson Hastie.
Catharine Boyd, M.A.
Thomas Wishart Davidson.
Robert Coubrough Hamilton.
Mary Poldores MacCunn.
William Douglas Hood.
Arthur Anderson, M.A.
James Wilson Hamilton.
Thomas Nicol.
James Hamilton Ramage.
Thomas Wilson Howie.
James Cook.

## III. Ordinary Degree.

Alexander Wallace Aird.
Margaret Alexander.
Robert Gavin Mason Alexander.
Isabel Pattie Allan.
Andrew Anderson.
William Cullen Andrew.
Margaret Russell Balloch.
Abraham Lewis Bernstein.
Catherine Alexandra Samuella Blair.
Mary MacQueen Bonnyman.
Mary Dickeson Allan Boyd.
Frederick Andrew Brown.
Robert Orr Bruce.
Barbara Adam Cameron.
Janet Russell Campbell.
Margaret Elizabeth Campbell.
Margaret Gilchrist Carrick.
Nora Teresa Cassidy.
Gladys Muriel Chapell.
Horace Collingbourne.
Margaret Eunice Colville.
Elizabeth Sym Cook.
John Stevens Cook.
Gerhardus Cornelius Cruijwagen.
John Spreull Currie.
Isabella Currie Darling.
Agnes Christian Johnstone Davidson, M.A.
Margaret Davidson.
James Dawson.
Isabelle Anderson Deans.
Agnes Foster Dickson.
Lilian Margaret Dickson.
James Smith Donaldson.
Alexander Finlayson Dunn.
Amy Margaret Fleming, B.Sc.
Elizabeth Hamilton Forrest.
Andrew Fyfe.
Archibald Gardiner.
Andrew Vint Garscadden.
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Margaret Brown Graham.
Ross Alfred Grant.
William Nicol Gray, M.A.
Hugh Graham Halliday.
Mary Nisbet Hendry.
George John Hutchison, M.A.
Elizabeth Groat Jamieson.
Jessie M'Leod Caldwell Jamieson.
Mary Kirk Jeffrey.
Tudor Jenkyn Jones.
Frederick Cairns Laing.
James Edwin Stanley Lee.
Alexander Leitch.
James Lindsay.
George Watt Lochhead.
Isabella Lumsden.
William Wilson Lundie.
Herbert M'Cluskey.
Joan Alexandra MacColl.
John Woods M'Conville.
Allan Anderson Macdonald.
Chrissiebelle Macdonald, M.A.
Thomas John M'Kail.
Kenneth Wallace MacKenzie.
Marion Allan M'Kenzie.
George MacKerracher.
Murdoch M'Lean.
Neil Alexander MacLean.
Samuel M'Mahon.
Robert Carmichael MacMurray.
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Marion Lesley M'Quaker.
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Doris M'Walter.
Agnes Lees Mair.
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Hugh Robertson Melville.
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William Thomson Mitchell.
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David Robert MacPherson Morrison.
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William Muir.
James Paton Neilson.
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James Nicholson.
James Leslie Orr.
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Louise Baker Pollok.
Thomas Core Porter.
Mary Elizabeth Proudlove.
Thomas Dryden Pyle.
John Steven Ramage.
Elizabeth Chapman Rodger.
Bessie Scott Ross.
Mrs. Sarah Ross.
John Russell.
Bertha Eileen Annetta Sharpe.
William Simpson.
Christina Reid Inglis Sloan.
Andrew Snaddon.
James Sommerville.
Jack Fingland Martin Stenhouse.
Effie Scott Stephen.
Mary MacPherson Stevenson, M.A.

Jeanie Margaret Strathie.
Thomas Tannahill.
Hamilton George Bryce Teggart.
Robert Wilson Todd.
Margaret Stevenson Watt.
Jemima Waugh.
Janet Anne Orrok Weir.
Alexander Fraser Whyte.
Catherine Barclay Wilson.
James Hugh Wilson, M.A.
John Wight Wilson.
Joseph Houston Wright.
Grace Margaret Young.
John Alistair Kennedy Young.

## BACHELORS OF SCIENCE (B.Sc.).

### In Applied Chemistry.

John Allan.
[1] Samuel David Carson.
[2] Munro Steel Fisher.
Robert Higgins, A.R.T.C.
James Muil Leitch.
Alexander Henderson Campbell Page.
Gilbert Bruce Robertson.

[1] With Special Distinction in General Technical Chemistry and Chemical Engineering.

[2] With Special Distinction in Metallurgy.

Mr. Robert Campbell Garry who graduated on 16th October, 1922, gains the Brunton Memorial Prize of Ten Pounds awarded to the most distinguished Graduate in Medicine of the Year.

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# PREFACE.

This work, first discussed in the laboratories of the London Hospital in the spring of 1919 has been carried out in 1920 and 1921 with much less than the undivided attention which one would have wished to devote to an investigation of this size and importance, owing to the heavy pressure of other work. I have to acknowledge very gratefully the great debt under which I lie to the staff of the Rowett Institute and to others. First, to Mr. Crichton in charge of the Animal Department on whose shoulders fell a large part of the burden of the lengthy feeding experiments. Next, to Mr. Husband, A.I.C. to whom I am also indebted for analytical data on pigs 43 and 83.

Finally to Dr. Orr, M.D., D.Sc., Director of the Institute my thanks are chiefly due. He was unfailingly fertile in suggestion, and his interest and energy were of the very greatest assistance in overcoming difficulties.

Walter Elliot.

# A STUDY IN MINERAL METABOLISM.

## INTRODUCTION.

Only two, or at most three, of the eighty-odd elements scattered up and down the octaves of Mendelejef's Law have received any detailed consideration from the biologist. Even after the destruction of the old division into "organic" and "inorganic" substances, the overwhelming discovery that all the energy-yielders of life could in general be reduced to carbon, hydrogen, and oxygen, with nitrogen thrown in to cover the proteins, led to the concentration of two generations of physiologists upon the riddles of the metabolism of carbon and nitrogen. They saw the body structures as a dark street of shuttered houses, from which came the music and rhythm of carbons and nitrogens, partnering in the linked procession of their interminable quadrilles, and they strove more and more to peer through and study the weavings of these vivid dancers alone, ignoring the other atoms of the universe as no more than the frame to a picture.

At the end of last century the bent of men's minds towards a conception of nitrogen and carbon as in some special way "essential" was reinforced anew by a great body of laborious and brilliant work such as that upon the amino-

acids by Emil Fischer and his colleagues. The study of metabolism became almost the study of nitrogen excretion with here and there a calorimeter apparatus to determine the production of $CO_2$. This attitude we can trace even yet, embalmed in the word "vitamin"; for having discovered some factor or rhythm essential to growth or, may be, to life itself, researchers labelled it "vitamine" in the sure and certain hope that it too would prove merely another manifestation of these alchemic molecules, the $NH_2$'s. Lopped of its final "e", the word persists; though the factors are now shown to be no more vital than many others, and whatever their constitution, to be apparently devoid of any trace of nitrogen.

The time has come to draw away from this attitude, and to stress the importance of the other elements of the body. There is a phosphorus metabolism as there is a nitrogen metabolism, a calcium metabolism, a magnesium metabolism, an iron, a chlorine, an iodine and still more a sulphur metabolism. The fact that phosphorus, the very weft of the nucleus, can be assimilated direct from mineral sources, rather increases than diminishes its importance. Again we are only beginning to learn the controlling part played in the tissues by the ions of calcium and their necessity ever to such intimate body-building as the healing of a wound. The conception of the bones as the mere girders of the body is obviously out of date, as the persistence of the osteoclastic mechanism should

have made us suspect; they have at least this additional function as reservoirs of salts as vital to the economy of the tissues as the glycogen of the liver. Indeed, when one considers that the exo-skeleton of the crustacean is merely an afterthought utilisation of a waste product, it is not impossible to conceive that the endo-skeleton of the vertebrate may have originated not so much in a pillar as in a fly-wheel. This much is certain; that the ionised mineral content of the blood is as essential to life as any other of its constituents; and that when protoplasm moved out from the salt-water beaches it carried, enclosed in its structure and bathing every fibre of its tissues, a mineral solution without which it could not persist and probably could never have originated.

Little of the physiology of these mineral metabolisms has been determined and their pathology must be discussed with the greatest reserve. The process of utilising so obvious an element as iron is still almost unknown, nor are we any clearer about chlorine. Iodine, with its ductless gland the thyroid, has attracted some degree of attention; it has here been forced on our notice how strikingly the minutest traces of a mineral substance may affect the development of the whole body. The particular mineral metabolism chosen for the purpose of this thesis is that of calcium and phosphorus in relation to the physiology and pathology of bone formation. The quantities of these two elements ingested and

retained are large. We have a histological knowledge of the normal process of retention in ossifying tissue, and by chemical analysis of the bones themselves we can further check our conclusions. For these reasons it was decided to compare the calcium and phosphorus metabolism in health and in the rickets syndrome. True, we know nothing of the mechanism by which these two elements are dealt out to the tissues, save that for calcium an endocrine regulator of some kind exists in the parathyroids; but we are no better off in regard to any of the other minerals.

The conditions discussed in this thesis are, for the most part, those arising in the feeding of the larger domestic animals. The mass of material here available for investigation is not perhaps sufficiently admitted by experimental biologists. The number of animals subject to review is enormous; the sheep population of these islands was in 1920, 23,000,000; the pig population 3,100,000; the cattle population nearly 12 million. Already a great body of accurate and continuous clinical observation exists, and masses of data carefully recorded though not according to laboratory formulae. Such documents as the stud-books, the milk-records, and the meat-market statistics can and should be utilised for consideration. The meat industry is really a gigantic series of feeding experiments where weighed quantities of food are fed to weighed animals, the results checked in every case by

a post-mortem, and checked again by a system of serial sections which has no parallel amongst any but the most exacting of our pathologists. Four hundred thousand store cattle, i.e. experimental animals, are purchased annually from Ireland alone; their progress daily examined by the skilled clinical eye of the expert stockman; and finally verified by the life-long experience of the butcher and the housewife.

What is more, huge numbers of these animals share with convicts, babies and sailors, the infliction of a ration prescribed from outside and selected rather by reason than by instinct. The baby, powerless to influence its diet or environment, is classically subject to disturbances of its calcium metabolism. The young pig or puppy, similarly confined, is a frequent victim of the same disorder. Even in adult humans a rigid though reasoned control of foodstuffs produced the deficiency disease of Eijkmann's prison patients, and the traditional scurvy of long sea voyages. It should therefore be instructive to examine in detail the metabolic disorders of the masses of imprisoned stock.

Man, economically dependent on, and physiologically symbiotic with, his flocks and herds, has not entirely neglected even the recondite question of their mineral requirement. The craving of graminivora for common salt is recognised both by the farmer who lays down rock-salt in his fields, and by the hunter who waits at the "salt-lick" for the game which

will certainly arrive. Bunge's ingenious interpretation of this craving, as inspired by the desire of the animal to substitute Na for the K which is present in excess in all green plants, probably explains why this particular need should be the most generally recognised. The requirements of stock for calcium and phosphorus however are seldom specifically mentioned. The ordinary field herbage, if the earth is adequately rich, usually contains a sufficient supply of both, and the strengthening of the soil by lime and phosphates, either singly or in combination, is one of the hall-marks of good husbandry.

Cases however do occur in which, through a deficiency in the soil, a deficiency in the plant and so in the animal nourished by it, is brought about. These will be discussed throughout this thesis as they arise.

The animal most usually suffering from a disordered calcium or phosphorus metabolism in this country is the pig, which is often cut off completely from access to the soil and fed largely on a grain diet in which the mineral proportions are extremely ill-balanced. It is very noteworthy that for this animal the supply of inorganic substances has been empirically recognised as of the first importance and that all pig-keeping manuals emphasise the importance of an "earth-box" containing a wide range of mineral substances.

The grass-eating ruminants have a more natural diet, and

the domesticated carnivores have usually access to large quantities of calcium, phosphorus, etc. in the bones of meat. The confinement of puppies and the selection of their diet, either for pedigree purposes or in hunting breeds, is associated with metabolic disorders which are rarely encountered when the dog is permitted to run free. Especially is this noticeable since the practice arose of keeping hounds on a large scale in kennels instead of, as previously, boarding them out with the farmers of the district.

Is it possible to determine the amounts of calcium and phosphorus required by domestic animals (1) in growth (2) in maturity? A direct attack on this problem has been made by Voit (1) and others, and many of these results are confirmed by later work and also by the data of this thesis. Indirect reasoning will also show the necessity for a minimum intake, especially for the period of growth. It is certainly not possible to say what absolute amounts will be sufficient, since the salt, though present in sufficient amount, may be in unsuitable combination, or wrongly balanced against another mineral, or may not be absorbed owing to some intestinal cachexia. In addition there is, so to speak, an endogenous and an exogenous mineral metabolism, and not all the mineral ingested can be retained. But it is possible to calculate from the carcass composition what quantities will be <u>insufficient</u>. Lawes & Gilbert (2), Tschirwinsky (3), Jordan (4),

Wilson (5) and others, carrying out complete analyses of cattle, sheep and swine, give the ash content of the body weight as ranging above 3.0 per cent. It is never less, unless in one or two cases of fattened swine, and in some cases of lean animals runs as high as 5 or 6 per cent. About half of this ash may be taken as CaO. and this corresponds with the Rothamsted analyses (6) of various farm animals which give calf carcass at 1.07 per cent CaO, sheep at 1.32 per cent CaO and pig at 1.07 per cent CaO. We may conclude that for these animals CaO to the amount of 1 - 1.5 per cent of the increase in body weight should be ingested between birth and maturity. This conclusion is closely confirmed by Loew's (7) finding that for the human infant 1.2 grams CaO is required for each 100 grams increase in body weight. It may be taken in general that a diet containing less than one grm. CaO or $P_2O_5$ per cent of increase in live weight secured, is a diet already very deficient in some of the essentials of life.

McCollum (7A) in his recent book points out that the seeds, such as wheat, fail to supply enough of many of the essential inorganic elements. In several recent dietary studies not only has this been ignored, but experimenters specifically state that the animal was receiving a sufficiency of calcium which is arithmetically impossible on the figures supplied - Mellanby, (8) dieting a puppy, provided a Ca intake which works out at 2.45 grms. CaO per week while the animal was increas-

ing in weight at 300 grms. a week. This would require the retention of ~~energy~~ every milligramme ingested and still leave a deficiency of .5 gram per week. This experiment is merely one chosen at random from his recent monograph on an extensive investigation involving the use of some 400 experimental animals. It is claimed by him (l.c.), that "this research has centred round calcium metabolism" which makes the subsequent statement (l.c.), that "the problem of deficient calcium intake has received comparatively little consideration in this research", the more extraordinary. Repeated references to the need for "a sufficiency of calcium", "plenty of calcium and phosphorus in the diet", etc. are summed up on p.55 in reply to the query "What then is an adequate calcium intake?" by the answer "There is no absolute amount that can be described as adequate.....it is all a question of balance." This omits the fact that since the body is unable to synthesise calcium, a theoretical minimum for a theoretical growth of bone corresponding to an actual increase of weight, is not impossible to estimate. This minimum is neglected by him in many of his experiments and these are therefore without value.

Weiser (9) showed that even practical farmers have frequently failed to observe that the mineral deficiency in pure grain diets fed to rapidly growing animals may reach very large amounts, and in certain mixtures frequently fed to pigs the

creature would require to consume upwards of 36 lbs. per day to obtain a calcium sufficiency.

Intensive scientific observation on mineral requirements commenced with Chossat in 1842. He gave food deficient in calcium to young dogs and produced a clinical picture very similar to rickets. Milne-Edwards (1861), Guerin (1862), Roloff (1866) (10), Miwa and Stoeltzner (1898) (11) and ~~Rumor~~ Reimers and Boye (1905) (12) obtained similar gross results. In these experiments they found swelling of the epiphyses with softening and various curvatures of the bones. In most of the work, however, histological results were either not made or did not coincide with the conditions found in true rickets.

Miwa and Stoeltzner fed a dog on fresh horse meat and bacon, with distilled water. A waddling gait developed as early as the 10th day and epi~~phesal~~ physeal swellings within 20 days. At the end of the eighth week the dog showed a rachitic rosary and bending of the long bones. Histological examination showed irregular lines of ossification; the layer of proliferating cartilage was extended, the arrangement of the columns of cartilage cells was irregular and the cartilage cells themselves were enlarged. The preliminary calcification of cartilage was however quite normal and the thick masses of tissue, entirely without lime, usually present in rachitis, were not found by them. The authors considered

the process as a generalised osteoporosis with rachitis-like changes in the periosteum and in the uncalcified proliferating cartilage.

In the experiments of Reimers and Boye (l.c.) the compact bone substance was found very small, but the extension of the proliferating cartilage was only slight. Proliferation of the osteoid tissue had not taken place at all.

Voit (13) however (1877) and Baginsky (14) (1881) after withholding calcium from dogs described histological changes very similar to those universally admitted to be rachitic. It may also be remarked that even in the case described by Miwa and Stoelzner the clinical picture would have led to its diagnosis as rickets in the human. If this syndrome is only to be distinguished by minute histological differences it would be well to consider whether rickets conforming in every detail to the description would prove any more frequent in the infant than in the puppy.

Mellanby, for instance, considers (l.c. p.12) that neither clinical nor radiographic evidence is diagnostic of rickets, nor is a histological picture showing hypertrophy of the proliferating cartilage, invasion of the cartilage by marrow vessels, and absence of calcification at the cartilage-bone junction, unless excess of osteoid tissue is also present.

With this view one may join issue. The result of recent

experiments gives reason to think that the present meticulous differentiation between osteoporosis and rickets will not bear the emphasis which it has received. It seems not impossible that one essential factor in producing the microscopic appearances is the lessened firmness of the bones. The subsequent motion of the body produces shocks in the bone-forming tissues and as these shocks are greater, or less, a traumatic inflammation of varying intensity is produced. Overgrowth of the osteoid tissue and proliferation of the epiphyseal cartilage ensue. The histological picture at any rate does not exclude such an interpretation.

The first possible cause of a disordered calcium metabolism therefore is a deficient intake and this must be ample before any further hypothesis can be entertained. The second is an intake adequate itself but interfered with by other causes. As long ago as 1872 Wegener (14) produced rachitic changes in animals by combined withdrawal of lime and feeding of phosphoric acid. These changes he attributed mainly to the irritant effect of the phosphoric acid upon the bone forming tissues. Putz in 1874 expressed a similar view. Kassowoitz in 1878 (1) (p.934) showed that merely enlarging the amount of phosphoric acid would produce rachitic symptoms without any change in the amount of lime salts. Klimmer and Schmidt (15) (1906) and Ingle (15a) (1907) again emphasised the import-

ance of a due balance between the mineral constituents.

Tangl (16) in 1912 feeding Hungarian swine on a maize ration alone reported a loss of calcium and phosphorus and a gain in magnesium, while if calcium carbonate were added the retention both of Ca and P became normal, with a corresponding decrease in the retention of magnesium. Weiser (l.c.) in 1912 in his investigations showed that the Ca content of maize was insufficient to maintain a calcium equilibrium in growing swine on this ration alone, and that the phosphorus balance was either negative or the retention distinctly subnormal. He showed that $CaCo_3$ added to a ration of barley alone would increase the retention not only of calcium but also of phosphorus. He considered that magnesium retention was decreased by the feeling of $CaCo_3$ but E.B. Forbes reviewing his figures shows that magnesium retention was really increased.

In 1914 Hart, Steenbock and Fuller (17) and E.B. Forbes (18) confirmed the inadequacy of maize to preserve a calcium balance and showed that the addition of calcium carbonate or tricalcic phosphate (in successive periods) caused an increased retention of both calcium and phosphorus.

Zaitschek (19) stated that on maize, pigs showed a negative calcium balance and a retention of magnesium, while on the addition of 5 grams calcium carbonate daily the magnesium retention decreased and the phosphoric acid retention rose from 13 per cent to 30 per cent. E.B. Forbes and others (19A)

also stated that an excess of magnesium to calcium in the diet caused loss of Ca.

Lamb and Eward (20) in 1919 investigated the effects of mineral acid supplement to a calcium-rich diet. They considered that the acids did not cause a significant loss of calcium. Their figures, however, examined by Forbes, when worked out on the basis of retention per kilo of live weight, show a decrease in the retention of calcium from 35% of the amount fed (basal ration) to 25% of the amount fed (sulphuric acid added).

Forbes and others (21) in an exhaustive investigation of the utilisation of calcium compounds fed to growing swine, supplemented a grain ration with (1) pulverised limestone, (2) precipitated bone flour, (3) rock phosphate finely ground, (4) special steamed bone flour, and (5) precipitated calcium carbonate.

They found that the pulverised limestone or precipitated calcium carbonate caused marked increase in calcium magnesium and phosphorus retention; that all the mineral supplements increased chlorine retention; that all caused an increased retention of magnesium; and that limestone, bone flour and calcium carbonate were equally good in promoting increased calcium retention, while ground rock phosphate was decidedly

inferior. Pigs on rock phosphate had actually weaker bones than those on the basal (unsupplemented) ration. Finally they state that the retention of absorbed mineral is controlled by nutritive requirements and is not increased by the limitations of the capacities of elimination since these limitations immediately react by increasing absorption.

Korouchevsky in 1921 (21A) in experiments which will be further discussed found that feeding young rats on a ration deficient in calcium produced osteoporotic changes and diminished calcium content. When the mother herself had been kept on this deficient diet during lactation the histological changes in the bones of the offspring continued on a calcium-poor diet gave a picture closely corresponding to that generally accepted for rickets.

It is thus evident that though we may be assured that a ration containing less than a certain minimum of salts is deficient, we cannot say that a ration in which this minimum is present, or even an amount considerably above this, will be adequate. A lack of balance in the salts supplied or an unsuitable compound may still be present. McCollum (l.c. p.21) attempts to strike the correct proportions by the use of a salt mixture based upon milk. This is good as far as it goes; but it must be pointed out that such a salt-mixture pre-supposes a knowledge of the ash-content of

the milk of each species investigated. This knowledge simply does not exist in the case of rats, mice and guinea pigs on which so much nutritional work has been done. Again the salt-mixture cannot be added *per se* but only after very delicate modification to correct the deficiencies of the diet fed. How delicate, may be seen from the work of Sherman and Pappenheim (22A), who solely by the substitution of 0.4 per cent $K_2HPO_4$, for one-seventh of the calcium lactate present in the diet, were able to prevent the onset of rickets in young rats though this had constantly been caused by the un-modified diet.

Evidence even exists that an excess of organic acid may cause a lowered calcium retention. A German experiment is described in 1897 (22B) (Reichs) where by the administration of 20 grammes oxalic acid per day a positive Ca balance was converted into a negative. There is no doubt that digestive disturbances of any origin may have this effect, either by decreasing absorption or by increasing elimination in the intestine. Possibly the observations of Haubner (22C), that animals feeding near the metal factories of Freiberg acquired rachitis fall into this category.

The smoke contained arsenic lead and sulphurous acid; when these were intercepted the disease ceased to occur.

The part played by microbic infection in modifying calcium metabolism cannot here be discussed. The metabolism of every element in the body is profoundly influenced by the change from health to sickness and the minerals are no exception to this rule. The purpose of this thesis however is to deal as far as possible with animals otherwise healthy in which mineral metabolic disorders have either arisen or been induced.

Hoppe Seyler and Van Noorden (25) showed that exercise alone might influence calcium metabolism, since the excretion of calcium rose in the human during confinement to bed. Kochmann and Petrysch (26) stated that in one very active experimental animal they could not produce an increased excretion of calcium and concluded that exercise aided retention. Findlay (27) and Findlay, Paton and Watson (28) concluded from experiments on puppies that confinement played a large part in the etiology of rickets, a conclusion confirmed by the investigation of Ferguson (29) upon the conditions of the poor in Glasgow. This hypothesis received interesting confirmation from an unpublished paper to which I had access by the courtesy of Dr. Findlay, by Major Hutchison I.M.S. on the confinement of children in "purdah" at Nasik, India. Confinement of

the pig appears to play little part in the development of porcine rickets, but the rigorous selection of breeds for lethargy and the production of fat will probably explain this difference.

Lastly, there is the question of an additional food factor the so-called anti-rachitic vitamin or Vitamin A. Hopkins[30] in 1906 and 1912 in his classical experiments upon the absence of A-factor describes, not a rachitic change but a sudden arrest and collapse of every vital activity. The American authors do not apparently consider Vitamin A such an essential factor in the specific metabolism of calcium as do many writers in England. Thus McCollum states in the Newer Knowledge of Nutritition p. 31, "There has been secured much experimental evidence in support of the view that scurvy and pellagra do not arise from deficiency in the diet of specific chemical substances in the sense in which Funk suggested. This seems to be true also of rickets." Hess, Mc Cann and Pappenheim (31) conclude "This vitamin (A) cannot be regarded as the anti-rachitic vitamin and if the diet is otherwise adequate its deficiency does not bring about rickets. Korouchevsky (l.c.) found that a lack of Vitamin A frequently produced only osteoporosis while rachitic symptoms most readily occurred if the deprivation included calcium as well.

The most definite claims regarding the influence of this hypothetical factor on rickets are found in the Medical Research Committee's Special Reports Nos. 38 and 61. In 1919 No. 38, summarising an experimental investigation undertaken by E. Mellanby states "Although incomplete, the results are sufficiently definite to place rickets among the deficiency diseases", and in 1921 No. 61 (32) sums up "It is evident that a vitamine, probably Fat-soluble A occupies a position of prime importance in the etiology of rickets." Such statements made at the public expense by the leading official body for medical research in Great Britain demand respectful attention, and an examination of these claims will form a large part of the remainder of this thesis.

# SECTION I: RICKETS IN THE PIG.

## ~~Part I.~~ GENERAL SURVEY

### Definition.

Rickets in the pig is defined by Hutyra and Marek as "a disease of young animals which is due to a disturbance in metabolism and is characterised by the persistence of new-formed bony tissue in osteoid condition, by proliferation of the cartilaginous tissue and by an irregular advance of the lines of ossification, the result being manifold deformities of the skeleton."

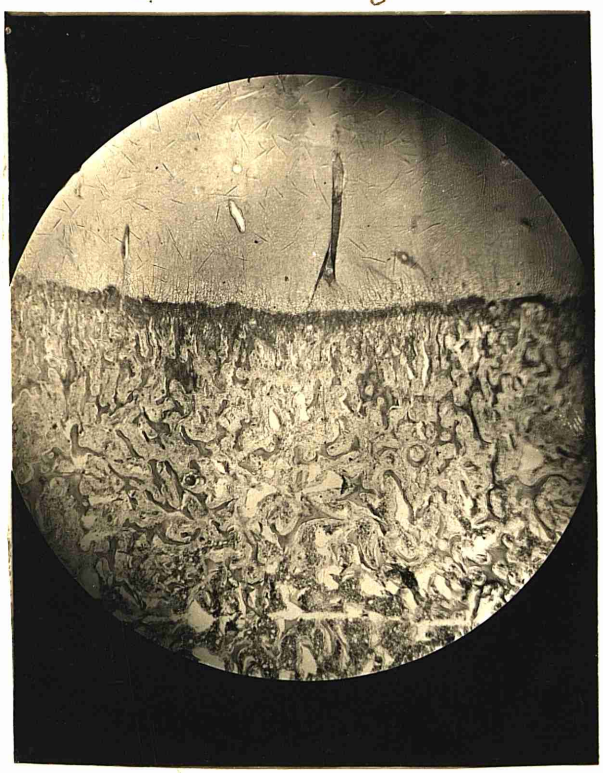
This is practically identical with the definition of the Medical Research Committee in 1918 for human rickets which runs:

> "A disease, the chief manifestation of which is in growing bones in which there is an excessive and irregular development of its earlier stages with arrest or delay in the formation of fully-formed and normally calcified bone."

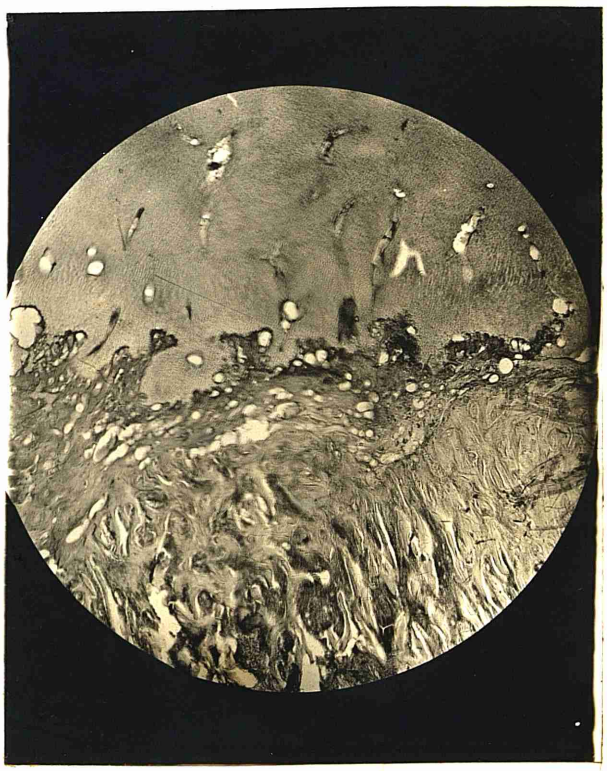
Histological examination of the bones of pigs suffering from this disease leaves no question that we are here dealing with a true rickets as above defined.

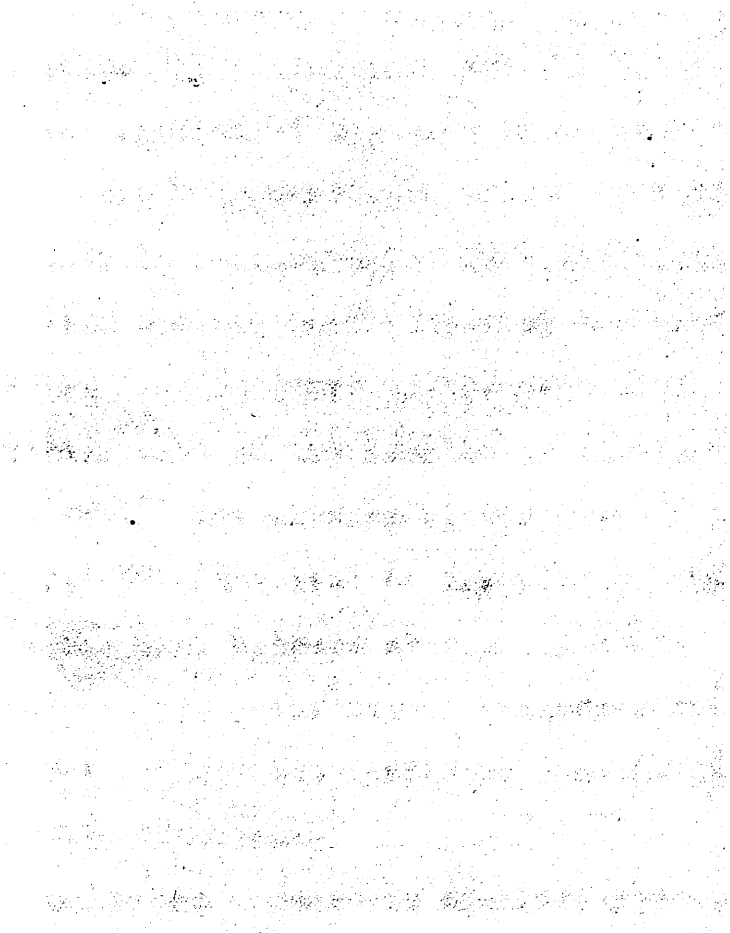
Normal

Commencing



Advanced

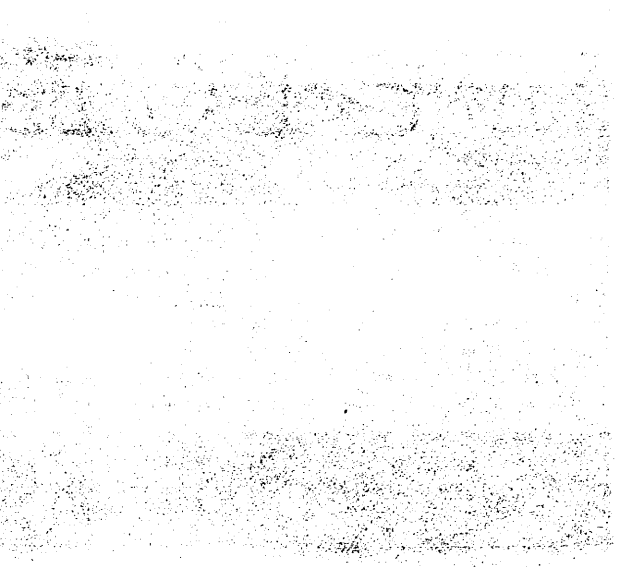




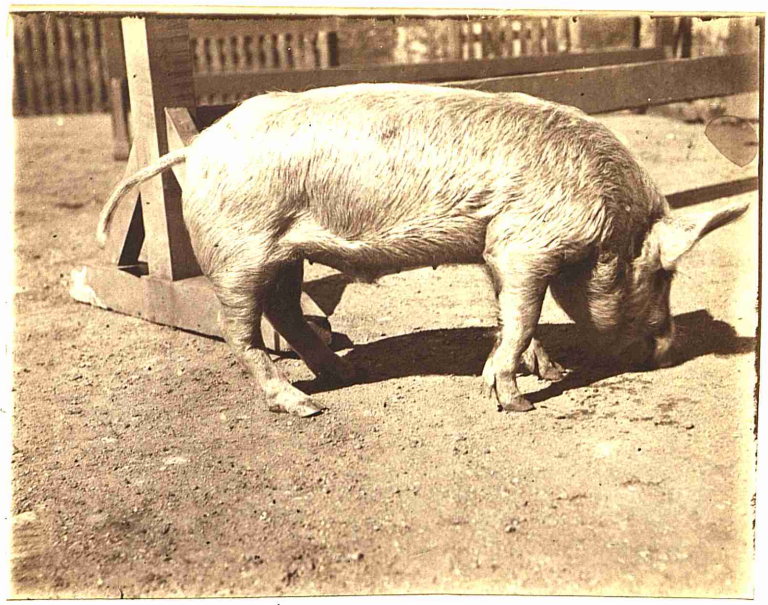
Description.

The first signs of the disease that are presented to the stockman, or clinician, are, in order of time, loss of appetite, slowing of the rate of growth, lethargy, and rough staring coat with excessive growth of hair. Next comes difficulty in locomotion. This difficulty consists at first of a stiff spastic gait affecting the hind quarters, and, later, of loss of power of the hind legs. Later still there are deformities of the long bones with a tendency to fracture of the ribs. The slowing of the rate of growth affects the head less than the rest of the body so that in the later stages the head appears abnormally large. Loss of appetite becomes complete, the animal becomes dirty, emaciated and paralysed and, if the condition remains untreated, death ultimately supervenes.

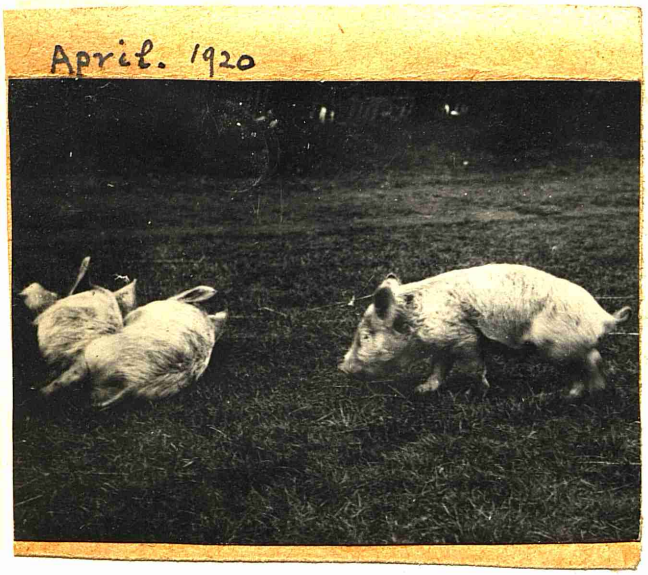
Though the disease is similar, especially in bone lesions, to human rickets there are points of difference, e.g. the spastic gait which has even led some observers to consider it as allied to beri-beri. Nerve changes, in the shape of a parenchymatous neuritis have also been described. by American observers. Wehrbein in 1916 (33) and Cary in 1920 (34)
(33) (34)



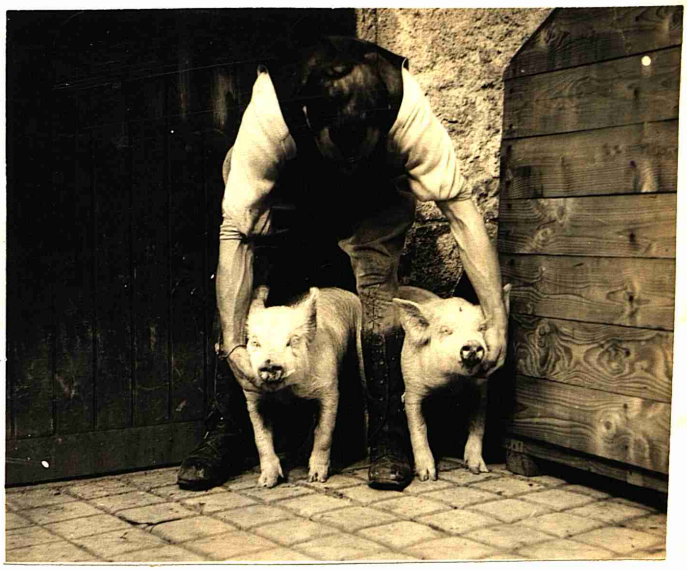
Showing attitude of hind limbs and rough staring coat



Showing large head




Showing bending of fore limbs



Incidence.

The disease is found chiefly in young pigs of from two to three months. It very often occurs during, or shortly after, the weaning period and is most prevalent in the winter months. In the young born in the beginning of winter, the disease is so common, that in the district around Aberdeen it is said by stockmen to affect as many as 50 per cent of the young animals. The condition is, like human rickets, rather associated with confinement, though this seems to be due to the fact that in confinement the diet of the pig is selected by its owner, largely on predetermined lines, rather than by the empirical test of the appetite of the animal.

Review.

In general, the bones of pigs running free are stronger than those of pigs in captivity. Where pig production is carried out by large-scale methods as in the United States the disease, or its prodromal state is especially prevalent. In view of its great economic importance a very large amount of research on this and allied conditions has been carried out in the Experimental Stations under the U.S. Department of Agriculture. Their Bulletins state that the weakening of the bones during fattening reaches such a pitch that pigs

very often suffer fracture of the bones or rupture of tendinous attachments during shipment to market (35). This is not surprising when the figures quoted for, say, the breaking strength of the humerus on a grain ration, as compared with a grain ration plus supplements, are considered, ranging as they do from 796 pounds on one ration to 1017 on another. These differences are not proved by one experiment only, but by many extending over a wide area and a long period of years.

This gross figure again, is but an indication of a profound cachexia involving the whole metabolism. The fact that the weak bone is also associated with a diminished rate of growth, and a large increase of the food required to lay on a kilo of body weight shows, as might be expected, that the bony alterations, though the most obvious, are not at all the most vital of the features of the syndrome.

The conditions, then, under which rickets in pigs arises, are not local but are very wide-spread. In view of the fact that swine are naturally adapted to a diet very similar to that of human beings and that, like the inhabitants of these islands they are frequently brought up from weaning on manufactured foods brought from great distances, and that they have by generations of selections been adapted

for a healthy existence in close confinement, it seemed probable that a close investigation of the origin of this disease would yield valuable results. In addition the economic importance of the problem has a twofold aspect. A solution of the problem would be of great financial importance to the stockmen of this country; figures are not available to show how great, but the United States Department of Agriculture in 1916, after a careful census, gives the losses that year amongst swine alone, from errors in feeding alone, as $6,500,000, a loss of which the condition which we are investigating forms a large percentage. The 50 per cent incidence reported amongst young winter pigs in the Aberdeen area shows, even allowing for exaggerations, that the figure quoted above for the U.S. (which is only 10 per cent of their losses from pig diseases during the year) is not without some parallel in Great Britain. Secondly the long-standing recognition of the economic problem showed that a mass of clinical observation must exist which if it could be brought into relation to an accurate and detailed investigation would repay the closest scrutiny. This was found indeed to be so.

General Statement of Experimental Results.

For the convenience of the reader the following general description of the results obtained is made, to give a general idea of the results subsequently detailed.

The surprising facts appear that the vitamin hypothesis of the origin of rickets has no relation whatever to the incidence or course of this disease amongst swine; that the absence of the so-called vitamin A does not ensure, nor does its presence prevent, the onset of a typical rachitis. Furthermore, a true rachitis, and not a mere osteoporosis, can be produced by withholding certain mineral salts; and this withholding is not merely a question of the gross quantity present in the diet, since the retention of one salt, which is being fed in fixed quantities, may be increased or diminished by the addition of another. These results were so contrary to the accepted opinions of the day that they have been withheld from publication till the investigation, which has now lasted for over a year and a half, was fully confirmed. Observations on over 100 animals have been made. For final security it was considered advisable to obtain an outside confirmation, and one of the documents attached is a report by Dr. Da Fano, of Kings College, London, on the histological condition of the

epiphyses of a pig which had been for seven months subsequent to weaning deprived completely of "fat-soluble A." The experiment is the more striking since the animal had reached a cachectic condition which was apparently averted in the control group by the administration of cod-liver oil. Throughout the experiments indeed, the therapeutic and prophylactic qualities of cod-liver oil in maintaining health have been constantly confirmed; but its specific antirachitic qualities have been as strikingly disproved.

PART I:

METHODS AND RESULTS OF INVESTIGATION.

# EXPERIMENTAL DATA.

~~QUALITATIVE~~.

## Part 1: FEEDING EXPERIMENTS.

### FEEDING EXPERIMENT I.

# EXPERIMENTAL DATA.

## PART I: FEEDING EXPERIMENTS. ~~QUALITATIVE.~~

### METHODS AND NATURE OF INVESTIGATION.

A feeding experiment carried out at the Rowett Institute in 1919-20 showed that young pigs in confinement on a diet of wheat offal, grains and potatoes develop the rickets syndrome as outlined above. It was thus possible to arrange a basal diet of these feeding stuffs which would with a degree of certainty produce the condition, and, by the addition of various substances to this diet, to determine what factors prevented the occurrence of the disease.

A consideration of the nature of wheat offal, grains and potatoes suggests (1) deficiency of "fat soluble A", and (2) a lack of correspondence between the mineral matter of the food, and the mineral requirements of the animal.

A series of experiments were carried out, in each of which, in a control group of animals, the supposed deficiency of the basal ration in both "fat soluble A" and mineral matter was roughly adjusted, and in the experimental group the deficiency in either "fat soluble A" or

mineral matter was allowed to remain, so that the influence of each of these factors in preventing the disease was tested separately. "Fat soluble A" was added in the form of cod-liver oil. The salt mixture used to adjust the mineral deficiency was a rough imitation of the ash of milk with either additional calcium, to make good the marked calcium deficiency of the basal diet and sodium hydrate to reduce the excessive acidity, or of a calcium-rich mixture of salts supplied in a separate box to which the animal had access. The details are given with the experimental data. Water soluble C was added to all the rations in the form of either lemon juice or swede turnip to avoid the possible complication of deficiency of this factor, though, as a matter of fact, the results of Experiment II. which was conducted to see whether the condition is allied to scurvy seem to indicate that the pig is very little susceptible to a deficiency of this vitamin. In all the experiments the animals were allowed to eat as much as they pleased.

## FEEDING EXPERIMENT I.

### Influence of Fat Soluble A.

In this experiment ample inorganic matter was given to both groups and one group was given additional fat

soluble A in the form of cod-liver oil, so that the influence of a relative deficiency of "fat soluble A" was determined in the other group.

Animals. Six young pigs, seven weeks old, of the same litter, four male, two female.

Method. The animals were housed in two open pens, three in each, on the ground floor of the laboratory. Area of pens 6 ft. by 11 ft. Straw bedding was provided. Each group was given a basal diet designed to supply all known requirements with the exception of Vitamin A which was supplied to Group I. in the form of cod-liver oil, Group II. receiving an isodynamic amount of lard.

Basal Diet. Two groups each of three animals all out of the same pen had the following ration:

| | Proportion. |
|---|---|
| Oatmeal . . . . . | 100 |
| White bread (replaced after first month by maize) . . . . | 200 |
| Cod muscle . . . . . | 200 |
| Swede turnip . . . . . | 100 |
| Marmite, 20 grm. per day | |
| * Salt mixture detailed below . . . | 2.9 |

~~The animals had constantly before them -~~

---

* Note:-

| | | |
|---|---|---|
| Salt Mixture, | Calc. Phosphate ..... | 2 grams. |
| | Mag. Sulp..... | 0.25 " |
| | Pot. Carb..... | 0.25 " |
| | Ferric. Chlor..... | 0.2 " |
| | Sod. Chlor..... | 0.2 " |

The animals had constantly before them

(1) Water.

(2) Box with ashes and sulphur.

(3) Box with salt mixture, Bone Meal. 10.
Mag. Sulph. 1.
Pot. Carb. 1.
Rock Salt. 2.
Chalk. 2.

The proportion of protein to carbohydrate and fat, at first 1 : 3 was reduced as the animals grew older to 1: 5. 5 in stages by reducing the proportion of fish.

To Group I was added 10 c.c. later 20 c.c. Cod Liver Oil per pig per day.

To Group II was added 10 grams, later 20 grams, lard per pig per day.

At the commencement of the experiment diarrhoea was present in all animals of both groups. To arrest this the fish was boiled from 13/1/21. This had no effect. From 4. 2. 21. onwards bread was replaced by maize meal and the diarrhoea ceased within 48 hours and did not recur.

Results.

1st Month.

Both groups suffered from diarrhoea from the beginning of the experiment. At the end of the first month diarrhoea was still continuing and appetite decreasing. The symptoms were rather more marked in Group I (Lard), than Group II, Cod Liver Oil. Pig No. 23 of Group II

showed some abdominal distension, roughness of coat very marked, loss of appetite and lethargy.

2nd Month.

During the second month after the change of maize for bread the growth and appetite of both groups were apparently normal though rather greater in Group I (Cod Liver Oil) than in Group II (Lard).

3rd Month.

At the end of the third month Group I was definitely larger and more active. Group II while showing no symptoms of disease were more lethargic with a tendency to lay on fat.

4th Month.

During the fourth month No. 23 of Group II (Lard) showed some stiffness in hind quarters and a tendency to a stilted gait. All the others of both Groups were normal but Group I had silkier coats and were slightly larger.

5th Month.

During the fifth month the relative condition and growth continued as during the fourth month.

End of Experiment.

On 13th May, 1921, the experiment was discontinued because (1) The sows had reached sexual maturity.

(2) No definite symptoms of disease other than the slight stiffness of gait of No. 23 was evident and this had shown no distinct development during the preceding six weeks.

No. 23 was continued on the same diet until 19. 5. 21. when it was killed.

Post-Mortem Examination of No. 23.

All the ~~signs~~ organs appeared on naked eye examination, normal.

Bones. Nothing abnormal was detected in the bones on naked eye examination. There were no haemorrhages under the periosteum. There was no enlargement of the joints. The epiphyseal line (lower end of femur) was regular and clearly defined and normal as compared with that of a pig of the same litter from Group II diet Cod Liver Oil.

Average Weights in Kilos.

Table 1.

| | Birth. | Beginning of Experiment. | | | | |
|---|---|---|---|---|---|---|
| | 16.11.20. | 1.1.21. | 1.2.21. | 1.3.21. | 2.4.21. | 15.4.2 |
| Group I. C.L.O. | 1.40 | 12.4 | 24.3 | 43.3 | 62.5 | 71. |
| Group II. Lard. | 1.44 | 12.2 | 21.5 | 37.9 | 53.9 | 63. |

On 15.4.21. No. 15 of Group I received a traumatic injury - a fracture of the femur. The following figures give the gains in weight from 15.4.21. to 13.5.21. of individual pigs.

| | | |
|---|---|---|
| Group I. (Cod Liver Oil) | (No. 14 - 11.8<br>(No. 16 - 10.8 | ) Average 11.3 |
| Group II. (Lard) | (No. 17 - 8.2<br>(No. 20 - 10.1<br>(No. 23 - 15.0 | ) Average 11.1 |

The animals of Group II (Lard) appeared normal and of full size for their age. The opinion of several practical men was that they were "fine healthy pigs" and in prime condition. When compared with Group I. however, they were inferior in size, the coat was less silky and glossy and the animals were less active.

Conclusions -

(1) The basal ration appeared to be sufficient to maintain health and growth in the pig from seven weeks until six months of age, i.e., the period of maximum growth.

(2) The pig appeared to be little susceptible to a deficiency of Vitamin A.

(3) Cod Liver Oil undoubtedly stimulated growth as against lard.

(4) The low intake of Vitamin A produced no effect on the bones.

(5) The results taken in conjunction with those of the next experiment when rickets was produced on a diet with

10 c.c. Cod Liver Oil per pig per day, suggested that rickets in pigs is not caused by a deficiency in Vitamin A.

It may be noted:

(1) The diarrhoea present in both groups in the first month had probably no connection with an avitaminosis.

(2) In criticism of the experiment it may be urged that -

(1) The amount of Vitamin A grains (maize) is unknown.

(2) That the animals may have eaten the straw bedding and this may have contained Vitamin A.

## EXPERIMENT II.

### Influence of Vitamin C.

This experiment was undertaken to test the influence of C factor (antiscorbutic) on the health and rate of growth of the pig.

Animals. Five pigs, seven weeks old, all out of same litter.

Group I, Control, Nos. 21 and 24,

Group II, Experimental, Nos. 18, 19 and 24.

Method.

The animals were housed in two open pens on the ground floor of the laboratory as in Experiment I. Each group was given a basal diet designed to contain all the factors except water-soluble C. (antiscorbutic). This was added to Group I. (control) in the form of turnips ½ lb., potatoes ¼ lb., per pig per day. For Group II. experiment the same quantities were taken but were boiled for four hours to destroy C. factor.

| | | Proportion. |
|---|---|---|
| Basal diet - | Oatmeal ..... | 100 |
| | Sharps ..... | 75 |
| | Blood Meal ..... | 175 |
| | Cod Liver Oil .. | 10 c.c. per day |
| | Marmite ..... | 20 grams " " |
| | Protein Ratio | ~~1 : 4.5~~ 1 : 4.5 |

No salts were given to either group.

Results. First Period, 61 days. (2. 1. 21. - 3. 3. 21.)

There was no apparent difference in the two groups. No definite signs of disease had appeared but all the animals were somewhat active and had less appetite than normal. The rate of increase in weight was rather under average. The average weight of all animals was 31.2 kilos. compared with 34.3 kilos for the average weight of nine pigs of the same age from the same litter fed on mixed uncontrolled diet.

Weights in Kilos.

Table 2.

| | | 2. 1.21. | 3. 3.21. | Increase. |
|---|---|---|---|---|
| Group I. | No. 21. | 8.9 | 28.0 | 19.1 |
| Basal Diet A B & C Vitamins. | No. 24. | 10.6 | 31.2 | 20.6 |
| Group II. | No. 18 | 9.4 | 30.4 | 21.1 |
| Basal Diet A & B Vitamins. | No. 19 | 13.3 | 35.1 | 21.8 |
| | No. 22 | 10.7 | 31.5 | 20.8 |

1st Month.

During the first month it was noted that about half a pound of plaster had been eaten from the wall of the pen of Group II. The wall was therefore boarded up.

## 2nd Month.

On 8.3.21. No. 18 was found dead in the morning. It had been inspected at 6 p.m. on the previous night on the usual evening visit and nothing abnormal noticed. It had eaten a full meal at 5 p.m.

Post Mortem.

This was carried out by Mr. Brown, M.R.C.V.S., Lecturer in Veterinary Hygiene at Marischal College.

The stomach was found full. There was pus in the mesenteric glands, and a small inflammatory patch at the lower end of the lung. There was also acute inflammation in the upper part of the small intestine. All the other organs were normal. The joints were normal. There were no sub-periosteal haemorrhages.

Case of Death was diagnosed as septicaemia. On 9. 3. 21. at about 10 a.m. No. 22 Group II. (No. C-factor) suddenly collapsed. The animal lay on its side with ears cocked and eyes staring. There was loss of power of the hind legs and complete loss of appetite. The animal was quite unable to walk within an hour (11 a.m.) All animals had had a special examination on the previous afternoon by Mr. Brown, M.R.C.V.S. and had appeared normal. They had also appeared normal at the morning visit on 9. 3.21.

The animal was isolated. A salt mixture as under was added to its food and to that of No. 19 the other animal of Group II. No. 22 gradually recovered. No. 19 developed no symptoms.

The experiment was accordingly altered as follows:-

Group I. was continued as before A.B. & C. Vitamins,
No salts.

Group II. was continued as amended A. & B. Vitamins.
No. C Vitamin.
Salt mixture added.

The basal diet remained unaltered.

Note to First Period of Experiment.

It is interesting to observe the rapid onset of the symptoms in No. 22. This is frequently found in feeding swine on deficient diets, and American observers have noted cases where movement precipitated the collapse, e.g., on the pig being taken out to be weighed.

The suddenness of the onset frequently leads to a diagnosis of poisoning and it is not improbable that similar cases in other farm stock have a similar cause. A French observer records several cases amongst horses in Paris. These are frequently attributed to H.C.N. which can be isolated from various glucosides naturally occurring in beans, etc. The evolution of hydrocyanic acid, however, is practically precluded under normal gastric conditions (acid nature of stomach contents, etc.)

The actual collapse is probably due to the rapid effusion of fluid into pleural and pericardial sacs, a fact often noted post-mortem. The part played by mineral salts in preserving the tone of the body tissues should be borne in mind.

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## EXPERIMENT II. Second Period.

- 63 days. 10.3.21. - 13.5.21.

### 1st Month.

In Group I.(A, B, & C, Vitamins, No. Salts); both animals steadily deteriorated in condition.

In Group II,(A + B Vitamins + salts), No. 22 (which fell ill on 9.3.21) gradually improved to nearly normal health. During this period it increased in weight at the rate of 0.256 kilos per day, which is less than half the normal rate of increase for a healthy pig of that age. No. 19 (which had never been actually ill) showed a marked improvement in condition and rapid increase in weight, 0.667 kilos per day, which is about the normal.

### 2nd Month.

In Group I. both animals continued to deteriorate in condition and made little weight. By 24.4.21. No. 21 was unable to walk, the bones were tender on handling, the skin was becoming dirty and the hair long and matted. No. 24 was showing the same symptoms but to a less degree.

In Group II. No. 22 (previously ill) had considerably improved and was putting on weight at the rate of 0.416 kilos per day. Slight stiffness of gait and reluctance to active movement persisted till the end of the Experiment. No. 19 continued to do well and to gain weight at the same

rate as formerly.

Weights in Kilos.

Table 3.

| | | 11.3.21. | 13.5.21. | Gain. |
|---|---|---|---|---|
| Group I. A,B & C Vitamins No added Salts | No. 21 | 30.7 | 33.6 | 2.9 |
| | No. 24 | 34.4 | 52.8 | 18.4 |
| Group II. A & B Vitamins Salts added. | No. 19 | 39.7 | 81.2 | 41.5 |
| | No. 22 | 32.0 | 53.6 | 21.6 |

No. 21 Group I. was killed on 13.5.21.

Post Mortem.

All organs were normal except -

(1) Slight thinning of the wall of the intestine (doubtful).

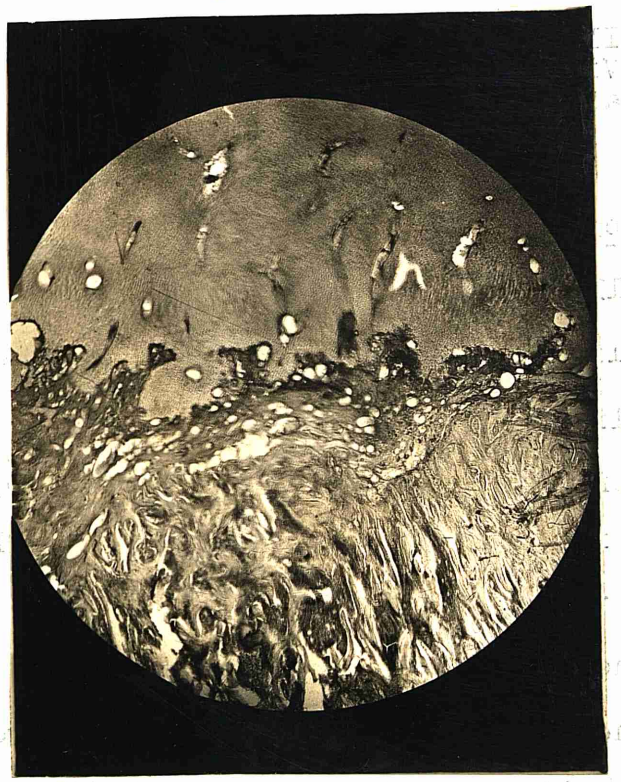
(2) Slight haemorrhages in pleura.

(3) Bones were very soft. No haemorrhages were observed in periosteum or at joints. The bone marrow was very red.

The femur and 8th rib were removed for analysis. The leg bones were cleaned and the marrow removed and the figures given are for the dry substance without the marrow. The ribs were dried without removal of the marrow.

The bones were then dried on the water bath, fragmented with bone forceps and 1 - 2 grams weighed in a small porcelain crucible. The sample was then ashed at a dull red heat till completely white. The crucible was again weighed and the contents dissolved in $\frac{N}{2}$ H.Cl. Ca.

Fig 21



was estimated as Ca. O by precipitation with ammonium oxalate and titration against potassium permanganate and P as $P_2 O_5$ by the uranium acetate method.

The following table shows the analysis of the bones:-

FEMUR.

Table 4.

| No. of Pig. | Percentage Composition of Dry Bone. | | | | Percentage Composition of Ash. | |
|---|---|---|---|---|---|---|
| | Organic Matter. | Ash. | Ca.O | $P_2O_5$ | Ca. O | $P_2O_5$ |
| No. 21. | 50.39 | 49.61 | 26.75 | 20.08 | 54.00 | 40.48 |
| | | | RIBS. | | | |
| No. 21. | 67.60 | 32.40 | 16.42 | 12.95 | 50.66 | 40.0 |

Histolological examination shows results as under.

Conclusions.

(1) The pig appears to be little susceptible to deficiency of Vitamin C.

(2) The condition produced, to a marked degree in No. 21, and to a less degree in No. 24, both of which animals were receiving Vitamins A, B, & C in abundance appears to be due to a deficiency of inorganic constituents in the food.

(3) This condition appears to be that known as "rickets" in pigs.

(4) This condition was not prevented by 10 c.c. per pig per day of Cod Liver Oil during the 124 days of the

experiment.

(5) The condition of rickets in pigs is histologically comparable to that of rickets in other animals.

## EXPERIMENT III; Influence of Mineral Matter.

This experiment was undertaken to determine the influence on the health and rate of growth of the pig of the addition of salts to a ration such as commonly fed, by practical stocksmen and to confirm or disprove the results of Experiment II. with regard to the association of rickets in pigs with a deficiency of inorganic constituents in the diet.

Animals. Six young pigs, three in each of two groups comparable in age and sex.

Method. The animals were housed in two wooden pens with brick floors, and outside runs also on brick floors. The pens were 6 ft. by 6 ft. the outside run 6 ft. by 5 ft. Each group was given a basal diet sufficient for growth and rich in Vitamins A, B, and C. Group II. received in addition a salt mixture; Group I. (control) received no salt mixture.

The basal diet was as follows:

| | | Proportion. |
|---|---|---|
| Sharps ..... | $\frac{2}{3}$ lb. | 100. |
| Oatmeal ..... | " | 120 |
| Blood Meal ..... | $2\frac{1}{3}$ oz. | 20 |
| Marmite ..... | $6\frac{2}{3}$ grams per day | |
| Turnips ..... | $\frac{1}{2}$ lb. | " " |
| potatoes ..... | $\frac{1}{4}$ " | " " |
| Cod Liver Oil .... | 10 c.c. | " " |

Water was given ad lib.

To Group II. ration was added per pig per day the following salt mixture.

| | |
|---|---|
| Calc. Phosph..... | 20 grams. |
| Mag. Sulph..... | 0.25 " |
| Pot. Carb..... | 0.25 " |
| Ferric Chloride ..... | 0.2 " |
| Sod. Chlor..... | 0.2 " |

There was in addition always before this group -

(1) A Box containing -

| | |
|---|---|
| Bone Meal ..... | 5 parts. |
| Rock Salt ..... | 1 part. |
| Chalk ..... | 1 part. |
| Mag. Sulph..... | ½ part. |
| Pot. Carb..... | ½ part. |

(2) A box with a mixture of earth, ashes, bones, rock salt and sulphur.

Results.

1st Month.

There was nothing to mark in the condition of the animals or to differentiate between them save that Group II. (Salts) appeared to be growing faster.

2nd Month.

Towards the end of the month there was a distinct difference between the two groups. Group I. (no salts) showed lack of lustre of coat, excessive growth of hair, dirty skin, lethargy, stiffness in gait and a slackening of the rate of growth.

3rd Month.

In addition to above signs of malnutrition, deformities of the limbs became apparent.

4th Month.

The above symptoms became more marked.

Average Weight in Kilos.

Table 5.

| | 10.1.21. | 11.2.21. | 11.3.21. | 11.4.21. | 11.5.21. |
|---|---|---|---|---|---|
| Group I. no salts. | 14.6 | 21.2 | 28.9 | 37.8 | 44.6 |
| Group II. salts added. | 14.2 | 27.0 | 39.0 | 55.4 | 74.4 |

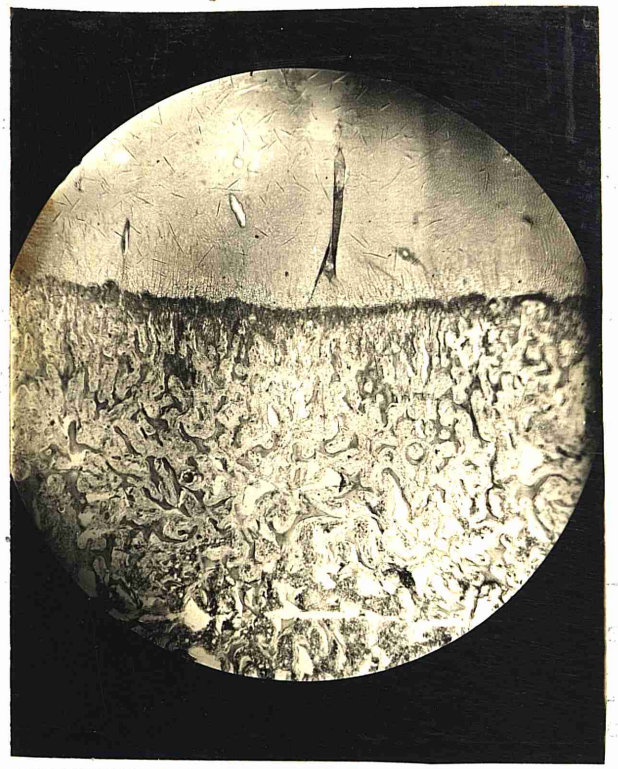
Pig No. 27 was killed on 13.5.21 showing the typical signs of rickets in the pig as previously described, though growth, though slow, had not ceased.

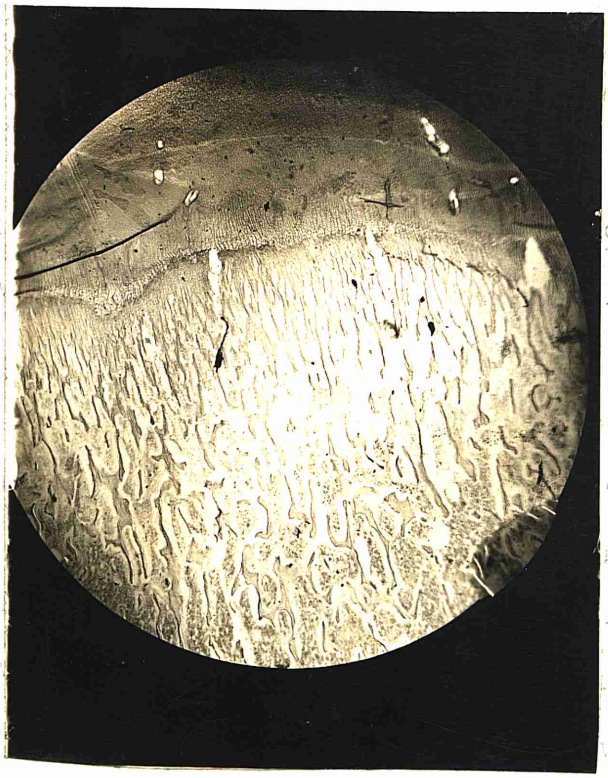
Post Mortem Examination showed all organs normal with the exception of a slight thinning of the wall of the intestines (doubtful).

The bones were soft, and could be cut with a scalpel. The marrow was markedly red. The femur and 8th rib were removed for analysis.

Pig No. 31, Group II. (salts added) was killed at the same time as a control. All organs were normal. The bones were hard and well calcified. The femur and ribs were removed for analysis. The rib bone contained a

Pig 27





Pig 31.

large well calcified callus, the relic of an earlier fracture.

The following table shows the result of the analysis. The bones were prepared as detailed in the previous experiment.

FEMUR.

Table 6.

| No. of Pig. | Percentage Composition of Dry Bone. | | | | Percentage Composition of Ash. | |
|---|---|---|---|---|---|---|
| | Organic Matter. | Ash | Ca.O. | $P_2O_5$ | Ca. O | $P_2O_5$ |
| No. 27 | 43.80 | 56.20 | 29.60 | 22.80 | 52.80 | 40.57 |
| No. 31 | 37.69 | 62.31 | 33.43 | 25.73 | 53.37 | 41.10 |

RIBS.

Table 7.

| No. of Pig. | Percentage Composition of Dry Bone. | | | | Percentage Composition of Ash. | |
|---|---|---|---|---|---|---|
| | Organic Matter. | Ash | Ca.O | $P_2O_5$ | Ca. O | $P_2O_5$ |
| No. 27. | 65.46 | 34.54 | 17.07 | 14.17 | 49.65 | 41.016 |
| No. 31. | 59.55 | 40.45 | 20.60 | 16.51 | 50.898 | 40.82 |

Histological Examination.

Microphotographs of the lower end of the femur of each pig are attached.

These show the commencing disorganisation of the epiphysical line which is so marked in No. 21 of the previous experiment. No. 27 presented all the clinical signs of "rickets in pigs", rough coat, slowing of growth, arching of back, deformity of long bones and reluctance to movement.

Conclusions.

(1) On a ration of grains, grain offal and potatoes such as a stocksman would commonly feed to growing pigs is a deficiency of inorganic material.

(2) This deficiency is one and probably the main factor in producing "rickets in pigs".

(3) This condition is histologically and physically very similar to human rickets.

(4) This condition is not prevented by Cod Liver Oil 10 c.c. per pig per day during the 120 days of the experiment.

## EXPERIMENT IV

In this experiment the basal diet was similar in nature to that on which Mellanby (1918) produced rickets in puppies, except that the fat was removed from the milk. Oatmeal and rice were fed in equal proportions according to appetite with an addition of 800 c.c. machine-skimmed milk per animal, i.e., about 80 c.c per kilo weight of the animal at the beginning of the experiment and 10 (ten) grm. sodium chloride per pig per day.

Four groups, each of four seven-week-old newly weaned pigs, were used. The groups as before were comparable with each other as to sex and litter, and the housing conditions were identical.

In comparison with the milk of either the bitch or the sow, Mellanby's basal diet is very poor in protein. Bitch's milk contains roughly, per cent. protein 10, fat 11, carbohydrate 3, and for the sow the respective figures are 7, 5 and 3. All the food-stuffs of the basal diet contain a much lower percentage of protein in proportion to the other energy-yielding constituents than that present in the milk of the species experimented on. To see whether deficiency of protein has any influence, additional protein in the form of blood meal, i.e., dried blood, was added to the basal ration of two of the groups to the extent of one-sixteenth of the total weight of oatmeal and rice given.

To the food of one of the two groups receiving the ration only and one of the two receiving the ration plus extra protein there was added per pig per day 20 grm. of the salt mixture used in Experiment 1 plus 5 grm. calcium carbonate. In addition there was added to every 6 pounds of mixture of rice and meal 80 c.c. of 10 per cent. sodium hydrate - the approximate amount necessary to neutralise the excessive acidity of the ration.

The diets were therefore as follows:

| Group I. | Group II. | Group III. | Group IV. |
|---|---|---|---|
| Ration only | Ration + salts. | Ration + protein | Ration + protein + salts. |

Water was always before the animals but no additional mineral matter was provided.

Results: During the first fortnight the rate of growth of the two groups receiving the extra protein exceeded that of the other two groups. During the second fortnight the signs of malnutrition noted in Experiment I became evident in Group III, and one of the animals died on the thirty-third day of the experiment. The post-mortem examination showed anaemia and patches of inflammation and adhesions in the pleura of the lungs. In the third fortnight the signs of disease noted in Group III appeared in Group I. Towards the end of the second month two of the three remaining animals of Group III were hardly able to walk and were reluctant even to stand. The remaining animal of the group had acquired the habit of drinking the urine of its companion. It was the last to show signs of disease.

The following table shows the average gains in weight in the different groups during the first 30 days. Owing to the death in Group III the averages for the second month during which the experiment was run are not comparable.

TABLE ~~VIII~~ 8.

Average Gains in Weight in Different Groups.

| Groups | Initial weight. Kilos. | Gain in weight. 1st 15 days. | Gain in weight. 2nd 15 days. |
|---|---|---|---|
| I. Ration only | 9.8 | 3.2 | 3.4 |
| II. Ration + salts | 9.8 | 3.1 | 4.4 |
| III. Ration + protein | 9.7 | 3.6 | 2.5 |
| IV. Ration + protein + salts | 9.8 | 3.5 | 4.9 |

On the sixty-ninth day one of the animals of Group III was killed. The following table shows the percentage calcium in the eighth rib compared with that found in a healthy animal of the same age.

TABLE ~~IX~~ 9.

Showing Percentage Composition of Dried Rib (Eighth)

| Organic matter | Ash. | CaO. | $P_2O_5$. |
|---|---|---|---|
| 59.3 | 41.7 | 21.7 | 17.3 No.99 (Group III) |
| 46.6 | 53.4 | 28.0 | 22.0 Normal animal same age. |

Conclusions.

(1). A diet was given by Mellanby for puppies, which will, as he says, rapidly and certainly induce rickets within six weeks in puppies, has a similar result in swine.

(2). The same diet with addition of certain inorganic constituents enables swine to grow rapidly and in splendid health for 75 days.

(3). The diet as framed by Mellanby contains an insufficiency of mineral matter for the growth of swine.

(4). Hypothetical substances such as Vitamin A. should not be invoked till all the known requirements of the animal have been completely fulfilled.

In the introduction it was pointed out that although Mellanby did not deny that an adequate Ca intake was essential for the development of growing bone he gave no indication whatever of the necessary amount. In Special Report No. 61 he also mentions phosphorus as a desirable constituent of the diet "plenty of phosphorus" p. 75, but there is no mention of any other of the mineral constituents. In his experimental diets the only inorganic substance, separately supplied, is sodium chloride. The fact that the supply of other necessary mineral constituents such as iron must be adequate beyond all cavil before any demands whatever can logically be made for further unproved "accessory factors" seems to have been overlooked. A comparison between the salts added by McCollum and by Mellanby respectively will emphasise the point.

| McCollum. | | | Mellanby. | |
|---|---|---|---|---|
| Na Cl..... | 5.19 | parts | Na Cl..... | 1-2 grams |
| $Mg.SO_4$ ..... | 7.98 | " | | |
| $NaH_2 PO_4$ ..... | 10.41 | " | | |
| $K_2 H PO_4$ ..... | 28.62 | " | | |
| $Ca H_4(PO)_2$ ..... | 16.20 | " | | |
| Ca Lactate ..... | 39.00 | " | | |
| Ferric Citrate.... | 3.54 | " | | |

Iodine trace.

The influence of Ca is discussed by Mellanby at some length in No. 61 but there is no discussion of the other mineral constituents of the dietary. Even the organic constituents are somewhat unusual in proportion; the protein ratio (1: 5½) is very low for a newly weaned animal, and he frequently refers to the striking improvement in condition caused by the addition of small quantities of lean meat. (p.37).

The original work specifically done on the possible influence of mineral matter, is given as an experiment on one animal (400 were used in all) though calcium phosphate occurs in certain other diets (influence of exercise, etc.)

In this experiment (No. 122) a puppy six weeks old was given 5 grams of $Ca_3 (PO_4)_2$ daily in addition to a basal diet of white bread ad lib. 175 c.c. separated milk, and 10 c.c. linseed oil. The puppy developed rickets within 10 weeks. Other observers, however,

working on swine have pointed out (Ohio Bulletin) (21), that certain forms of calcium phosphate produced no increase in the strength of the bones of swine though this could be readily secured by the use of other mineral supplements (calcium carbonate, etc.) Considering the length of time that a puppy will devote to mineral-containing substances such as bone, when left to its own devices, further investigation of the part played by the inorganic constituents of the food of this animal would have seemed justified.

[illegible]

[illegible] comparable in age and [illegible].

[illegible] The animals were housed in two [illegible] on the ground floor of the laboratory. [illegible] used and no bedding given. The animals were fed on a basal ration devoid of A. Factor. To Group 1. (control) 10 cc. cod-liver oil per pig per day was given. To the experimental Group 10 c.c. of linseed oil.

Basal Diet

| | |
|---|---|
| Crushed Oats. | ¼ lb. |
| Bran | ¼ lb. |
| [illegible] | 1 oz. per [illegible] |

## EXPERIMENT V.

This experiment was undertaken to investigate the undoubted good effects of cod-liver oil in promoting growth and to determine whether the superiority of the growth curves in pigs fed on a ration containing this substance, as against others receiving a vegetable oil, over long periods of time, would continue. If so, would the vegetable oil result in cachexia? and if it did would the characteristics of the rachitic syndrome be eventually evoked?

Animals. Eight pigs were taken, four each in two groups comparable in age and sex.

Method. The animals were housed in two neighbouring pens on the ground floor of the laboratory. Cement floors were used and no bedding given. The animals were fed on a basal ration devoid of A. factor. To Group 1. (Control) 10 cc. cod-liver oil per pig per day was given. To the Experimental Group 10 c.c. of linseed oil.

Basal Diet

| | |
|---|---|
| Crushed Oats. | ¾ lb. |
| Bran | ¾ lb. |
| Blood Meal | 1 oz. per pig per day. |

| | |
|---|---|
| 10% NaOH | 20 c.c. |
| Calcium Carbonate | 5 grams. |

Salt mixture in food.

| | | |
|---|---|---|
| Bone Meal | 15 grams. | |
| Mag. Sulph. | 1.875 grams. | per pig per day. |
| Pot. Carb. | 1.875 grams. | |
| Ferric Chloride | 1.5 grams. | |

On 27/6/13 the crushed oats $\frac{3}{4}$ lb. was altered to $\frac{3}{8}$ lb. crushed oats and $\frac{3}{8}$ lb. oatmeal as there was rather too much fibre in the previous diet.

On 7/7/13 50 c.c. lemon juice per pig per day was added to supply (C. anti-scorbutic factor) though previous experiments had shown the requirements of the pig to be minimal in this respect.

Results. First Month.

The control group I. (cod-liver oil) increased at a distinctly slower rate than the experimental group II. (linseed oil). Both groups were in good health.

Second Month.

The control group I. (cod-liver oil) increased at almost as great a rate as group II. (linseed oil). Both groups were in good health.

## Third Month.

There was a considerable increase in the rate of growth of both groups though group II. was still doing better than group I. Both groups were in good health.

## Fourth Month.

During this month group I. (cod-liver oil) was making rather better progress than group II. (linseed oil) though below group II. in total weight.

The linseed oil was omitted from the ration of group II. for the last 10 days of this month to determine whether the deprivation of this small amount of fat (10 c.c. per pig per day) would disproportionately affect the growth curves. The pigs appeared to suffer from its withdrawal and in the continued experiment (5th month) olive oil was substituted.

As both groups had now run for 120 days in perfect health, the experiment was changed. The subjoined tables give the results to date. Pig No. 106 (cod-liver oil) and pig No. 108 (linseed oil) were killed and the bones analysed as in Table.

Table 10.

Weights in Kilos.

| Initial 15/6/21. | Initial Weights. | 1st month to 15/7/21. | 2nd month to 14/8/21. | 3rd month to 13/9/21. | 4th month to 13/10/21. |
|---|---|---|---|---|---|
| Pig No. 112 Group I. Cod-liver oil. | 10.4 | 12.2 | 19.8 | 32.8 | 49.6 |
| Pig No. 110 Group II. Linseed oil. | 10.6 | 14.3 | 22.7 | 37.6 | 52.3 |

Average Increase in Kilos per pig per day.

Table 11.

| No. of Pig. | 1st Month | 2nd Month | 3rd Month | 4th Month |
|---|---|---|---|---|
| Pig 112 | 0.06 | 0.253 | 0.433 | 0.56 |
| Pig 110 | 0.128 | 0.28 | 0.497 | 0.49 |

Pigs 106 and 108. Post-mortem examination showed nothing abnormal. The bones of each pig appeared perfectly normal. The bone analyses are subjoined.

Table 12.

Bone Analyses.

Rib (Dry Bone)

| Percentages. | Ash. | CaO. | P O. | Ash Content. CaO. | Ash Content. P O. |
|---|---|---|---|---|---|
| Pig. 106 (Cod-liver oil). | 44.8482 | 23.79 | 19.344 | 53.04 | 45.14 |
| Pig. 108 (Linseed Oil). | 49.7153 | 26.22 | 20.79 | 52.94 | 41.82 |

Femur.

| | Ash. | CaO. | P O. | Ash Content. CaO. | Ash Content. P O. |
|---|---|---|---|---|---|
| Pig 106 (Cod-liver Oil) | 63.98 | 34.68 | 26.24 | 53.64 | 41.01 |
| Pig 108 (Linseed Oil). | 64.39 | 34.21 | 26.58 | 53.14 | 41.28 |

The experiment was continued using olive oil. This was added (20 c.c. each pig) on 22nd October (9 days into 2nd month). The change was made since certain workers had claimed to have detected traces of vitamin A. in linseed oil. This change did not apparently suit the pigs, and group II.

after an initial improvement began to lose ground in comparison with group I.

Fifth Month. 13/10/21 to 12/11/21.

There were now three pigs in each group. On 7/11/21 the experimental group did badly on olive oil and a re-adjustment of the diet was made as under

| | |
|---|---|
| Bran. | 1lb. |
| Oatmeal | 2½ lbs. |
| Calc. Carbonate | 24.25 grams. |
| Calc. Chloride | 7 grams. |
| Sod. Hydrate | 3 grams. |

Sixth Month.

On 1/12/21 one of the pigs of the experimental Group II. which had gone off its feed was transferred to group I. (cod-liver oil) and after several days slowly began to improve.

On 13/12/21 Pig No. 110, which had become cachectic and had ceased to grow, was killed. Specimens were taken to Dr. Da Fano of King's College, London, for a full histological report which is appended (Appendix I.). On this date the remaining pig of this group, which had now gone off its feed, was transferred to group I. This animal also, after some days began slowly to improve.

Table 13.

Average Weights as before.

| No. of Pig. | 13/10/21 | 12/11/21 | 12/12/21. |
|---|---|---|---|
| Pig 112 Cod-liver Oil | 49.6 | 65.6 | 87.1 |
| Pig 110 Olive Oil | 52.3 | 57.3 | 59.1 |

Table 14.

Average Increase in Kilos.

| No. of Pig | Fifth Month 13/10/21 to 12/11/21. | Sixth Month 12/11/21 to 12/12/21 |
|---|---|---|
| Pig 112 | 0.56 | 0.72 |
| Pig 110 | 0.17 | 0.06 |

Table 15.

Pig 110. The subjoined table gives the bone analysis.

Rib.

| Dry Bone (Percentage Composition) | | | | Ash (Percentage Composition) | |
|---|---|---|---|---|---|
| Organic Matter. | Ash. | CaO. | $P_2O_5$. | CaO. | $P_2O_5$ |
| 57.42 | 42.58 | 22.31 | 18.04 | 52.40 | 42.38 |

Femur.

| Organic Matter. | Ash. | CaO. | $P_2O_5$ | CaO. | $P_2O_5$ |
|---|---|---|---|---|---|
| 38.65 | 61.35 | 32.43 | 25.51 | 52.86 | 41.6 |

These results show no divergence from the normal composition of the bones of swine.

The very full report of Dr. Da Pano (Appendix I.) shows that the nerve and bone tissues were perfectly normal and in particular that not a trace of any rachitic lesion could be discovered. He comments, indeed, upon the hardness of the bones; a point of interest, since the bone analysis makes it evident that this was not due to an unusual proportion of lime salts. The bone analysis is also interesting since it shows that the animal though receiving added calcium salts for many months, did not pass the optimum point of deposition of lime in bone.

Conclusions.

(1) The basal diet of grains and grain offal plus cod-liver oil permitted growth and health for an indefinite period.

(2) The basal diet plus linseed oil permitted growth and health for an indefinite period.

(3) The cachectic condition caused by olive oil in group II. and cured by cod-liver oil had nothing in common with the rachitic syndrome.

(4) The pig, though a rapidly growing animal laying down much bone, is apparently able to do with very little vitamin A, if the mineral constituents of the food supply its requirements.

## GENERAL REVIEW OF EXPERIMENTS I - V.

These experiments proved qualitatively that the rations usually fed to swine may be deficient in mineral matter, that this deficiency produces a rachitic condition, and that this condition does not arise, however long or severe the deprivation of the hypothetical "Vitamin A." if mineral matter be supplied in appropriate quantities.

These results receive confirmation from the work done on swine by Zilva and others (36) who found that the pig can continue to grow for at least 70 days on a synthetic diet with no A. Factor.

## PART 2.

## ~~Quantitative~~ Metabolic Experiments.

A series of experiments was undertaken to test various points which had arisen in the course of the previous work, as under:

I. To determine the calcium retention of the Pig.

II. To determine the effect of added calcium salts on the calcium retention.

III. To determine the effect of added calcium salts on the phosphorus retention.

IV. To determine the effect of added phosphorus salts on the calcium retention.

V. To determine the effect of added organic substances (fats) on the calcium retention.

METHOD: The same method was used throughout the metabolic experiments and the following description *mutatis mutandis* will apply to all.

The pig was placed in a metabolic cage. It was fed by hand thrice daily, and distilled water to appetite was given at these times. The ration was made up in amounts which would be entirely consumed. The substance whose action was to be investigated was added to the ration on the morning of administration and the food made up to a semi-fluid consistency. By giving

the drinking water in repeated washings of the food trough a complete consumption of the food was always obtained. Only in one experiment (low phosphorus diet) was any difficulty encountered in getting the animal to consume its full ration.

URINE: The urine was collected through the false floor of the cage, draining into a jar in the usual way.

FAECES: The faeces which were firm and well-formed especially during the administration of Ca salts were collected from the floor of the cage. An anal bag was tried in the first experiment, but subsequently discarded as it annoyed the animal. The pig, left to its own devices, usually defaecates in one corner of the cage and pays scrupulous attention to cleanliness.

WEIGHT: The animal was removed every morning and weighed; meanwhile the cage floor was washed down with distilled water and the washings added to the contents of the urine jar for the previous day.

ANALYSES: A complete balance of excretion was determined for each day. The foods to be used for the period were analysed and the basal intake thus ascertained. Total nitrogen, calcium and phosphorus were determined for both urine and faeces with certain other determinations for other concurrent experiments. The nitrogen is given throughout as N; the Ca as CaO; the P. as $P_2O_5$. Total nitrogen was determined by the Kjeldahl method - Calcium by precipitation with ammonium oxalate and

titration - phosphorus by the uranium acetate method or precipitation with ammonium molybdate and subsequent weighing.

Metabolic Experiment I.

OBJECT: 1. To determine the calcium retention of the pig.

2. To determine whether the acid nature of the food adversely affected the Ca retention.

METHOD as above:

Animal - Pig No. 48. Weight at commencement of experiment 55.0 kilos.

Weight at completion of experiment 60.6 kilos.

Ca O given in food 1.792 grams.

Ca O added (as $CaCl_2$) 2.576 grams.

The Table subjoined shows the amount retained over a 16 days estimation.

Table 16.

| | Days | Ca O Retained (grams per day) |
|---|---|---|
| Pre-period CaO intake 1.79 grams per day. | 1 | + 0.118 |
| | 2 | - 0.02 |
| | 3 | + 0.56 |
| | 4 | + 3.47 |

E.D. 42 A

| | days. | Ca O Retained (grams per day) | |
|---|---|---|---|
| | 5 | 3.49 | |
| | 6 | 2.55 | |
| | 7 | 3.42 | |
| | 8 | 2.93 | Food neutralised by added NaOH |
| | 9 | 2.43 | |
| Experimental Period | 10 | 2.45 | |
| | 11 | 3.21 | |
| CaO intake 4.37 grams per day. | 12 | 3.07 | |
| | 13 | 3.50 | |
| | 14 | 3.18 | |
| | 15 | 2.75 | |
| | 16 | 3.58 | |

From the 7th to the 11th day inclusive, the food, which was strongly acid, was neutralised by the addition of Na OH to determine whether the calcium balance was thus affected. As will be seen by the table of averages below (Table 17) the retention was rather adversely affected, and this was accordingly discontinued.

Table 17.

Retentions. Averages for the periods of the experiment

| I | II. | III. | IV. |
|---|---|---|---|
| Pre-period (Low Ca Intake) | Added Ca O | Added CaO (Food neutralised) | Added CaO (Food not neutralised.) |
| Grams. 0.2204 | 3.1698 | 2.883 | 3.2153 |

RESULTS: 1. A pig of 55 kilos weight can retain, over a period of more than two weeks, at least 2.5 to 3 grams of CaO per diem.

2. The neutralising of the acid food adversely affects this balance.

Note: The decreased retention of calcium on the addition of NaOH indicates that the acids, being organic, are mostly destroyed in the stomach, and that the extra sodium tends to remove calcium from the tissues or to prevent its absorption.

Full details of this experiment are given in Appendix II.

DISCUSSION: This experiment showed that a growing pig could retain large absolute quantities (3 grams and upwards) of Ca O per day. On theoretical grounds this seemed not improbable since the amount of Ca O retained per 100 grams of live weight increase is reckoned by Oscar Loew (l.c.) at 1.2 grams for the human infant. Taking 1% as a rough approximation, a pig laying on .5 kilos

per day ( a very ordinary gain) would require sooner or later CaO at the rate of 5 grams per day ~~of this increase~~ for full satisfaction of its calcium requirements, and the insistence laid by pig-keeping manuals on the necessity of an earth box for growing swine shows that this point had not escaped the notice of the clinicians.

Since those large retentions were theoretically possible and indeed imperative, experiment II was undertaken to determine whether retentions up to the theoretical maxima took place in actual fact.

E.D. 45.

Metabolic Experiment II.

OBJECT: To determine the effect of added calcium salts on the calcium retention of a growing pig.

METHOD: The pig was kept in a metabolism cage as above.

BASAL DIET:

| | | |
|---|---|---|
| Oatmeal | 250 | grams |
| "Sharps" (Grain Offal) | 250 | " |
| Maize | 250 | " |
| Blood Meal | 50 | " |
| Potatoes | 250 | " |
| Cod Liver Oil | 10 | c.c. |

The foodstuffs were analysed and their mineral and nitrogenous content determined. The pig weighed 15.2 kilos at the commencement of the experiment and 19.4 at the close, 24 days.

E.D. 46.

RESULTS: The results are shown in Table II. subjoined.

Table 18.

No. 83 Pig.

| Day | CaO Fed | CaO Retained. | Intake. |
|---|---|---|---|
| 1 | .652 | - 0.0027 | |
| 2 | .652 | + 0.1900 | |
| 3 | .652 | + 0.0484 | Diet only. |
| 4 | .652 | + 0.1557 | |
| 5 | .652 | + 0.1300 | |
| 6 | 3.152 | 2.5938 | |
| 7 | 3.152 | 2.0352 | |
| 8 | 3.152 | 2.2430 | Diet plus $CaCl_2$ 10 grams |
| 9 | 3.152 | 2.2165 | (2.5 ~~gr~~ CaO) |
| 10 | 3.152 | 2.3941 | grams |
| 11 | 3.152 | 1.4651 | |
| 12 | 4.402 | 3.0438 | |
| 13 | 4.402 | 2.8583 | |
| 14 | 4.402 | 2.4036 | Diet plus $CaCl_2$ 15 grams |
| 15 | 4.402 | 2.6369 | 3.75 ~~gr~~ CaO. |
| 16 | 4.402 | 2.6031 | grams |
| 17 | 5.652 | 3.5776 | Diet plus $CaCl_2$ 20 grams |
| 18 | 5.652 | 2.8470 | |
| 19 | 5.652 | 3.0200 | 5 grams CaO. |
| 20 | 0.652 | - 1.5563 | |
| 21 | 0.652 | - 0.105 | |
| 22 | 0.652 | + 0.1379 | Diet only. |
| 24 | 0.652 | + 0.1032 | |
| 25 | 0.652 | + 0.1524 | |

Table 19.

| Averages | | Intake. | Retention. |
|---|---|---|---|
| | Period I | (0.652 grams) | 0.1043 |
| | " II | (3.152 grams) | 2.0708 |
| | " III | (4.402 grams) | 2.5655 |
| | " IV | (5.652 grams) | 2.9335 |
| | " V | (0.652 grams) | 0.1292 |

CONCLUSIONS: A growing pig of between 15 and 20 kilos is able to retain as much as 3 grams of CaO per day.

DISCUSSION: It will be observed that of the first increment of CaO (2.5 grams) 80% was retained; of the second (1.25) 40%; of the third (1.25) 30%; showing that although retention was lessening for the later increments, the limit of coloration had not been reached.

It is noteworthy that the prompt return of the excretion to a figure similar to that of the pre-period shows that the large amounts of Ca retained were at least temporarily in combination with the tissues.

I have permission to quote from a joint experiment by Dr. Orr and Mr. Husband the following unpublished figures from a "fish-meal" feeding experiment in general support of the contention as to the large amounts of CaO retained by growing swine.

## Table 20.

Pig No. 83.

| | Ca O given in food. | Ca O Balance. | |
|---|---|---|---|
| 1. | 0.652 | 0.1032 | |
| 2. | 0.652 | 0.1518 | |
| 3. | 12.244 | 11.0442 | 100 grams per day. "Fish-meal" added. Fish meal contains about 20 per cent calcium phosphate. |
| 4. | 12.244 | 6.5436 | |
| 5. | 12.244 | 5.8802 | |
| 6. | 12.244 | 4.6124 | |
| 7. | 12.244 | 4.7471 | |
| 8. | 0.652 | - 5.5484 | |
| 9. | 0.652 | - 1.8446 | |
| 10. | 0.652 | - 0.0078 | |

There were of course many other factors influencing the retention besides the extra quantities of Ca; the phosphorus intake for instance was raised by the addition of the fish meal from 6.86 grams to 17.37 grams. The proportion of the two elements, Ca and P, was probably in close approximation to that required for the optimum retention since their presence in fish-meal is derived from the finely ground fish-bones. The point is here that this large amount of CaO was actually being retained, since on the third day of the post-period the excretion had fallen to 0.66 grams and the negative balance to 0.0078 as against an excretion for the pre-period of 0.54 and a positive balance of 0.125.

Table III.

A further table from a joint experiment, (Elliot, Husband & Orr) (on the addition of calcium lactate, sodium Phosphate and fats) illustrates further the reality of these high retention.

TABLE 21.

| Day of Experiment. | CaO Intake. | CaO retained. | Pig 83. |
|---|---|---|---|
| 1. | 0.76 | -0.2478 | |
| 2. | 0.76 | -0.09 | Diet only. |
| 3. | 0.76 | -0.1122 | |
| 4. | 4.51 | 3.4276 | |
| 5. | 4.51 | 3.3452 | Diet plus 20 grams |
| 6. | 4.51 | 3.0645 | Calcium Lactate. |
| 7. | 4.51 | 3.3422 | 3.75 grams CaO. |
| 8. | 4.51 | 3.2458 | |
| 9. | 8.26 | 7.102 | Diet plus 40 grams |
| 10. | 8.26 | 6.3928 | Calcium Lactate. |
| 11. | 8.26 | 5.6013 | 7.5 grams CaO. |
| 12. | 8.47 | 5.9277 | Diet plus 40 grams Cal- |
| 13. | 8.47 | 6.33 | cium Lactate, Plus 10 |
| 14. | 8.47 | 6.57 | grams $Na_2PO4$, Plus in- |
| 15. | 8.47 | 6.01 | creased food. |
| 16. | 0.97 | -2.2936 | |
| 17. | 0.97 | -0.0468 | Diet only. |

The rapid decline in Ca excretion is noteworthy, proving again that as the faecal contents were eliminated in the first 2 days of the post-period, the excretion of Ca returned to a figure comparable to that of the pre-period - i.e. that these large retentions remained built into the body tissues.

## Experiment III.

OBJECT: To determine the effect of added calcium salts on the phosphorus retention.

METHODS: The methods used were as above and the determinations were carried out during Experiment II of which they formed part. They will be found together with Experiment II in the Appendix, but have been here separated for the sake of clearness.

The food contained 6.869 grams $P_2O_5$ per day, which was a constant. The variations therefore for Periods I, II, III, IV and V are attributable to the varied amounts of Ca salts added to the food.

RESULTS:

Table 22.

Influence of CaO on a P constant Diet.

| | | $P_2O_5$ in Food | $P_2O_5$ retained. | Pig 83. |
|---|---|---|---|---|
| Period I | 1. | 6.869 | 4.45 | |
| | 2. | " | 4.68 | |
| | 3. | " | 4.20 | CaO in Food 0.652 |
| | 4. | " | 4.11 | |
| | 5. | " | 4.14 | |
| Period II | 6. | " | 5.19 | |
| | 7. | " | 4.44 | |
| | 8. | " | 5.03 | |
| | 9. | " | 5.15 | CaO in Food 3.152 |
| | 10. | " | 5.43 | |
| | 11. | " | 4.22 | |

Table 22 (Contd.)

| | | $P_2O_5$ in Food. | $P_2O_5$ retained. | |
|---|---|---|---|---|
| | 12. | 6.869 | 4.90 | |
| Period III | 13. | " | 5.24 | |
| | 14. | " | 5.11 | CaO in Food 4.402. |
| | 15. | " | 5.13 | |
| | 16. | " | 5.11 | |
| | 17. | " | 5.07 | |
| Period IV | 18. | " | 4.61 | CaO in Food 5.65. |
| | 19. | " | 4.90 | |
| | 20. | " | 4.31 | |
| | 21. | " | 3.42 | |
| Period V | 22. | " | 3.93 | CaO in Food 0.652 |
| | 23. | " | 3.55 | |
| | 24. | " | 3.78 | |

Average Retention $P_2O_5$

| Period | | | | |
|---|---|---|---|---|
| | I | 4.32 | IV | 5.22 |
| | II | 4.85 | V | 3.75 |
| | III | 5.12 | | |

DISCUSSION:

It is extremely interesting to note that the $P_2O_5$ retention not only shows a clearly marked rise with increase of CaO ingested but falls off rapidly when the ingestion of these large amounts is stopped. Thus the retention in Period IV (CaO in food 5.65) is 5.22 and comes down suddenly to 3.75 on the withdrawal of the added Ca in Period V (CaO = 0.652) a reduction of 1.47 grams or nearly 30%.

## EXPERIMENT IV.

OBJECT: To determine the effect of added phosphorus salts on the calcium retention.

METHOD: The methods were used as above. Special difficulties presented themselves in this case owing to the impossibility of producing a low Phosphorus diet which is not at the same time a low calcium diet. This difficulty has been encountered by all previous workers and notably by the Ohio School (Bulletin 6, 1914). This difficulty was met by adding a fixed quantity of CaO in the form of calcium chloride (2.933 grams CaO). As it was possible that the subsequent addition of sodium phosphate would lead to the formation of calcium phosphate in the food, and unintended results in the power of absorption of the salt, the phosphate, in the form of sodium acid phosphate, was added to the ration over night and allowed to soak well in while the $CaCl_2$ solution was added just before feeding.

A further difficulty, which is inseparable from the low phosphorus ration, was encountered in that the pig is extremely intolerant of a low phosphorus ration, and tends to go off his feed. More than one diet had therefore to be tried, and 14 days prior to the day taken as commencement of the experiment were discarded. The pig was thus under full excretory analysis for 24 days during all of which time he was on a low phosphorus diet with

the exception of the 5 days of added phosphorus. During these 5 days he improved greatly in condition and became voraciously hungry, though he fell off again 2 days after the withdrawal of the phosphorus. The low condition is shown in the weight curve which only rose from 23.8 kilos on the 1st day to 28.7 on the 24th an increase of less than 5 kilos. At the conclusion of the experiment the sodium phosphate was restored to his ration and in 7 subsequent days he increased by over 1 kilo per day, relapsing again when the added phosphate was withdrawn.

RESULT: The result of adding increased phosphorus on the constant Ca intake is shown in attached table 23.

Table 23.

Influence of $Na_2 HP O_4$ on a Ca constant diet.

| | CaO in Food. | CaO retained. | |
|---|---|---|---|
| 1. | 3.3167 | 2.110 | |
| 2. | " | 1.016 | $P_2O_5$ in Food 4.863 grams |
| 3. | " | 1.046 | |
| 4. | " | 1.578 | |
| 5. | " | 2.335 | |
| 6. | " | 1.374 | |
| 7. | " | 1.832 | $P_2O_5$ in food 8.843 grams |
| 8. | " | 2.035 | |
| 9. | " | 1.853 | |

| | CaO in Food. | CaO retained. | |
|---|---|---|---|
| 10. | 3.3167 | 1.576 | |
| 11. | 3.3167 | 1.882 | |
| 12. | " | 1.593 | $P_2O_5$ in food 4.863 grams |
| 13. | " | 1.997 | |
| 14. | " | 1.813 | |

Average Retention CaO - 5 days periods.

| | | |
|---|---|---|
| Period I | 1.687 | |
| Period II | 1.886 | Increase 0.119 |
| Period III | 1.772 | Decrease 0.114 |

DISCUSSION: The table of averages shows a slight but quite definite increase of the Ca balance or the substitution of high phosphorus for low phosphorus (0.199 i.e. practically 0.2 grams CaO per day) and evident decline is manifest on the withdrawal of the added phosphorus (0.114 grams CaO) for the 5 day period. The experiment could not have been prolonged beyond this as the pig was already showing loss of appetite, and indeed declined in weight for two days after conclusion of the experiment.

For greater exactitude the data for the last 3 days of each period have been analysed since the first two days are inseparable from the fallacy that the intestinal contents of the lower end of the canal, which are passing out, are different.from those of the upper end, which contain the salt added. This fallacy tends to cancel

itself out in the subsequent period but, as will be seen in the subjoined table, the actual curve rises more steeply than the 5 day period would reveal. It is also more gradual in its fall, and this again is in accord with the clinical signs which show a rapid improvement on the administration of phosphorus compounds and a more gradual decline on their withdrawal.

Table 24.

Average Retention CaO - Last 3 days periods of each section omitting the first 2 for adjustment of faecal excretion. CaO intake constant, phosphorus added in Period II.

| | | |
|---|---|---|
| Period I | 1.5465 | |
| Period II | 1.9079 | Increase 0.4604 grams per day. |
| Period III | 1.8008 | Decrease 0.1061 grams per day. |

## Experiment V.

OBJECT: To determine the effect of added organic substances (fats) on calcium retention.

METHODS: The methods were used as above. A low Ca intake was devised, in order to show more clearly the results of adding fats. It is noteworthy that the pig showed great tolerance of this low Ca intake, continued to grow, and remained in good condition.

DIET:

| | Grams. |
|---|---|
| Sharps | 400 |
| Oatmeal | 400 |
| Maize | 400 |
| Potatoes | 400 |
| Blood Meal | 50 |

WEIGHT OF PIG: The pig weighed 27.8 kilos on the first day and 40.3 kilos on the 33rd (concluding) day of the experiment. The results are shown in the subjoined table; owing to the length of the experiment (33 days) averages only are given.

In Period I basal diet was given; in Period II basal diet and cod liver oil; in Period III basal diet and lard; in Period IV basal diet and linseed oil; in period V basal diet only.

The calcium intake was not altered and remained at 1.0618 grams CaO throughout the experiment.

Table 25.

| Grams CaO. | Basal Diet. Period I | Cod Liver Oil. Period II | Lard. Period III | Linseed Oil. Period IV | Basal Diet Period V. |
|---|---|---|---|---|---|
| Grams CaO Average Intake per day. | 1.0618 | 1.0618 | 1.0618 | 1.0618 | 1.0618 |
| Grams CaO Retention per day. | 0.1769 | 0.4531 | 0.5465 | 0.7820 | 0.6389 |

CONCLUSIONS:

(1) The influence of fats in promoting a Ca retention is strikingly manifest.

(2) The superiority of cod liver oil is not apparent and indeed linseed oil in this experiment coincided with the largest retention.

DISCUSSION:

The increase of retention, by the administration of fats might almost be said to show Ca as a fat soluble, growth-promoting substance.

The high retention on Linseed oil may be due to a cumulative improvement in the animal's condition. It will be observed that the high Ca retention persists

throughout the three days of the post-period.

It is interesting as a general point to contrast the long tolerance of this pig for a low Ca diet as against the previous experiment where a much shorter period of low phosphorus caused signs of ill-health.

One is led to remark on the strange coincidence that animals are supposed to have the power of storing Vitamin A in considerable quantity while Vitamin B cannot be stored. It is mathematically certain that this pig could not have laid down a due proportion of fully calcified bone during this period since he was only receiving in all, during the whole period, .27 grams CaO in his food for every 100 grams increase of body weight added and the utilisation was only about 50-60% of this.

## NOTE TO METABOLIC EXPERIMENTS.

In these tables only the gross data have been dealt with. Appendix II contains the complete metabolic data for the five experiments. The other data from the tables showing the influence of the alteration of salts both in absolute and in relative quantities on the method of excretion are also of the greatest interest. Graphs showing these will be found in Appendix III.

## SECTION II.

## OSSEOUS LESIONS IN OTHER ANIMALS WITH A NOTE ON RICKETS IN THE HUMAN.

## SECTION II.

## OSSEOUS LESIONS IN OTHER ANIMALS.

Osseous Lesions of a chronic progressive nature occur in many species of domesticated animals. Hutyra and Marek (l.c.) give the animals most frequently affected with "rickets" as "young pigs, puppies, lambs, and kids; calves and rabbits much more rarely; birds may also suffer from this disease."

The data recorded concerning the physiology and dietetics of the young of these animals, both by clinical and by laboratory methods are second in extent and in importance only to those recorded for the human, and in many cases not even surpassed by those. There are literally millions of experimental facts recorded about milk cows and cow-milk, yet in spite of the fact that there are many thousands of years of trial and error to guide us in the problem of using the milk of this species for the young of our own, the problems are not yet all cleared up, though human milk may be at will obtained for comparison.

Compared with these, the physiology of the laboratory animals is unexplored territory. There is not a single exact estimation recorded as to the milk of the

rat, mouse, or guinea-pig, on which so many growth, i.e. weaning, experiments have recently been performed. The amount, specific gravity, fat content, protein ratio, ash content, carbohydrate quantity, are all alike unknown.

Observations made on these small animals are therefore relatively of little importance for nutritional work on problems of growth, since we have neither clinical nor experimental evidence as to the food to which we desire to approximate. This review therefore will deal first with the pathological bone changes in animals of whose physiology we have at least a partial knowledge, before passing to the lesions of animals concerning whom the rudimentary physiological data are not as yet ascertained.

## I. SHEEP.

Next to the pig the farm animal most frequently affected in this country is the sheep. The condition in this animal is of great importance since the numbers in these islands are very large, (23,000,000) and are kept in conditions more closely approximating to the normal habitat of the species than those of any other domesticated animal.

Investigation of the bone lesions of the sheep has proceeded for the purposes of this research for the last eighteen months. So far only a general survey has been accomplished since all available time was devoted to the foregoing work on the pig. In addition, suitable experimental animals for growth investigations come on the market normally in autumn, and by this time the sheep are large and grow slowly. It was not thought justifiable to take lambs and feed artificial diets to them before the time at which this is usually done by practical men, since this involves losing the benefit of the great mass of observations, which farmers have collected, on sheep passing through the dietary transitions which, by the process of trial and error

for many hundred years, have been worked out as desirable.

It was only in autumn, 1921, therefore that a sufficient naked-eye and low-power survey had been completed to warrant the use of a higher magnification. Sixteen sheep, in three groups and a control, have been under experiment since October, 1921, but results are not anticipated till April or May.

The following facts, however, have been ascertained in this preliminary survey.

(1) The condition occurs in young growing animals both in sheep kept in the open, on grass, and in sheep houses, artificially fed.

(2) The signs presented to the shepherd are loss of appetite, lethargy and bending of the long bones. The lethargy is not excessive. There are few signs of pain.

(3) Recovery is usual, and animals which have recovered, though permanently deformed, are still highly esteemed and in the case of rams are used without misgiving for breeding.

(4) Male sheep are the most frequently affected.

(5) The condition is universally attributed by

shepherds to dietary errors.

Two cases, of a number collected, may be considered.

Normal

See page 97.



Sheep A.


Sheep B

Sheep A + B. See page 96

These two rams born April 5, 1920, were reared in the country without artificial food. They were sent at the age of 7 months to winter in the Tay-side area. They formed part of a lot of 30, all of whom were kept out-of-doors all winter and re-received no artificial food of any kind. A large proportion developed rickets, the condition appearing insidiously between the ages of 7 and 12 months.

In the spring they returned to their own farm and recovered. These sheep were used for stud purposes in November.

The sheep were on "heavy land", 1st year young grass, all winter.

In other cases sheep (mostly young rams) being fed under cover with concentrated food, may develop rickets. These conditions do not differ essentially from those under which the disease arises in the pig and in the infant.

Conclusions.

(1) It is not yet certain whether rickets in the sheep bears a close resemblance histologically to rickets in the human.

(2) Rickets in the sheep can arise on a diet in all possible Vitamines (grass).

(3) Rickets in the sheep can have no relation to an avitaminosis.

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The special feature of interest in the case of the sheep is the case of out-of-doors, grass-fed rickets. Since neither vitamines nor housing can have any influence it is interesting to consider the possible influence of mineral matter. There is only one source of mineral matter for the sheep (since it is not, like the pig, a rooting animal) and that is the herbage which it consumes. The plant, however, is the analysis of the soil on which it grows, and deficiencies or excesses of any mineral constituent in the soil are forthwith reflected in the vegetation upon it. The plant, which is a biological unity, is not a chemical unity and even the cereals vary within very wide limits. Indian corn is given in the official ash analyses of the U.S.A. department of agriculture (37) (Exper. Station record 1898, P.372), as totally devoid of chlorine. (0.00). It is given in the Ohio State analysis (38) as containing 0.073 Cl. per cent of dry substance. The importance of such a factor in

estimating the vitamin destroying power of $Na_2CO_3$ and Na Cl respectively in making corn bread as was done in the Spartanburg County enquiry, (38A) may be imagined. The Ohio workers give a table of mineral elements of wheats variously fertilised showing variations even in such an essential constituent as organic phosphorus from .318 per 100 of dry substance (for wheat from a nitrogen-potassium fertiliser) to .380 for unfertilised wheat, a variation of nearly 20%. These mineral differences, though tiny in themselves, reach considerable amounts, in the cumulative result of many months feeding.

Since these variations exist even in the seed, the leaves naturally show a much wider range of variation. One table alone will demonstrate the point, an analysis carried out on carrots at Riga. (37).

CARROTS: ASH ANALYSIS.

| | Ash | Pot Oxide | Sod- ium Oxide | Cal- cium Oxide | Mg.O. | Fe.O. | Phosp. A. | Sulph. A. | N. |
|---|---|---|---|---|---|---|---|---|---|
| N. Fertiliser. | | | | | | | | | |
| Leaves | 15.727 | .66 | 1.89 | 4.58 | 1.13 | .5 | .54 | .47 | 3.27 |
| Roots | 4.62 | .62 | .75 | .25 | .28 | .02 | .48 | .25 | 1.95 |
| Potassium Chloride. | | | | | | | | | |
| Leaves | 18.06 | 5.13 | .31 | 4.52 | .61 | .1 | .38 | .76 | 3.19 |
| Roots | 9.07 | 4.10 | .22 | .30 | .18 | .15 | .50 | .26 | 2.02 |
| Sodium Chloride. | | | | | | | | | |
| Leaves | 20.6 | .82 | 4.75 | 4.46 | 1.07 | .15 | .61 | .66 | 3.61 |
| Roots | 7.79 | .93 | 1.92 | .36 | .34 | .02 | .72 | .34 | 2.43 |

Note: Constancy of Ca and Mg. and wide variation of K. also to some extent of Fe in leaves. Also replacement of Na by K.

Buckner in the Journal of the American Chemical Society gives two analyses of crab grass (Digitaria Sanguinalis), one of a plant growing on a garden soil and the other of a plant growing on a limestone roadway. The plant on the limestone roadway contained 18.8% less $K_2O$, 22.7% more $P_2O_5$, 44% more Ca O, and 27.6% more $M_3O$. The total ash was 16% less. There was no observable difference in their external appearance. The work of Theiler (39) and others on lamziekte in South Africa, demonstrates another point. They found grass in the same area but at different seasons of the year to vary from 0.08% of $P_2O$ to 0.56%, a difference in phosphorus content of 700 per cent.

It is impossible to do more at present than to indicate the lines which it is hoped may lead to results, but the fact that from the mineral standpoint the plant is the analysis of the soil and the graminivore the analysis of the plant, is full of significance, considering the extraordinarily heavy death rates, from obscure nervous and other disease, of sheep on certain soils in Scotland.

In The West Highlands of Scotland, for instance, sheep stocks have a death-rate so far above that of normal areas that specially great importance is attached to the usual contracts entered into between landlord and tenant binding the tenant to hand over, and the landlord to receive, a given population of sheep at the end of the lease. If the "acclimatised" stock were removed from the area, the incidence of sickness upon a new stock introduced would not only absorb the entire surplus for perhaps 12-15 years, but would require the purchase of large annual contingents merely to maintain the existing numbers. The value of the small and ill-bred stocks which can alone maintain themselves in these areas is at the end of a lease loaded with an "acclimatisation figure" of as much as 30% above their utmost value in a market.

It may be argued that this suggests much more a process of minimisation against microbic infection than a possible deficiency disease. The geological composition of good or bad sheep-land is, for all that, distinctly suggestive in the association of granite with disease and limestone with health.

Much work has been carried out and a great deal of light thrown on the possible infectious nature of these widespread and costly diseases, but the influence of a dietary factor has not so far been broached.

Again, in their natural habitat the graminivora are accustomed to migrate over long distances to obtain change of food. It is interesting to note that in certain areas in this country, on Border sheep-farms, similar migrations are still carried out, though by rail. The sheep are moved into England; not for wintering but for the "change", and it is the verdict of men making their living by the care of flocks that but for this migration the animals would die in large numbers, although apparently provided with ample food supplies. The change in climate or in amount of food is practically negligible; a change in quality of food seems at present the most useful hypothesis, and as has been shown, the mineral constituents of herbage are those which show the widest variations.

Cattle. It is convenient here to consider calves and oxen generally. The most striking fact here is a negative one. Rickets is very seldom met with in the calf. This is more than surprising, considering the rigorous confinement and drastic withdrawal of butter fat to which this animal is subjected. There were in Scotland alone during the year 1920, 350,000 cows in milk (Trans. High Soc. Vol. XXXIII, 1921), and the dairy industry derives a large part of its revenue by abstracting the fat from the food of the calf and selling it for the use of other species.

The existence of this industry requires more attention from the exponents of Vitamin A. than it has hitherto received. The fact that dairy farmers habitually rear calves successfully on skim, or separated, milk to which starch, rolled oats, or linseed oil has been added, is not commented on in the copious Vitamin literature, save that from an experiment on puppies Mellanby (l.c.) dismisses a mixture of this kind called "Marylebone Cream", (then being distributed in the Infant Welfare Centres in London) as "a concoction", and "not possessing the anti-rachitic effect."

In the Aberdeen area the conditions of calf-rearing approach a laboratory test. Owing to the value of the Aberdeen-Angus breed for beef purposes, winter calves are not slaughtered but continued on a separated milk diet for many weeks, with, eventually, added turnip-roots, and survive successfully, though naturally not as good animals as those born in the summer.

Even cod-liver oil has been known for many years to the dairy farmer, and after repeated tests the agriculturist will not commit himself further than to say that it may be safely recommended as a cream substitute. Examination of the growth surves of calves supplied with cod-liver oil as against those on vegetable oils show usually a superiority for the cod-liver oil ration but in no such striking disproportion as the addition of Vitamin A. would demand, the ratio running about 7 to 5 in favour of cod-liver oil as against linseed.

A bone disease of ~~calves~~ *cattle* described by Ostertag and Zuntz and summarised by Theiler (40) is of considerable interest from the point of view of mineral deficiency. This disease is a chronic disease of cattle whose chief symptom is an abnormal craving for

unusual foods, mortar, stones, wood, etc. There are disturbances of digestive and nervous systems, wasting of the tissues, excessive anaemia, and abnormal brittleness of the long bones, with wasting of the cortex.

The disease is chiefly noted in heifers, cows, and young animals. It is peculiar to certain areas and appears chiefly in the winter. It is produced by the feeding of hay from these areas.

Analysis of the hay showed an increase of potassium and a reduction of lime as compared with normal hay. A seasonal differentiation was attempted by feeding separately the first, second, and third cuttings from the same area in the same year. The first hay did not produce any disease; the second and third produced the disease. Stutzer's analysis (41) gives the percentage composition of ash in good quality hay as 9.5 CaO and 4.3 $P_2O_5$ in parts per 1000; while for second crop hay the percentage is 5.1 CaO to 4.1 $P_2O_5$.

Metabolism experiments showed a greatly diminished retention of both lime salts and phosphoric acid.

The addition of lime salts and phosphoric acid failed to prevent the disease, but treating the hay

with boiling water produced some benefit (calves increased in weight by 16.5% in 5 months but sickened and died in 8 months.)

It is interesting to note the point as to the excess of K. K salts in excess have a very deleterious effect upon health and in the United States, millet hay, which has a high K/Na ratio (K 133 Na9) as against alfalfa hay (K 83 Na 48) is well known for its toxic effects on stock. (Forbes Analyses of Foods, l.c.) Not improbably the treatment with hot water would remove some of the excess of K.

Thieler states that this disease bears a strong resemblance to "lamziekte" in South Africa though this disease produces no wasting of the bones. It is now considered by him and his associates to have its origin in a phosphprus starvation leading to a phosphorus craving so intense that the animal will consume any P-containing substance, even putrid bones. From these bones are absorbed toxic substances producing the acute effects.

The mineral metabolism of milch cows is a subject too large to enter on within the present limits. The very large quantities of mineral needed for a cow

in full milk require emphasis. Hals (42) states that a milch cow with a daily yield of 10 kg. milk would require 70 grams CaO and 45 grams $P_2O_5$ per day, and he, too, points out that concentrated feeds are low in line and high in phosphoric acid.
In Bihar, India, again, where the $P_2O_5$ content of the soil and of the crops is extremely low - less than 1% of the European soil standard for certain areas,- the native cattle yield very little milk. The cattle of Bihar yield from 3 to 5 seers of milk per day, those of Surat 16 seers, those of Kathiawar 15 to 20 seers, and those of Delhi 25 seers. The influence of breed must, of course, be considered; but the yield of even the best milch cows rapidly decreases on being brought into Bihar State. (43)

It has recently been stated (1920) by Hess and Unger (44) that the vitamin content of milk is altered by various diets. They also give the percentage composition of the ash of milk on a green food ration as against a ration of meal and molasses.

| | Green Food | Meal & Molasses |
|---|---|---|
| CaO | 0.165 | 0.138 |
| $P_2O_5$ | 0.190 | 0.158 |

On the other hand, the percentage of sulphur and magnesium was higher in the milk of cows fed on the meals. All these facts go to show that the differential qualities of the mineral contents of milk must be excluded before any hypothetical substance is invoked.

Horses. Foals rarely suffer from rickets. The adult horse however suffers from some bone lesions not without interest from the point of view of mineral metabolism. The fact that a sufficiency of phosphorus must be present to permit the utilisation of the calcium which might be present in abundance was brought out in Metabolic Experiment IV (pig). Disturbance of the balance on the other side, however, is equally fatal to health. Hutyra and Marek (l.c.) and other authors describe a cachexia and osteoporosis known as "bran-sickness" and caused by excessive amounts of bran in the food where the excess of the P & K in the Bran eventually produces ill-health.

In this connection the disease known as "kumri" may be considered.

This disease is described by C.H.K. Macalister (45) in 1917. He defines it as -

"A chronic incurable disease of horses characterised by inco-ordination and loss of power in the hind limbs", and other observers state that the bones are soft and easily cut.

The range of the disease is very widespread, occurring as it does in India, Ceylon, the Federated Malay States, the Dutch Indies and China. A similar

disease is described in the Belgian Congo, with well marked lesions in colts of 8-9 months (46).

It seems to have points of close resemblance to Bighead of horses described in Florida (47) and to an Osseous Cachexia of Horses of Brazil (48) and in New Caledonia (49). It is especially rife in Bihar Province, India. In this connection it is interesting to note that the soil of Bihar may contain as low as 1% only of the European standard content of phosphorus and that the rice of Bihar runs in many cases to a phosphorus content of as low as 0.29% as against 0.47% for normal rice.

Cures are reported from the Belgian Congo (46.l.c.) by the administration of Ca in the food. It is noteworthy that the soils on which this disease arises have been found by analysis both in New Caledonia and in the Congo to be very deficient in lime, while in neighbouring areas rich in lime this disease does not occur.

## THE DOG.

The dog occupies an intermediate position between farm stock and the laboratory animals. It is kept in close association with man and receives much observation. At the same time, owing to this association it has a varied diet, and in particular a diet in which bones are generally available to supply any possible calcium or phosphorus deficiency. As has been pointed out, there is no such great clinical body of observation on the feeding of dogs in confinement as is available in the case of the staple meat-supplying flocks and herds.

Reference has been made in the Introduction to the work of Voit and others. It is impossible to ignore this mass of patient investigation in the cavalier fashion of some recent experimenters. During his work he made estimations of the calcium physiologically necessary to the growth and health of the animal whose pathology he had under review. He worked this out (l.c.) at figures greater than are usually allowed to-day. Voit found that a dog of moderate size required .5 grams Ca O per day and a large dog .75 grams. Taking the previously established approximation of 1% of the increase in body weight as due to retention of Ca O this would correspond, even if all were

retained, to an increase of 50 grams per day for a small, and 75 grams for a large dog, a not impossible growth. An experiment performed for the purpose of this thesis (detailed below) gave a retention over 31 days of 1.7 gram Ca O per 100 grams increase in live weight in a small, slowly growing dog. The daily retention worked out at just over .21 grams per day - which for the size of the dog (4.4 kilos) is in close agreement with the work of forty years ago.

The earliest work done in this country is that of Findlay, 1908 (l.c.) continued and expanded by Paton and others till 1921 (l.c. and 50); their studies are mainly concerned with the influence of the exercise factor and of the organic constituents of the food, though Paton has also investigated the endocrine regulation of calcium metabolism which limits of space forbid a discussiom of here. The most extensive dietary studies in this country on dogs are those of Mellanby's (l.c.) and his work, on over 400 animals has, he claims, "centred round calcium metabolism". But one searches almost in vain for any attempt to meet, or even to calculate the needs of his animals for anything but the hypothetical vitamin - A. The fundamental work of Voit on the calcium requirements of dogs is neither repeated nor disproved, but

simply ignored. The question of deficient intake is dismissed in the sentence: "It will be generally admitted that experimental results on animals and clinical experience are opposed to calcium deficiency as being the main cause of rickets", and he continues: "The problem of deficient intake of calcium has received comparatively little consideration in this research" - a claim which is fully confirmed on examination of the work. Thus, discussing the influence of bread he gives a puppy (No. 335) (l.c. No. 61 p. 43) as increasing in weight in ten weeks by 2,990 grams. The basal diet provided an intake of Ca O at the rate of .35 grams per day. This is far below Voit's standard even for a small dog (.5 grams). Reckoned in percentage of increase this rate of growth - 299 grams per week - would demand the retention of 2.9 grams per week, whereas the ingestion is only 2.45. It seems undoubted that so long as the minimum needs of known constituents are not supplied there is no justification for "the slow crystallisation of the idea that excess of bread might be responsible for the exaggeration of the rickets syndrome" (l.c.) In face of these figures the generalisations, e.g., that "a sufficiency of calcium must be given" or that "plenty of calcium and phosphorus in the diet" tend to prevent rickets in puppies have no meaning. In very many of the experiments these needs are not fulfilled

and the experiments are therefore without value.

The following experiment was made to examine the findings of Voit and to test some later statements.

A dog of 4.4 kilos was placed in a metabolic cage; the methods were the same as in experiments previously detailed. The basal diet was 50 grams oatmeal, 50 grams rice, 2 grams Na Cl and 250 c.c. goat's milk per day. Calcium was added in the form of 6 grams Calcium lactate = 1.058 grams Ca O.

The subjoined table shows the balances.

Table 26.

| Day | Ca O Intake in food | Ca O Balance | |
|---|---|---|---|
| 1 | 1.637 | - 0.33 | |
| 2 | " | + 0.82 | |
| 3 | " | + 0.57 | |
| 4 | " | - 0.66 | |
| 5 | " | + 0.01 | 5 grams $Na_2 HPO_4$ added |
| 6 | " | + 0.68 | = 1.06 gram $P_2O_5$ |
| 7 | " | + 0.53 | |
| 8 | " | - 0.78 | |
| 9 | " | + 0.84 | |
| 10 | " | - 0.48 | |
| 11 | " | + 0.61 | |
| 12 | " | + 0.09 | 5 grams $Na_2 HPO_4$ added |
| 13 | " | - 0.33 | + 10 c.c. cod liver oil. |
| 14 | " | + 0.09 | |

Table 26. (Continued)

| Day | Ca O Intake in food | Ca O Balance | |
|---|---|---|---|
| 15 | 1.603 | + 0.94 | 5 grams $Na_3 PO_4$ added |
| 16 | " | - 0.17 | = 1.03 grams $P_2O_5$ |
| 17 | " | - 0.22 | + 10 c.c. cod liver oil. |
| 18 | " | + 0.79 | |
| 19 | " | + 0.74 | 5 grams $Na_3 PO_4$ |
| od 4 20 | " | - 0.72 | added |
| 21 | " | + 0.27 | |
| 22 | " | + 0.96 | + 10 grams lard. |
| 23 | " | + 0.72 | |
| 24 | " | - 0.40 | |
| 25 | 0.5786 | - 0.05 | |
| od 5 26 | " | + 0.17 | No calcium or |
| 27 | " | + 0.40 | phosphorus added. |

The results are clearer when averaged out for the whole periods, as has been done in the table following No 27 (page 116)

Table 27.

| | Ca O Intake | Ca O Balance | Diet + |
|---|---|---|---|
| Period 1 | 1.637 | + 0.35 | 1.05 grams Ca O |
| Period 2 | 1.637 | + 0.02 | Diet + Ca O<br>+ $Na_2 HPO_4$ 5 grams<br>+ 10 c.c. cod liver oil. |
| Period 3 | 1.62 | + 0.16 | Diet + Ca O 1.60 grams<br>+ $Na_3 PO_4$ 5 grams<br>+ 10 c.c. cod liver oil. |
| Period 4 | 1.62 | + 0.33 | Diet + Ca O 1.60 grams.<br>+ $Na_3 PO_4$ 5 grams<br>+ 10 grams lard. |
| Period 5 | 0.57 | 0.18 | Diet only<br>no added calcium<br>or phosphorus. |

The agreement with Voit's work is close, in view of the fact that the dog only increased from 4.4 to 4.8 kilos during the 31 days of the experiment. It will be observed that the addition of sodium phosphate actually lowered the calcium retention. This may be due to the fact that both calcium lactate and sodium phosphate were added together and it may be, possibly, the resultant compound is less easy to absorb. It is interesting to note

that cod liver oil gave a lower Ca absorption than lard, and this has been not unusual in the work of this thesis. Mellanby in his monograph mentions one dog whose diet was supplemented with tricalcic phosphate (l.c. p. 54) and developed rickets. He states that abundance of calcium in the diet will not prevent rickets, but it is obvious that the calcium must be in a form utilisable by the animal, and the experiment detailed above shows that apparently by purely chemical means the calcium in the food may become unavailable for the dog's needs.

Rodents.

The fact that nothing whatever is known of the natural food of the young rat, mouse or guinea-pig (milks of these species) makes it difficult to treat with due seriousness observers demanding wide and sweeping dietetic changes on the strength of experiments on these undertermined requirements. For instance, although it does not actually come within the scope of this paper, reference may be made in passing to the typical absurdity of using the needs of the guinea-pig, to determine the properties of the milk of the cow administered to the young of the Primates. Considering that the young guinea-pig is born in such an advanced state of dentition that it can practically do without milk and that in any case lactation does not last for more than 14 days, the conclusion that "milk", i.e., cows' milk, has a very low power of protection against the syndrome known as "guinea-pig scurvy" is not surprising.

The brilliant work of Hopkins in 1906 - 1912 concerned itself with the general failure of the whole economy and not of one particular metabolism alone. McCollum and others (51) (52) in 1921 observed in their work upon rats that changes resembling rickets more frequently occurred when the rations were deficient in calcium and phosphorus as well as in Vitamin A. Hess and Pappenheim (53) in

1921 found that a diet deficient in vitamin A did not produce rickets though there was a cessation of active bone-formation. Sherman and Pappenheim (l.c.) noted that by substituting only one-seventh of the calcium lactate in their diet by secondary potassium phosphate ($K_2 HPO_4$) they prevented the onset of rickets in a diet deficient in Vitamin A, and moreover a diet which had constantly caused rickets in young rats previously. They also emphasise the importance off the balance in all dietary factors both organic and inorganic. Koronchevsky (l.c.) considered that a combined deficiency in both Vitamin A and mineral matter was most likely to produce the rachitic syndrome. He records, however, a condition very closely resembling rickets in animals on calcium - poor diet, born of a calcium-starved mother, in all four of the animals on whom the experiment was made. In 7 out of 12 rats on a vitamin-A poor diet born of a vitamin A starved mother "the picture is "suggestive of rickets" while only two are given as "typical rickets".

The difficulty with all these experiments is as has been mentioned, that we are attempting to approximate to an unknown optimum. Several attempts were made for the purpose of this thesis to obtain by some means either rat's milk or mouse's milk or guinea-pig's milk for the purpose

of an ash analysis. It proved impossible either by manipulation, or by cutting the ducts and administering pituitrin under anaesthetic, to obtain more than a few milligrammes. Eventually by the courtesy of the Imperial Cancer Research Institute of London two lactating mothers were obtained, separated from their young for three hours, and the young after feeding removed and killed. The stomach contents gave at least an approximate curd of rats' milk. This was found to have an ash content of 2.87% ($2CaO : 3P_2O_5$). The ash content of the diet administered by Koronchevsky and the American workers is between 5 and 6 per cent, i.e., more than double that of the young rats' normal food. The mere fact that a surplus of all salts is, or may be, present, does not improve matters since in mineral metabolism an excess is often as harmful as a deficiency.

The recent work on guinea-pigs is summarised by Hume (54) in a table showing the growth-promoting properties of certain natural food-stuffs, added to a basal diet. The table is reproduced below with a calculation of the quantity of Ca in the amount of food-stuff added. It will be noted how closely their growth promoting properties are paralleled by their calcium content and the Theory of Probabilities would show an almost unlimited bias against this being merely an accidental coincidence.

Table 28.

| Amount of Ca O added. | | Ca O grms. | Percentage increase in weight. | Histology Pathological Changes. |
|---|---|---|---|---|
| Orange Juice | 10 c.c. | 0.002 | - 10.7 | Definite to acute. |
| Onion | 30 grams | .0078 | - 0.2 | Definite. |
| " | 60 " | .0146 | - 3.6 | " |
| White cabbage leaf | 15 " | 0.0066 | - 3.3 | " |
| Fresh Milk | 20 c.c. | 0.034 | - 2.1 | " |
| Green cabbage leaf. | 5 grms. | 0.053 | 29.9 | Incipient to definite. |
| Glaxo = 60 cc. Raw milk | | 0.102 ? | 37.7 | Incipient. |
| Aut. milk | 60 c.c. | 0.102 | 44.8 | Nearly normal. |
| Raw milk | 60 " | 0.102 | 46.9 | Nearly normal. |
| Green cabbage leaf | 15 grms. | 0.159 | 40.1 | Normal. |
| Green cabbage leaf | 30 " | 0.318 | 60.4 | " |

The Glaxo was made up so that the liquid contained 3.5 per cent of fat.

As Glaxo is stated to contain dried milk plus butter fat the calcium content would certainly be lower in a solution of Glaxo containing 3.5 per cent of fat than in a fresh milk with the same content.

Hume concludes that the diets which produced growth were not superior in their protein, salt or calorific value, to those which did not; the difference must therefore lie in their Vitamin content; water soluble B and C were adequately supplied in all cases. the only known dietary constituent which could have been lacking is thus the Vitamin A. The above table, however, shows how widely even parts of the same plant (cabbage) may vary in mineral content and the need for checking all factors in the case.

In general, the work on the rodents has not begun at the bottom, and in looking for a factor which is only presupposed, and considering that its absence is shown by a disease, we are liable to fall into grave error without the experimental, long continued traditional knowledge of the needs of the healthy creature to guide us.

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## A NOTE ON RICKETS IN THE HUMAN.

The slow growth and the interminable infancy of the human species make the determination of its mineral requirements a matter of great difficulty. Thus, a young rabbit doubles its weight in 6 days, a puppy in 9 days, a calf in 47 days and an infant in 180 days (Loew. Kalkbedarf von Mensch and Tier 1919). The calcium content of the milks of these species is low or high in proportion to these periods. The milk of the rabbit contains 0.89% CaO, of the dog 0.46% CaO, of the cow 0.17% and of the human 0.05% CaO. (Loew. l.c.) The quantities of calcium required for the infant per day are thus very much smaller, and the time required for changes to be manifest twenty times longer, than the corresponding figures for the puppy. The quantity of food required for the production of a kilo of body weight works out about six times as high for the human as for other species so that the mineral concentration on all these grounds need not be high. Metabolic experiments on the infant would therefore require periods perhaps twelve to twenty times as long as those required for the familiar metabolic animals, if even the rough accuracy obtained on them is to be reached. Direct experiments on this scale are unknown. We are therefore

thrown back upon clinical evidence.

Clinical experience of disordered mineral metabolism in the infant centres almost exclusively around the subject of rickets. The main incidence of this disease in children is between 6 and 18 months i.e. at the period of weaning. It is a suggestive coincidence that at this period the child is transferred from a staple diet rich in calcium (cows' milk = 0.16% CaO) to a staple diet remarkably deficient in all minerals (white bread = 0.014% CaO). It is further interesting to note that clinicians have observed that lime water is of benefit during the infantile disorders of this period.

It is not of course intended to suggest that rickets in the child is simply calcium starvation. The literature of this disease is encyclopaedic in extent and theological in acrimony. The deficiency, however, now fully proved, of a grain ration and still more of its highly-milled constituents, show that nothing is easier than to select from the bakers' shops of large cities a cheap energy-yielding ration extremly incomplete in bone-building material.

The importance of the mineral metabolism of infants has been obscured by rather sensational assertions on behalf of the improved vitamin theory. A mission, for instance, was sent by the Medical Research Committee to Vienna for field work in 1919. They reported a great

shortage in that area of vitamin A. and consequent ravages of rickets amongst the young. Dalyell is quoted as giving the incidence at 100 per cent of the infants of nine months old (55) and Mellanby (l.c. p.67) mentions "the extraordinary amount and severity of rickets that has recently developed in Vienna and other towns in Central Europe during the war." He makes the quite gratuitous statement that "this depends largely, no doubt, on the absence of fats and milk in these countries" an assertion disproved by the investigation of Gribbon and Ferguson (56) to which subsequent reference will be made. As a matter of fact the clinical observations of the M.R.C. mission were made by an Australian lady doctor, whose experience of the slums of European cities was by no means life-long. Personally in a visit to examine this work, in January 1920, I saw no evidence of any such sweeping affection and can, for what my observation is worth, corroborate Findlay (57) who in 1921, also after personal inspection, concluded on clinical grounds that the disease was rather less prevalent there than in Glasgow. His opinion receives a more valuable confirmation from the vital statistics of Vienna city (58). The infantile death rate per 1,000 from rickets in 1910 was 0.4. It was in 1920 0.3; having shared the general decline in infant mortality from 216.8 (1910) to 168.6 in 1920. It may be claimed that this represents a great

triumph for the anti-rachitic campaign and the missionary effort of the Medical Research Committee's workers on behalf of vitamins. Gribbon and Ferguson (l.c.) found, however, that even amongst the rachitic, of the 29 families investigated only a few of the children were having cod liver oil. They further point out that in 29 rachitic as against the 20 non-rachitic families there was little difference in the amount of fat consumed. The energy value of the food consumed, however, was no less than 440 calories per man per day lower for the rachitic than for the non-rachitic families.

It will be seen, to quote the somewhat bitter words of the Committee's own Report No. 38 (l.c.), "how unreliable are many views on etiology when the basis is supposition and conjecture unconfirmed by scientific observation."

Further, in this country the rigid restriction during the war of animal fats, for the vitamin-poor margarine, coincided with no increase, and even with a striking decrease, in the infantile mortality from rickets. The following table shows the figures since 1905. (59)

TABLE 29.

Deaths from Rickets.

Rate per 1,000 for children under 1 year.

| 1905 | 1906 | 1907 | 1908 | 1909 | 1910 | 1911 | 1912 | 1913 | 1914 | 1915 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.58 | 0.60 | 0.50 | 0.46 | 0.47 | 0.44 | 0.39 | 0.34 | 0.44 | 0.39 | 0.39 |

| 1916 | 1917 | 1918 | 1919 |
|---|---|---|---|
| 0.41 | 0.41 | 0.35 | 0.24 |

It will be seen that after a slight preliminary rise in 1916 and 1917, the rate fell rapidly, and ~~that~~ this at a time when margarine consumption was becoming more universal. It may be argued that rickets is not a killing disease and that these figures are not reliable, but their remarkably consistent movement over long periods of time gives reason to consider that we have here, if no more, a valuable indicator of increase or decrease of the disease. The fact that the sharp fall is parallel to the increased addition of the mineral of the grain to war bread seems again more than a mere coincidence. Much work has been done in America on the association of vitamin A with rickets by Hess and Unger and others. Hess and Unger (60) summarising work with 100 children of eighteen months duration

concluded: "Fat-soluble A is not the controlling influence in rickets for in several cases children developed rickets while receiving fat-soluble A while other cases had no rickets though deprived of it for months." Hess, McCann and Pappenheimer (~~41~~) again state in 1921 "This vitamine (fat-soluble A) cannot be regarded as the antirachitic vitamine and if the diet is otherwise adequate, its deficiency does not bring about rickets."

The mineral metabolism of the teeth is still acutely controversial and the statements made are too contradictory to discuss usefully until research has further ascertained definite facts.

## GENERAL REVIEW.

The work of the great chemists of the earlier part of the nineteenth century was overshadowed by the subsequent realisation of the tremendous significance of the linked complexes of carbon and nitrogen which occur in bodies such as the purins or the amino-acids, and from which protein derives its vital importance in animal economy. Of all this work Emil Fischer's name may be taken as typical; and it is only natural that biology, having encountered the fundamental fact that the animal world was parasitic upon the vegetable world for its utilisable supplies of the two essential atoms C and N, should for a decade and more devote itself to grasping the significance of this discovery. Of recent years, however, the balance has swung too far to this side. The other elements present in so-called inorganic substances are equally important to the very existence of this labile compound which we call protoplasm. The fact that of all the elements of the body we are parasitic upon plants for carbon hydrogen and oxygen compounds and through them upon bacteria, for nitrogen, should not lead us to neglect those atoms which the cell, even of the vertebrates, can utilise

direct, though supplied in inorganic form.

It may seem platitudinous to reiterate these well-known facts, but the parallel "salt mixtures" of McCollum and Mellanby for growing animals show it not to be without a bearing on the subject of skeletal lesions and the mineral constituents of the diet, taken for this thesis.

| McCollum. | Mellanby. |
|---|---|
| Na Cl. | Na Cl. |
| Mg. $SO_4$ | |
| $Na_2$ H $PO_4$ | |
| $K_2$ H P $O_4$ | |
| Ca $H_4$ ($PO_4$) | |
| Ca. Citrate | |
| Ferric Citrate. | |
| Iodine. (trace) | |

Indeed, much of the so-called "biological analysis" gains its results by a previous neglect of the old-fashioned art of chemical analysis. The question of the Ca content of the food necessary to skeletal development has already been touched on. It is note-worthy that it is unusual to find substances rich in Vitamin A which are not at the same time rich in Ca and Mellanby himself comments on the fact, quoting the following figures from Albu and Neuberg (Mineralstoffwechsel 1906)

| Deficient in anti rachitic factor. | % Ca. | Rich in anti rachitic factor. | |
|---|---|---|---|
| White Bread (less than) | 0.014 | Cow's milk | 0.16. % Ca |
| Sugar | None. | Egg Yolk | 0.4 " |
| Margarine | None. | Cabbage | 0.45 " |
| Potato | 0.025 | Cauliflower | 0.14 " |
| Polished Rice | 0.016 | | |

In a biological analysis of foods therefore, by the use of puppies, for the existence of a hypothetical substance, it would seem unjustified to use a staple of white bread with small quantities of cows' milk without at the same time making sure that Ca was added in ample and utilisable form.

Again, the biological analysis carried out for the presence or absence of the hypothetical Vitamin B. are made by the use of pigeons. Polished rice is highly deficient in mineral matter, and it is logically necessary to supply all the known needs before proceeding to postulate an unknown. In Canada (62) it was found that pigs fed on polished rice sickened and died but that this could be long postponed or prevented by the addition of phosphates to the diet. In view of the fact that the $P_2O_5$ deficiency of rice is used as an index of its anti beri-beri power, so that a content

of less than 0.28% is regarded as dangerous, and a greater content as safe, this seems especially necessary. It is interesting to note that Theiler (63) and others found, in an attempt to produce Vitamin B. deficiency in farm stock, that diets causing the death of birds in the regulation 30 days had some though little ill effect upon swine and no effect whatever, in 13 lunar months, upon oxen. These results find a suggestive coincidence in the following blood ash analyses (64) (Table 30)

Percentages of Ash.

| | CaO | $P_2O_5$ |
|---|---|---|
| Ox | 1.1 | 5.27 |
| Pig | 1.55 | 12.52 |
| Hen | 1.08 | 26.62 |

The susceptibility of the fowl to the admitted mineral deficiencies occur in polished rice might easily differ totally from the susceptibility of the ox, since the blood of the hen has 26 times as much $P_2O_5$ as CaO while that of the ox has only 5 times as much.

The permissibility of using the needs of the guinea-pig, whose young scarcely need milk at all, as a "biological analysis" of the usefulness of milk in preventing deficiency disease in the human species

has already been questioned.

On clinical grounds the existence of a specific anti-rachitic factor must be a matter for repeated and unshakable demonstration. If labelled "health-factor" it would at once excite universal derision; yet one is scarcely more unlikely than the other. There are only eight notes in the musical octave. Innumerable combinations of these have been made, and the selection of any one as the "harmony factor" would lead to absurdities. One positive result is equal in value to many scores of negatives; and even the few experiments recorded here, proving the unlimited tolerance of one species to absence of any substance hitherto proved to contain this specific factor, would require attention. The Vitamin hypothesis, as has been well said by Orr, of Aberdeen, is at present as unproved as the phlogiston theory, to which indeed it bears a great resemblance.

FINIS.

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APPENDIX I.

UNIVERSITY OF LONDON.

KING'S COLLEGE.

HISTOLOGICAL LABORATORY.

January 9th, 1922.

# REPORT ON SPECIMENS FROM PIG SUPPLIED BY CAPT. ELLIOT, B. Sc.

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## Introductory Remarks.

It appears expedient to point out at once that in none of the specimens examined either macroscopic or microscopic alterations of a pathological character were found. The few and trivial changes noted here and there are to be referred either to the conditions in which the material was received or to the technique employed or to the somewhat hurried manner in which the investigation had to be carried out. What follows should be, therefore, considered more as explanatory of the methods used than as a description of specimens which do not appear to differ in any way from those one might have obtained from a normally fed animal of the same size and age.

## Collection and macroscopic examination of the material.

The material was brought into the laboratory about 20 hours after death had taken place but no cadaveric alterations were seen anywhere; on the contrary the material seemed, and as a fact was, in an excellent state of preservation.

The collection of specimens proceeded systematically from the great sciatic nerve which was isolated first and fixed in different fluids. The vertebral canal was then opened along its whole length and the spinal cord, after having been macroscopically examined, removed and subdivided for microscopic examination by various methods. The same was done with the medulla oblongata, cerebellum and cerebrum which were preserved in suitable fluids but not made, for the time being, into microscopic preparations as no particular place was found altered or congested or oedematous or such as to warrant a certainly laborious and practically useless investigation.

The pituitary body was also collected and examined by a special method to which reference will afterwards be made.

As one proceeded to expose the central nervous system, the bones forming the vertbral column and skull, as well as portions of the ribs, were macroscopically

examined. No material was preserved as no alterations could be seen and the only feature common to all of them was their extreme hardness.

The bones of the left hind limb were all dissected out and then sawn lengthways in order to make sure that not even some small localised lesion might escape attention. In this way the marrow was also examined and everywhere found to be of a white colour with all the characteristics of pigs' adipose tissue.

Parts of the sawn bones were put into Kaiserling's fluid for preservation as museum specimens, and parts were put aside for microscopic research after decalcification. Before being definitely mounted the Kaiserling specimens were photographed; from the negatives three lantern slides were obtained which by themselves already show the absence of any rarefaction, softening or rachitic change.

Microscopic examination.

1. Specimens from the sciatic nerve. This was investigated by ordinary histological methods, Cajal's photographic formulae 2 and 3, the processes of Schridde, Marchi-Busch, and Heller and Gumpert. No degeneration of the myelin sheath, alterations of the axis cylinders or changes of an infiltrative character were found in any of the many specimens examined.

In transverse and longitudinal sections of fragments fixed in formol-Muller and stained either with Ehrlich's haematoxylin and eosin, or with Weigert's haematoxylin and picro-fuchsin (van Gieson) the sustaining tissue of the nerve trunk and the nerve-fibres have quite normal features.

In the preparations made according to Cajal's formulae 2 the axis-cylinders of the large medullated fibres are hardly stained at all; but bundles of thin, probably non-medullated, fibres are brought to view. These thin fibres, which are generally considered as sympathetic in character, also have a normal aspect. The preparations made by Cajal's formulae 3 are similar to the former ones but here and there they show more clearly the axis-cylinders of large medullated fibres with features identical to those of normal specimens of peripheral nerves stained in the same way.

In the specimens treated according to the method of Schridde, the medullary sheaths have in many places a granular aspect which might be mistaken for a pathological phenomenon. But as a matter of fact such a granular aspect can be observed also in normal peripheral nerves stained by the same method or simply treated with a solution of osmic acid. It might be difficult to give a satisfactory explanation of this

granular aspect of the medullary sheath of peripheral nerve fibres, but it appears sufficient to point out here that it is probably connected with the mode of penetration of the osmic acid and subsequent operations of clearing and mounting. In addition one should bear in mind that according to recent investigations the myelin sheath of peripheral nerve-fibres, normally consists of a reticular stroma and of a semi-fluid fatty-like material situated in the meshes of the stroma under the form of minute spheres or drops.

Lastly the absence of pathological changes is confirmed by the specimens made by the Marchi -Busch method in which no trace of true myelin degeneration is found, as well by the specimens treated according to the method of Heller and Gumpertz. These preparations also require some care in their interpretation. Indeed when seen under a low magnifying power of the microscope they have the rather poor look of badly stained specimens; but under high power one can easily recognise the intact, somewhat transparent and almost greenish myelin sheaths. In some places there are darker portions but these are not the expression of any degenerative phenomenon and are simply due to the way in which the mordanting and differentiating processes, which are an essential part of the method of Heller and

Gumpertz are, perforce, carried out.

In considering the specimens of the sciatic nerve one should not forget that it was collected 20 hours after death and that some of the results and slight artefacts described above may as easily be connected with quite initial phenomena of decomposition which sets in very rapidly in peripheral nerves and are very liable to affect the impregnating and staining properties both of myelin sheaths and axis-cylinders.

2. Specimens from the spinal cord. The absence of degenerative phenomena in the peripheral nerve investigated is likewise proved by the observation of the specimen from the spinal cord. In fact had there been a pathological alteration of at least some of the bundles of fibres forming the sciatic nerve, one ought to have found chromatolytic changes in some of the large nerve cells of the anterior horn of the lumbar cord. The specimens from this region stained with toluidin blue, thionin and Giemsa's fluid convincingly show that this is not the case. In fact they do not differ in any way from the specimens of the dorsal and cervical regions of the same cord.

With the object of rendering the investigation of the spinal cord as complete as possible, portions were

treated also according to the methods of Bielschowsky, Cajal and Weigert-Pal. The Bielschowsky method did not give satisfactory results probably owing to the fact that the material had not been left for a sufficiently long time in the fixative. The method of Cajal was only partially successful but those cells which took the silver do not show any appreciable alteration of the neurofibrils, whilst the extracellular interlacement of non-medullated fibres appears in these preparations intact. The Weigert-Pal specimens of the three principal regions of the spinal cord are like those one could have obtained from any normal material of the same kind. There are, here and there, some parts lighter than others, but this fact is due to the way in which the pieces have been hurried through the bichromate solution and mordant, not certainly to any, even slight, systematic degeneration.

3. Specimen from spinal ganglia and pituitary body. These were treated by Da Fano's cobalt nitrate method for the demonstration of Golgi's internal apparatus, because this peculiar cell organ appears sometimes to suffer a little from conditions known as deficiency diseases. In this case, however, no alteration is seen either in the spinal ganglia or in the pituitary

body. There are, here and there, some autolytic changes of the apparatus, but it is well known that these are frequently met with in tissues collected a few hours after death and that they have no morbid significance.

The specimens from the pituitary body may present some difficulty of interpretation because in some of its deepest parts mitochondria have become impregnated along with or independently from the apparatus, but this confirms the normal condition of the pituitary body in the case under examination. It is true that it sometimes appears affected by the absence of either the A or B accessory food factors but only when experiments are persistently carried to a point at which the animals (rats, pigeons) would not recover even if the treatment were suspended. This fact was borne in mind when examining the pig's pituitary body but no changes of the apparatus, no undue enlargement of the blood vessels, no cytoplasmic or nuclear alterations could be noticed.

4. Specimens from decalcified bones. Sections could be obtained only from the phalanges, metatarsal bones and a fragment of fibula. The pieces of the larger bones of the hind limb were not fit for cutting after having been left for more than 10 days in the decalci-

fying fluid. This fact is pointed out here because it also has a certain bearing on the statement made at the beginning of this report as to the absence of any pathological alteration. The same conclusion can be drawn from even a quite superficial observation of the microscopic preparations obtained from the cut bones. The epiphyseal and articular cartilages are not enlarged or irregular in aspect; there is no fresh production of an osteoid tissue, no abnormal reabsorption of bone, no condition in any way similar to osteoporosis, osteomalacia, rickets, either as observed in the human being or as experimentally obtained in animals.

## Conclusion.

Material from the animal experimented upon is still available and could be made, in course of time, into microscopic specimens, but it does not appear advisable to persist in an investigation which, judging from the results so far obtained, would only lead to conclusions completely negative from a histo-pathological point of view. If the pig under examination was fed on a diet deficient in some accessory food factor and the experiment had been carried far enough, some lesions of the kind recently described by other observers might have, perhaps, been brought about. However, in the case in question, they are not present even in a very mild form.

C. DA FANO.

# APPENDIX II.

# APPENDIX 2.

## METABOLIC DATA.

### EXPERIMENT 1.

### URINE.

| Days | Amount | Acidity in c.c. NaOH | Grams T.N. | Grams CaO. | Grams $P_2O_5$ |
|---|---|---|---|---|---|
| | Period 1. | Commencement May 9th 1921. | | | |
| 1 | 1,500 | 900 | 17.598 | 0.1092 | 5.4 |
| 2 | 2,600 | 1,118 | 20.821 | 0.1832 | 7.2 |
| 3 | 2,600 | 936 | 15.652 | 0.0874 | 6.37 |
| | Period 2. | Diet + 2.576 grams CaO. ($10$ grams $CaCl_2$) May 12th. | | | |
| 4. | 2,000 | 660 | 13.216 | 0.1288 | 4.80 |
| 5 | 4,400 | 1,628 | 25.995 | 0.1725 | 9.77 |
| 6 | 3,000 | 990 | 18.48 | 0.1344 | 6.81 |
| | Period 3. | Diet + 2.576 grams CaO. Food neutralised May 15th. | | | |
| 7 | 3,400 | 646 | 19.61 | 0.1428 | 6.80 |
| 8 | 3,600 | 288 | 20.16 | 0.1008 | 5.04 |
| 9 | 3,000 | 330 | 20.664 | 0.1008 | 6.96 |
| 10 | 4,300 | 301 | 21.5516 | 0.0843 | 7.84 |
| 11 | 3,800 | 342 | 20.216 | 0.1084 | 6.88 |
| | Period 4. | Diet + 2.576 grams CaO. May 20th. | | | |
| 12 | 4,300 | 903 | 26.2472 | 0.1083 | 8.82 |
| 13 | 3.600 | 1,116 | 24.4944 | 0.1714 | 8.21 |
| 14 | 3,250 | 1,007 | 20.475 | 0.1547 | 7.93 |
| 15 | 4,000 | 1,160 | 24,752 | 0.168 | 8.92 |
| 16 | 3,800 | 1,064 | 23,408 | 0.1383 | 8.70 |

### EXPERIMENT 1.

### FAECES.

| Amount | Grams T.N. | Grams CaO. | Grams $P_2O_5$ |
|---|---|---|---|
| 855 | 12.1239 | 1.5646 | 6.5322 |
| 940 | 14.15 | 1.626 | 7.40 |
| 656 | 12.34 | 1.142 | 6.156 |
| 493 | 9.367 | 0.77 | 4.5213 |
| 383 | 8.6137 | 0.70 | 3.689 |
| 932 | 17.71 | 1.687 | 8.00 |
| 482 | 9.5677 | 0.8016 | 3.964 |
| 775 | 14.88 | 1.3376 | 6.2775 |
| 736 | 13.91 | 1.84 | 8.0221 |
| 700 | 13.37 | 1.8368 | 7.6468 |
| 530 | 10.335 | 1.048 | 4.71 |
| 685 | 12.1245 | 1.19 | 5.538 |
| 412 | 8.0422 | 0.688 | 3.2264 |
| 634 | 11.1742 | 1.034 | 4.8469 |
| 926 | 15.5568 | 1.4464 | 6.9561 |
| 426 | 7.4124 | 0.6454 | 3.1613 |

## Analytical Data to Experiment 1.

*Amounts in Grams*

| Days | T. N. in food | Excreted in urine | Excreted in faeces | Total excreted. | T. N. Balance | Total CaO. in food. | Excreted in urine | Total CaO excreted in faeces | Total excreted. | CaO Balance | $P_2O_5$ in food | Excreted in urine | Excreted in faeces | Total excreted | $P_2O_5$ Balance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 52.625 | 17.598 | 12.1239 | 29.722 | 22.90 | 1.7912 | 0.1092 | 1.5646 | 1.6738 + | 0.1174 | 18.5145 | 5.40 | 6.5322 | 11.932 + | 6.5821 |
| 2 | | 20.821 | 14.150 | 34.971 | 17.65 | | 0.1832 | 1.626 | 1.8092 − | 0.018 | | 7.20 | 7.40 | 14.60 | 3.9145 |
| 3 | | 15.652 | 12.340 | 27.99 | 24.63 | | 0.0874 | 1.142 | 1.2294 + | 0.5618 | | 6.37 | 6.156 | 12.526 | 5.9885 |
| 4 | | 13.216 | 9.367 | 22.583 | 30.04 | 4.3672 | 0.1288 | 0.77 | 0.8988 | 3.4684 | | 4.80 | 4.5213 | 9.3213 | 9.1932 |
| 5 | | 25.995 | 8.6137 | 34.609 | 18.016 | | 0.1725 | 0.70 | 0.8725 | 3.4947 | | 9.768 | 3.689 | 13.457 | 5.0575 |
| 6 | | 18.48 | 17.71 | 36.19 | 16.435 | | 0.1344 | 1.687 | 1.8214 | 2.5458 | | 6.81 | 8.00 | 14.81 | 3.7045 |
| | Period 3. Food neutralised. | | | | | | | | | | | | | | |
| 7 | 52.625 | 19.61 | 9.5677 | 29.178 | 23.447 | | 0.1428 | 0.8016 | 0.9444 | 3.4228 | 18.5145 | 6.80 | 3.964 | 10.764 | 7.7505 |
| 8 | | 20.16 | 14.880 | 35.04 | 17.585 | | 0.1008 | 1.3376 | 1.4384 | 2.9288 | | 5.04 | 6.2775 | 11.3175 | 7.197 |
| 9 | | 20.664 | 13.91 | 34.574 | 18.051 | | 0.1008 | 1.84 | 1.9408 | 2.4264 | | 6.96 | 8.0221 | 14.9821 | 3.5324 |
| 10 | | 21.5516 | 13.37 | 34.9216 | 17.7034 | | 0.0843 | 1.8368 | 1.9211 | 2.4461 | | 7.482 | 7.6468 | 15.1288 | 3.3857 |
| 11 | | 20.216 | 10.335 | 30.551 | 22.074 | | 0.1084 | 1.048 | 1.1564 | 3.2108 | | 6.878 | 4.71 | 11.588 | 6.9265 |
| | Period 4. Food not neutralised. | | | | | | | | | | | | | | |
| 12 | 52.625 | 26.2472 | 12.1245 | 39.3717 | 13.2533 | | 0.1083 | 1.1900 | 1.2983 | 3.0689 | 18.5145 | 8.815 | 5.538 | 14.353 | 4.1615 |
| 13 | | 24.4944 | 8.0422 | 32.5366 | 20.0884 | | 0.1714 | 0.688 | 0.8594 | 3.5078 | | 8.208 | 3.2264 | 11.4344 | 7.0801 |
| 14 | | 20.475 | 11.1742 | 31.6474 | 20.9776 | | 0.1547 | 1.034 | 1.1887 | 3.1785 | | 7.93 | 4.8469 | 12.7769 | 5.7376 |
| 15 | | 24.752 | 15.5568 | 40.3088 | 12.3162 | | 0.168 | 1.4464 | 1.6144 | 2.7528 | | 8.92 | 6.9561 | 15.8761 | 2.6384 |
| 16 | | 23.408 | 7.4124 | 30.8204 | 21.8046 | | 0.1383 | 0.6454 | 0.7837 | 3.5835 | | 8.702 | 3.1613 | 11.8633 | 6.6512 |

## EXPERIMENTS 11 and 111.

### Urine

| Weight of pig. Kilos. | Days | Volume c.c. | T. N. gms. | CaO. gms. | $P_2O_5$ gms. |
|---|---|---|---|---|---|
| | Period 1. | | Diet. | | |
| 15.2 | 1 | 1,000 | 3.76 | 0.0224 | 0.8 |
| 15.5 | 2 | 1,500 | 3.57 | 0.0336 | 0.945 |
| 15.6 | 3 | 1,500 | 3.948 | 0.021 | 0.855 |
| 15.7 | 4 | 1,500 | 4.305 | 0.0147 | 1.215 |
| 15.9 | 5 | 1,500 | 4.494 | 0.021 | 1.14 |
| | Period 2. | | Diet + 10gms. $CaCl_2$. = | | (2.5 gms. CaO). |
| | 6 | 2,000 | 4.704 | 0.0784 | 0.28 |
| 16.3 | 7 | 2,000 | 5.348 | 0.1512 | 0.26 |
| 16.5 | 8 | 1.500 | 4.41 | 0.0882 | 0.24 |
| 16.8 | 9 | 1.500 | 4.284 | 0.1176 | 0.36 |
| 17.0 | 10 | 1.500 | 4.788 | 0.1075 | 0.27 |
| | 11 | 1.500 | 5.376 | 0.168 | 0.15 |
| | Period 3. | | Diet + 15gms. $CaCl_2$. = | | (3.75 gms. CaO). |
| 17.2 | 12 | 1.500 | 4.767 | 0.2394 | 0.09 |
| 17.3 | 13 | 1.600 | 5.1072 | 0.3181 | 0.112 |
| 17.5 | 14 | 1.500 | 5.376 | 0.3978 | 0.105 |
| 17.6 | 15 | 1.500 | 4.956 | 0.3801 | 0.12 |
| 18.0 | 16 | 1.800 | 5.2416 | 0.3579 | 0.126 |
| | Period 3. | | Diet + 15 gms. $CaCl_2$. = | | (3.75 gms. CaO). |
| 18.2 | 17 | 1.800 | 6.0228 | 0.6653 | 0.108 |
| 18.5 | 18 | 2.100 | 5.5272 | 0.735 | 0.105 |
| | 19 | 2.000 | 5.264 | 0.7952 | 0.11 |
| | Period 5. | | Diet (no Calcium) | | |
| 18.8 | 20 | 2.000 | 5.264 | 0.1232 | 0.54 |
| 19.0 | 21 | 2.000 | 5.712 | 0.0532 | 1.64 |
| 19.0 | 22 | 2.300 | 5.313 | 0.05152 | 1.564 |
| 19.3 | 23 | 2.000 | 5.152 | 0.0504 | 1.70 |
| 19.4 | 24 | 2.000 | 5.488 | 0.0364 | 1.60 |
| | Averages for different Periods. | | | | |
| 0.14 | Period 1 | | 4.02 | 0.0225 | 0.991 |
| 0.23 | 2 | | 4.841 | 0.1265 | 0.256 |
| 0.2 | 3 | | 5.1702 | 0.3610 | 0.116 |
| 0.3 | 4 | | 5.3956 | 0.7651 | 0.107 |
| 0.15 | 5 | | 5.4160 | 0.0479 | 1.6261 |

## EXPERIMENTS 11 and 111.

### Faeces

| Weight in grms. | T. N. gms. | CaO. gms. | $P_2O_5$ gms. |
|---|---|---|---|
| 180 | 4.5144 | 0.6323 | 1.6164 |
| 140 | 3.416 | 0.4284 | 1.2394 |
| 200 | 4.99 | 0.5826 | 1.806 |
| 190 | 4.4023 | 0.4816 | 1.539 |
| 195 | 4.641 | 0.501 | 1,5844 |
| | | | |
| 157 | 3.7366 | 0.4798 | 1,4039 |
| 260 | 5.46 | 0.9656 | 2.1684 |
| 180 | 4.009 | 0.8208 | 1.602 |
| 170 | 3.893 | 0.8179 | 1.36 |
| 150 | 3.0375 | 0.6504 | 1.173 |
| 300 | 6.54 | 1.5189 | 2.4972 |
| | | | |
| 250 | 5.475 | 1.1188 | 1.9075 |
| 182 | 3.9494 | 1.2256 | 1.5142 |
| 205 | 4.469 | 1.6006 | 1.6488 |
| 175 | 4.2665 | 1.385 | 1.6205 |
| 195 | 4.095 | 1.441 | 1.6231 |
| | | | |
| 225 | 4.608 | 1.4091 | 1.69 |
| 225 | 4.5225 | 2.07 | 1.1515 |
| 200 | 4.05 | 1.8368 | 1.852 |
| | | | |
| 220 | 4.444 | 1.9851 | 2.015 |
| 230 | 4.77 | 0.7038 | 1.8067 |
| 180 | 4.1958 | 0.4626 | 1.3734 |
| 200 | 4.658 | 0.4984 | 1.614 |
| 200 | 4.566 | 0.4638 | 1.482 |
| | | | |
| 181 | 4.393 | 0.5252 | 1.557 |
| 212 | 4.5879 | 0.9547 | 1.7601 |
| 192 | 4.2735 | 1.4755 | 1.6308 |
| 213 | 4.2863 | 1.9534 | 1.502 |
| 193 | 4.8066 | 0.4749 | 1.4898 |

## Analytical Data to Experiments 11 and 111.

Amounts in Grams

| Days | T. N. in Food. | Excreted in urine | Excreted in faeces | Total excreted. | T. N. Balance | Total CaO. in food | Excreted in urine | Total CaO. excreted in faeces | Total excreted. | CaO. Balance. | $P_{2,5}O_5$ in food | Excreted in urine | Excreted in faeces | Totsl excreted | $P_2O_5$ Balance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 21.7378 | 3.78 | 4.5144 | 8.2944 | 13.4434 | 0.652 | 0.0224 | 0.6323 | 0.6547 | 0.0027 | 6.869 | 0.8 | 1.6164 | 2.4164 | 4.4526 |
| 2 | | 3.57 | 3.416 | 6.986 | 14.7518 | | 0.0336 | 0.4284 | 0.4620 | 0.1900 | | 0.945 | 1.2394 | 2.1844 | 4.6846 |
| 3 | | 3.948 | 4.99 | 8.938 | 12.7998 | | 0.021 | 0.5826 | 0.6036 | 0.0484 | | 0.855 | 1.806 | 2.6610 | 4.208 |
| 4 | | 4.305 | 4.4023 | 8.7073 | 13.0305 | | 0.0147 | 0.4816 | 0.4963 | 0.1557 | | 1.215 | 1.539 | 2.754 | 4.115 |
| 5 | | 4.494 | 4.641 | 9.135 | 12.6028 | | 0.021 | 0.501 | 0.522 | 0.130 | | 1.14 | 1.5844 | 2.7244 | 4.1446 |
| 6 | 21.7378 | 4.704 | 3.7366 | 8.4406 | 13.2972 | 0.652 | 0.0784 | 0.4978 | 0.5882 | 2.5938 | 6.869 | 0.28 | 1.4039 | 1.6839 | 5.1869 |
| 7 | | 5.348 | 5.460 | 10.8080 | 10.9298 | | 0.1512 | 0.9656 | 1.1168 | 2.0352 | | 0.26 | 2.1684 | 2.4284 | 4.4416 |
| 8 | | 4.410 | 4.009 | 8.4190 | 13.3188 | | 0.0882 | 0.8208 | 0.909 | 2.2430 | | 0.24 | 1.602 | 1.842 | 5.027 |
| 9 | | 4.284 | 3.893 | 8.1770 | 13.5608 | | 0.1176 | 0.8179 | 0.9355 | 2.2165 | | 0.36 | 1.36 | 1.72 | 5.149 |
| 10 | | 4.788 | 3.0375 | 7.8255 | 13.9123 | | 0.1075 | 0.6504 | 0.7579 | 2.3941 | | 0.27 | 1.173 | 1.443 | 5.426 |
| 11 | | 5.376 | 6.540 | 11.9160 | 9.8218 | | 0.1680 | 1.5189 | 1.6869 | 1.4651 | | 0.15 | 2.4972 | 2.6472 | 4.2218 |
| 11 | 21.7378 | 4.767 | 5.475 | 10.242 | 11.4958 | 4.402 | 0.2394 | 1.1188 | 1.3852 | 3.0438 | 6.869 | 0.09 | 1.9075 | 1.9975 | 4.8715 |
| 12 | | 5.1072 | 3.9494 | 9.0566 | 12.6912 | | 0.3181 | 1.2256 | 1.5437 | 2.8583 | | 0.112 | 1.5142 | 1.6262 | 5.2428 |
| 13 | | 5.376 | 4.469 | 9.8450 | 11.8928 | | 0.3978 | 1.6006 | 1.9984 | 2.4036 | | 0.105 | 1.6488 | 1.7538 | 5.1152 |
| 14 | | 4.956 | 4.2665 | 9.2225 | 12.5153 | | 0.3801 | 1.385 | 1.7651 | 2.6369 | | 0.12 | 1.6205 | 1.7405 | 5.1285 |
| 15 | | 5.2416 | 4.095 | 9.3366 | 12.4012 | | 0.3579 | 1.441 | 1.7989 | 2.6031 | | 0.126 | 1.6321 | 1.7581 | 5.1109 |
| 16 | 21,7378 | 6.0228 | 4.608 | 10.6308 | 11.1070 | 5.652 | 0.6653 | 1.4091 | 2.0744 | 3.5776 | 6.869 | 0.108 | 1.69 | 1.798 | 5.071 |
| 17 | | 5.5272 | 4.5225 | 10.0497 | 11.6881 | | 0.735 | 2.07 | 2.8050 | 2.8470 | | 0.105 | 2.1515 | 2.2565 | 4.6135 |
| 18 | | 5.264 | 4.05 | 9.314 | 12.4238 | | 0.7952 | 1.8368 | 2.6320 | 3.0200 | | 0.11 | 1.852 | 1.9620 | 4.907 |
| 19 | 21,7378 | 5.264 | 4.444 | 9.7080 | 12.0298 | 0.652 | 0.1232 | 1.9851 | 2.1083 | 1.4563 | 6.869 | 0.54 | 2.015 | 2.555 | 4.314 |
| 20 | | 5.712 | 4.77 | 10.4820 | 11.2558 | | 0.0532 | 0.7038 | 0.757 | 0.105 | | 1.64 | 1.8067 | 3.4467 | 3.4223 |
| 21 | | 5.313 | 4.1958 | 9.5088 | 12.2290 | | 0.0515 | 0.4626 | 0.5141 | 0.1379 | | 1.564 | 1.3734 | 2.9374 | 3.9316 |
| 22 | | 5.152 | 4.658 | 9.81 | 11.9278 | | 0.0504 | 0.4984 | 0.5488 | 0.1032 | | 1.70 | 1.614 | 3.3140 | 3.555 |
| 23 | | 5.488 | 4.566 | 10.054 | 11.6838 | | 0.0364 | 0.4638 | 0.5002 | 0.1524 | | 1.60 | 1.482 | 3.082 | 3.787 |
| | | | Averages. | | | | | | | | | | | | |
| Period | 1 | 4.02 | 4.3930 | 8.4130 | 13.3248 | 0.652 | 0.0225 | 0.5252 | 0.5477 | 0.1043 | 6.869 | 0.991 | 1.5570 | 2.5480 | 4.3210 |
| | 2 | 4.841 | 4.5879 | 9.4289 | 12.3089 | 3.152 | 0.1265 | 0.9547 | 1.0812 | 2.0708 | 6.869 | 0,256 | 1.7601 | 2.0161 | 4.8529 |
| | 3 | 5.1702 | 4.2735 | 9.4437 | 12.2941 | 4.402 | 0.361 | 1.4755 | 1.8365 | 2.5655 | 6.869 | 0.116 | 1.6308 | 1.7468 | 5.1222 |
| | 4 | 5.3956 | 4.2863 | 9.6819 | 12.0559 | 5.652 | 0.7651 | 1.9534 | 2.7185 | 2.9335 | 6.869 | 0.107 | 1.5020 | 1.609 | 5.2160 |
| | 5 | 5.4160 | 4.8066 | 10.2226 | 11.5152 | 0.652 | 0.0479 | 0.4749 | 0.5228 | 0.1292 | 6.869 | 1.626 | 1.4898 | 3.1158 | 3.7532 |

Analytical Data to Experiments 11 and 111.
Amounts in Grams

EXPERIMENT IV.

Urine.

| Weight of pig Kilos. | Days. | Volume cc. | T.N. gms. | CaO. gms. | $P_2O_5$. gms. |
|---|---|---|---|---|---|
| Period 1. Diet + 50 cc. $CaCl_2$. (approx 25%) = 2.933 gms. CaO. | | | | | |
| 25.0 | 1 | 1,600 | 6.1376 | 0.5690 | 0.0760 |
| 25.4 | 2 | 1,400 | 6.7032 | 0.8114 | 0.1686 |
| 25.2 | 3 | 2,000 | 7.6720 | 0.9856 | 0.2160 |
| 25.8 | 4 | 1,800 | 7.3836 | 0.8921 | 0.1260 |
| Period 2. Diet 50 cc. $CaCl_2$. (approx.25%) = 2.933 gms. CaO. 200 cc. $Na_2HPO_4$ (approx.20%) = 3.98 gms. $P_2O_5$. | | | | | |
| 27.0 | 5 | 2,000 | 8.1760 | 0.2352 | 0.6594 |
| 26.9 | 6 | 2,500 | 8.9250 | 0.1820 | 2.2500 |
| 26.9 | 7 | 2,500 | 7.7640 | 0.1360 | 2.4330 |
| 27.4 | 8 | 2,000 | 7.3400 | 0.1008 | 2.6437 |
| 27.3 | 9 | 2,800 | 7.6480 | 0.1411 | 2.8532 |
| Period 3. Diet 50 cc $CaCl_2$. (approx. 25%) = 2.933 gms. CaO. | | | | | |
| 27.5 | 10 | 3,000 | 7,1344 | 0.2940 | 0.428 |
| 27.8 | 11 | 2,800 | 8.3104 | 0.2705 | 0.2744 |
| 28.3 | 12 | 3,300 | 8.778 | 0.3003 | 0.3660 |
| 28.6 | 13 | 3,300 | 9.3324 | 0.3049 | 0.2092 |
| 28.7 | 14 | 2,800 | 11.1440 | 0.2862 | 0.1287 |

EXPERIMENT IV.

Faeces.

| Weight in grms. | T.N. gms. | CaO. gms. | $P_2O_5$. gms. |
|---|---|---|---|
| 160 | 5.9922 | 0.6370 | 1.8992 |
| 270 | 10.0505 | 1.0897 | 3,2314 |
| 330 | 11.0689 | 1.2847 | 3.16 |
| 218 | 7.7368 | 0.8469 | 1.9790 |
| 180 | 6.4580 | 0.7465 | 1.6398 |
| 350 | 11.3701 | 1.7605 | 3.2095 |
| 310 | 9.765 | 1.3485 | 3.0070 |
| 265 | 8.2812 | 1.1806 | 2.9415 |
| 240 | 7.584 | 1.3223 | 3.223 |
| 310 | 9.8270 | 1.4471 | 3.3200 |
| 238 | 7.4280 | 1.2642 | 2.5347 |
| 300 | 9.3435 | 1.3236 | 2.802 |
| 215 | 7.1412 | 1.0148 | 1.9221 |
| 205 | 7.1060 | 1.2177 | 2.1119 |

EXPERIMENT V.

Urine.

| Weight of Pig Kilos. | Days. | Volume c.c. | T.N. grms. | CaO. grms. | $P_2O_5$ grms. |
|---|---|---|---|---|---|
| | Period 1. | Diet. | | | |
| 28.1 | 1 | 2,500 | 10.3600 | 0.1330 | 4.7500 |
| 28.1 | 2 | 2,500 | 11.9800 | 0.1120 | 2.6500 |
| 28.0 | 3 | 2,100 | 9.9960 | 0.0823 | 5.9850 |
| 28.2 | 4 | 2,100 | 10.4664 | 0.1117 | 5.5650 |
| 28.5 | 5 | 2,200 | 9.6272 | 0.0862 | 5.2800 |
| 29.0 | 6 | 2,400 | 10.0800 | 0.0672 | 5.6340 |
| | Period 2. | 25 cc. Cod-liver oil. + Diet. | | | |
| 29.3 | 7 | 2,200 | 9.8560 | 0.0739 | 5.4120 |
| 29.9 | 8 | 2,000 | 9.5760 | 0.0560 | 5.5600 |
| 30.2 | 9 | 2,300 | 9.6600 | 0.0451 | 4.8300 |
| 31.4 | 10 | 2,100 | 10.5840 | 0.0471 | 4.5780 |
| 31.7 | 11 | 2,400 | 10.8864 | 0.0739 | 5.0880 |
| 31.8 | 12 | 2,700 | 10.7352 | 0.1172 | 5.0220 |
| 32.1 | 13 | 2,800 | 10.5056 | 0.1019 | 6.6080 |
| 32.5 | 14 | 2,500 | 9.9400 | 0.0700 | 5.5500 |
| | Period 3. | Diet. + 25 gms. Lard. | | | |
| 32.9 | 15 | 2,800 | 10.0744 | 0.0784 | 5.7120 |
| 33.2 | 16 | 2,800 | 10.5056 | 0.0784 | 6.1040 |
| 33.7 | 17 | 3,200 | 11.1104 | 0.0672 | 5.9520 |
| 34.3 | 18 | 2,900 | 10.3936 | 0.0609 | 6.3220 |
| 34.4 | 19 | 2,900 | 10.9620 | 0.0893 | 6.0900 |
| 35.1 | 20 | 3,400 | 11.7096 | 0.0952 | 6.5960 |
| 35.8 | 21 | 3,200 | 12.0960 | 0.0672 | 6.1440 |
| 36.1 | 22 | 3,200 | 10.7520 | 0.0538 | 5.5680 |
| | Period 4. | Diet + 25 cc. Linseed oil. | | | |
| 364- | 23 | 3,500 | 10.6820 | 0.0686 | 6.0200 |
| 37.1 | 24 | 3,600 | 10.9368 | 0.0655 | 6.1200 |
| 37.4 | 25 | 3,200 | 11.2000 | 0.0672 | 6.1440 |
| 37.7 | 26 | 2,800 | 10.4272 | 0.0706 | 6.2720 |
| 38.1 | 27 | 3,700 | 11.1888 | 0.0570 | 6.4380 |
| 38.9 | 28 | 3,100 | 11.2840 | 0.0608 | 5.6420 |
| 39.9 | 29 | 2,600 | 11.1748 | 0.0582 | 3.8480 |
| | Period 5. | Diet | | | |
| 40.0 | 30 | 3,300 | 12.6588 | 0.0693 | 5.7420 |
| 40.0 | 31 | 3,100 | 12.1520 | 0.0651 | 5.7040 |
| 40.3 | 32 | 3,300 | 11.5038 | 0.0647 | 6.2040 |

EXPERIMENT V.

Faeces.

| Weight in gms. | T.N. gms. | CaO. gms. | $P_2O_5$ gms. |
|---|---|---|---|
| 255 | 3.3836 | 0.4458 | 1.3959 |
| 695 | 9.6202 | 1.3058 | 5.1513 |
| 430 | 6.1193 | 0.9092 | 3.3531 |
| 600 | 8.3100 | 0.9768 | 3.7034 |
| 400 | 5.7269 | 0.6628 | 2.5100 |
| 430 | 6.1795 | 0.6233 | 2.4351 |
| 425 | 6.0758 | 0.6115 | 2.3175 |
| 470 | 6.2618 | 0.5748 | 2.4266 |
| 490 | 6.7640 | 0.5581 | 2.4819 |
| 460 | 6.0545 | 0.5354 | 2.8318 |
| 420 | 5.7221 | 0.6178 | 3.1588 |
| 490 | 6.5356 | 0.7561 | 3.4442 |
| 520 | 7.0278 | 0.6362 | 2.9697 |
| 530 | 7.1317 | 0.6137 | 2.9134 |
| 390 | 5.3894 | 0.3820 | 1.9816 |
| 450 | 6.0359 | 0.3767 | 2.2248 |
| 490 | 6.7556 | 0.3350 | 2.3104 |
| 470 | 6.6890 | 0.3004 | 2.0694 |
| 520 | 7.1677 | 0.2905 | 2.0332 |
| 550 | 7.1616 | 0.3247 | 2.2315 |
| 460 | 6.1760 | 0.7199 | 3.3060 |
| 570 | 6.7876 | 0.5746 | 3.1002 |
| 440 | 5,5994 | 0.2728 | 1.8797 |
| 430 | 5.9125 | 0.2589 | 1.7807 |
| 550 | 7.5576 | 0.2697 | 2.3639 |
| 450 | 5,9850 | 0.1814 | 1.8347 |
| 380 | 5.0187 | 0.1763 | 1.5276 |
| 470 | 6.3098 | 0.2022 | 1.8795 |
| 430 | 5.3759 | 0.2111 | 2.0928 |
| 550 | 7.2936 | 0.4796 | 4.5694 |
| 500 | 5.7985 | 0.3685 | 3.1390 |
| 440 | 5.6703 | 0.2213 | 1.9316 |

Analytical Data to Experiment V.

Amounts in Grams.

| Days. | T.N. in food. | Excreted in urine | Excreted in faeces. | Total excreted. | T.N. Balance. | Total CaO in food. | Excreted in urine. | Total CaO excreted in faeces. | Total excreted. | CaO Balance. | $P_2O_5$ in food. | Excreted in urine. | Excreted in faeces. | Total excreted. | $P_2O_5$ Balance. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Period 1 Diet. | | | | | | | | | | | | | | |
| 1 | 32.5320 | 10.3600 | 3.3836 | 13.7436 | 18.7884 | 1.0618 | 0.1330 | 0.4458 | 0.5788 | 0.4830 | 12.4754 | 4.7500 | 1.3959 | 6.1459 | 6.3295 |
| 2 | | 11.9800 | 9.6202 | 21.6002 | 10.9318 | | 0.1120 | 1.3058 | 1.4178 | 0.3560 | | 2.6500 | 5.1513 | 7.8013 | 4.6741 |
| 3 | | 9.9960 | 6.1193 | 16.1153 | 16.4167 | | 0.0823 | 0.9092 | 0.9915 | 0.0703 | | 5.9850 | 3.3531 | 9.3381 | 3.1373 |
| 4 | | 10.4664 | 8.3100 | 18.7764 | 13.7556 | | 0.1117 | 0.9768 | 1.0885 | 0.0267 | | 5.5650 | 3.7034 | 9.2684 | 3.2070 |
| 5 | | 9.6272 | 5.7269 | 15.3541 | 17.1779 | | 0.0862 | 0.6628 | 0.7490 | 0.3128 | | 5.2800 | 2.5100 | 7.7900 | 4.6854 |
| 6 | | 10.0800 | 6.1795 | 16.2595 | 16.2725 | | 0.0672 | 0.6433 | 0.7105 | 0.3513 | | 5.6340 | 2.4351 | 8.0691 | 4.4063 |
| | Period 2. Diet 25 cc. Cod-liver oil | | | | | | | | | | | | | | |
| 7 | 32,5320 | 9,8560 | 6.0758 | 15.9318 | 16.6002 | 1.0618 | 0.0739 | 0.6115 | 0.6854 | 0.3764 | 12.4754 | 5.4120 | 2.3175 | 7.7295 | 4.7459 |
| 8 | | 9.5760 | 6.2618 | 15.8378 | 16.6942 | | 0.0560 | 0.5748 | 0.6308 | 0.4310 | | 5.5600 | 2.4266 | 7.9866 | 4.4888 |
| 9 | | 9.6600 | 6.7640 | 16.4240 | 16.1080 | | 0.0451 | 0.5581 | 0.6032 | 0.4586 | | 4.8300 | 2.4819 | 7.3119 | 5.1635 |
| 10 | | 10.5840 | 6.0540 | 16.6385 | 15.8935 | | 0.0470 | 0.5354 | 0.5424 | 0.5194 | | 4.5780 | 2.8318 | 7.4098 | 5.0656 |
| 11 | | 10.8864 | 5.7221 | 16.6085 | 15.9235 | | 0.0739 | 0.6178 | 0.6917 | 0.3701 | | 5.0880 | 3.1588 | 8.2468 | 4.2286 |
| 12 | | 10.7352 | 6.5356 | 17.2708 | 15.2612 | | 0.1172 | 0.7561 | 0.8733 | 0.1885 | | 5.0220 | 3.4442 | 8.4662 | 4.0092 |
| 13 | | 10.5056 | 7.0273 | 17.5334 | 14.9986 | | 0.1019 | 0.6362 | 0.7381 | 0.3237 | | 6.6080 | 2.9697 | 9.5777 | 2.8977 |
| 14 | | 9.9400 | 7.1317 | 17.0717 | 15.4603 | | 0.0700 | 0.6137 | 0.6837 | 0.3781 | | 5.5500 | 2.9134 | 8.4634 | 4.0120 |
| | Period 3. Diet 25 gms. Lard. | | | | | | | | | | | | | | |
| 15 | 32,5320 | 10.0744 | 5.3894 | 15.4638 | 17.0682 | 1.0618 | 0.0784 | 0.3820 | 0.4604 | 0.6014 | 12.4754 | 5.7120 | 1.9816 | 7.6936 | 4.7818 |
| 16 | | 10.5056 | 6.0359 | 16.5415 | 15.9905 | | 0.0784 | 0.3767 | 0.4551 | 0.6067 | | 6.1040 | 2.2248 | 8.3288 | 4.1466 |
| 17 | | 11.1104 | 6.7556 | 17.8660 | 14.662 | | 0.0672 | 0.3350 | 0.4022 | 0.6596 | | 5.9520 | 2.3104 | 8.2624 | 4.2130 |
| 18 | | 10.3936 | 6.6890 | 17.0826 | 15.4494 | | 0.0609 | 0.3004 | 0.3613 | 0.7005 | | 6.3220 | 2.0694 | 8.3914 | 4.0840 |
| 19 | | 10.9620 | 7.1577 | 18.1297 | 14.4023 | | 0.0893 | 0.2905 | 0.3798 | 0.6820 | | 6.0900 | 2.0332 | 8.1232 | 4.3522 |
| 20 | | 11.7096 | 7.1616 | 18.8712 | 13.6608 | | 0.0952 | 0.3247 | 0.4199 | 0.6419 | | 6.5960 | 2.2315 | 8.8275 | 3.6479 |
| 21 | | 12.0960 | 6.1760 | 18.2720 | 14.2600 | | 0.0672 | 0.7199 | 0.7871 | 0.2747 | | 6.1440 | 3.3060 | 9.4500 | 3.0254 |
| 22 | | 10.7520 | 6.7876 | 17.5396 | 14.9924 | | 0.0538 | 0.5746 | 0.6284 | 0.4334 | | 5.5680 | 3.1002 | 8.6682 | 3.8072 |
| | Period 4. Diet. 25 cc. Linseed oil. | | | | | | | | | | | | | | |
| 23 | 32.5320 | 10.6820 | 5.5994 | 16.2814 | 16.2506 | 1.0618 | 0.0686 | 0.2728 | 0.3414 | 0.7204 | 12,4754 | 6.0200 | 1.8797 | 7.8997 | 4.5757 |
| 24 | | 10.9368 | 5.9125 | 16.8493 | 15.6827 | | 0.0655 | 0.2589 | 0.3244 | 0.7374 | | 6.1200 | 1.7807 | 7.9007 | 4.5747 |
| 25 | | 11.2000 | 7.5576 | 187576 | 13.7744 | | 0.0672 | 0.2697 | 0.3369 | 0.7249 | | 6.1440 | 2.3639 | 8.5079 | 3.9675 |
| 26 | | 10.4272 | 5.9850 | 16.4122 | 16.1198 | | 0.0706 | 0.1814 | 0.2520 | 0.8098 | | 6.2720 | 1.8347 | 8.1067 | 4.3687 |
| 27 | | 11.1888 | 5.0187 | 16.2075 | 16.3245 | | 0.0570 | 0.1763 | 0.2333 | 0.8285 | | 6.4380 | 1.5276 | 7.9656 | 4.5098 |
| 28 | | 11.2840 | 6.3098 | 17.5938 | 14.9382 | | 0.0607 | 0.2022 | 0.2629 | 0.7989 | | 5.6420 | 1.8795 | 7.5215 | 4.9539 |
| 29 | | 11.1748 | 5.3759 | 16.5507 | 15.9813 | | 0.0582 | 0.2111 | 0.2693 | 0.7925 | | 3.8480 | 2.0928 | 5.9408 | 6.5346 |
| | Period 5. Diet | | | | | | | | | | | | | | |
| 30 | 32.5320 | 12.6588 | 7.2936 | 19.9524 | 12.5796 | 1.0618 | 0.0693 | 0.4796 | 0.5489 | 0.5129 | 12.4754 | 5.7420 | 4.5694 | 10.3114 | 2.1640 |
| 31 | | 12.1520 | 5.7985 | 17.9505 | 14.5815 | | 0.0651 | 0.3685 | 0.4336 | 0.5282 | | 5.7040 | 3.1390 | 8.8430 | 3.6324 |
| 32 | | 11.5038 | 5,6703 | 17.1741 | 15.3579 | | 0.0647 | 0.2213 | 0.2860 | 0.7758 | | 6.2040 | 1.9316 | 8.1356 | 4.3398 |

Analytical Data to Experiment V.

Amounts in Grams

EXPERIMENT VI.

| Weight of Dog. grms. | Days. | Urine. Volume. c.c. | Urine. T.N. grms. | Urine. Total CaO. grms. | Urine. Total $P_2O_5$. grms. | Faeces. Weight of dried faeces. | Faeces. T.N. grms. | Faeces. Total CaO grms. | Faeces. Total $P_2O_5$. grms. |
|---|---|---|---|---|---|---|---|---|---|
| Period 1. | | Diet + 6 grms. Calcium Lactate = 1.0584 grms. CaO. | | | | | | | |
| | 1 | 600 | 2.3688 | 0.0983 | 0.2040 | 10.4 | 0.3627 | 1.1853 | 0.9546 |
| 4,420 | 2 | 1,000 | 2.1000 | 0.0686 | 0.2600 | 15.3 | 0.5418 | 1.5100 | 1.2843 |
| 4,430 | 3 | 1,000 | 2.5480 | 0.0798 | 0.2000 | 11.0 | 0.4096 | 1.3706 | 1.1184 |
| 4,420 | 4 | 1,000 | 2.5760 | 0.0742 | 0.2100 | 6.8 | 0.2545 | 0.7740 | 0.6724 |
| 4,430 | 5 | 1,000 | 2.2120 | 0.0742 | 0.1900 | 16.8 | 0.6460 | 1.8984 | 1.5504 |
| 4,450 | 6 | 1,000 | 2.3660 | 0.0728 | 0.1700 | 6.3 | 0.2513 | 0.7438 | 0.6295 |
| 4,460 | 7 | 1,000 | 2.4640 | 0.0672 | 0.2300 | 8.3 | 0.2688 | 1.0000 | 0.8200 |
| Period 2. | | Diet + 6 grms Calcium Lactate+5 grms $Na_2HPO_4$. = 1.0854 grms. CaO. + 1.06 grms $P_2O_5$. | | | | | | | |
| 4,450 | 8 | 1,000 | 2.3100 | 0.0224 | 0.6800 | 19.73 | 0.6899 | 2.2802 | 2.0251 |
| 4,460 | 9 | 1,000 | 2.5200 | 0.0182 | 0.7700 | 13.00 | 0.4589 | 1.6084 | 1.6539 |
| 4,480 | 10 | 1,000 | 2.4780 | 0.0238 | 0.7200 | 7.43 | 0.2898 | 0.9303 | 0.9475 |
| 4,450 | 11 | 1,000 | 2.4080 | 0.0238 | 0.8400 | 7.81 | 0.2615 | 1.0850 | 1.1123 |
| 4,470 | 12 | 1,000 | 2.5620 | 0.0182 | 0.8300 | 19.42 | 0.7079 | 2.3969 | 2.4382 |
| 4,490 | 13 | 1,000 | 2.7160 | 0.0210 | 0.8300 | 5.65 | 0.2152 | 0.7752 | 0.8158 |
| 4,510 | 14 | 1,000 | 2.2400 | 0.0154 | 0.8200 | 15.84 | 0.5896 | 2.1011 | 2.1146 |
| Period 3. | | Diet + 6 grms Calc. Lact. + 5 grms. $Na_2HPO_4$ 10 cc. Cod-liver oil. | | | | | | | |
| 4,540 | 15 | 1,000 | 2.2400 | 0.0210 | 0.8300 | 7.54 | 0.2800 | 1.0049 | 1.0556 |
| 4,590 | 16 | 1,000 | 2.1280 | 0.0168 | 0.8000 | 11.93 | 0.4420 | 1.5278 | 1.5606 |
| 4,600 | 17 | 1,000 | 2.3100 | 0.0168 | 0.7800 | 14.91 | 0.5212 | 1.8578 | 1.0338 |
| 4,630 | 18 | 1,000 | 2.3140 | 0.0168 | 0.8800 | 12.55 | 0.4614 | 1.5300 | 1.5842 |
| 4,660 | 19 | 1,000 | 2.3240 | 0.0182 | 0.9200 | 5.15 | 0.1757 | 0.6408 | 0.6759 |
| 4,670 | 20 | 1,000 | 2.3562 | 0.0169 | 0.9240 | 15.16 | 0.5529 | 1.7642 | 1.8082 |
| 4,650 | 21 | 1,000 | 2.3240 | 0.0210 | 0.9100 | 14.88 | 0.5565 | 1.8033 | 1.8845 |
| Period 4. | | Diet + 6 grms Calc. Lact. + 5 grms. $Na_2HPO_4$ +10 grms Lard (new solution $Na_2PO_4$.) | | | | | | | |
| 4,690 | 22 | 1,000 | 2.5480 | 0.0196 | 0.8800 | 7.47 | 0.2631 | 0.7958 | 0.8785 |
| 4,720 | 23 | 1,000 | 2.2260 | 0.0154 | 0.9200 | 7.54 | 0.2621 | 0.8503 | 0.9598 |
| 4,750 | 24 | 1,000 | 2.4080 | 0.0168 | 0.8100 | 19.93 | 0.7335 | 2.3123 | 2.3390 |
| 4,770 | 25 | 1,000 | 2.5060 | 0.0154 | 0.9700 | 12.10 | 0.4335 | 1.3198 | 1.4541 |
| 4,790 | 26 | 1,000 | 2.1560 | 0.0126 | 0.8900 | 6.10 | 0.2346 | 0.6284 | 0.7480 |
| 4,820 | 27 | 1,000 | 2.2400 | 0.0154 | 0.8900 | 7.10 | 0.2706 | 0.8678 | 0.9685 |
| 4,850 | 28 | 1,000 | 2.1700 | 0.0210 | 0.8100 | 18.2 | 0.6926 | 1.9890 | 2.1883 |
| Period 5. | | No Calcium or Phosphorus. | | | | | | | |
| 4,820 | 29 | 1,000 | 2.3100 | 0.0182 | 0.6700 | 8.80 | 0.3584 | 0.6284 | 0.7152 |
| 4,820 | 30 | 1,100 | 2.3100 | 0.0185 | 0.6930 | 10.64 | 0.4244 | 0.4091 | 0.6448 |
| 4,800 | 31 | 1,000 | 2.5480 | 0.0210 | 0.8200 | 8.87 | 0.3618 | 0.1478 | 0.5788 |

Analytical Data to Experiment V1.

Amounts in Grams

| CAO in food | CaO in Urine | CaO in faeces | Total CaO excreted | | CaO balance | $P_2O_5$ in food | $P_2O_5$ in Urine | $P_2O_5$ in Faeces | Total $P_2O_5$ excreted | | $P_2O_5$ balance |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Period 1. Diet 6 grams Calc. Lact. = 1.0584 grms CaO.** | | | | | | | | | | | |
| 1.6370 | 0.0983 | 1.1853 | 1.2836 | | 0.3534 | 1.8566 | 0.2040 | 0.9546 | 1.1586 | | 0.6980 |
| | 0.0686 | 1.5100 | 1.5786 | | 0.0584 | | 0.2600 | 1.2843 | 1.5443 | | 0.3123 |
| | 0.0794 | 1.3706 | 1.4500 | | 0.1870 | | 0.2000 | 1.1184 | 1.3184 | | 0.5382 |
| | 0.0742 | 0.7740 | 0.8482 | | 0.7888 | | 0.2100 | 0.6724 | 0.8824 | | 0.9742 |
| | 0.0742 | 1.8984 | 1.9726 | | 0.3356 | | 0.1900 | 1.5504 | 1.7404 | | 0.1162 |
| | 0.0728 | 0.7438 | 0.8166 | | 0.8204 | | 0.1700 | 0.6295 | 0.7995 | | 1.0571 |
| | 0.0672 | 1.0000 | 1.0672 | | 0.5698 | | 0.2300 | 0.8200 | 1.0500 | | 0.8066 |
| | | | | Av. | 0.3500 | | | | | A. | 0.6432 |
| **Period 2. Diet 6 grams Calc. Lact. 5 grms. $Na_2HPO_4$ - 1.0854 grms. CaO. 1.06 grms. P O** | | | | | | | | | | | |
| 1.6370 | 0.0224 | 2.2802 | 2.3026 | | 0.6656 | 2.9166 | 0.6800 | 2.0251 | 2.7051 | | 0.2115 |
| | 0.0182 | 1.6084 | 1.6266 | | 0.0104 | | 0.7700 | 1.6539 | 2.4239 | | 0.4927 |
| | 0.0238 | 0.9303 | 0.9541 | | 0.6829 | | 0.7200 | 0.9475 | 1.6675 | | 1.2491 |
| | 0.0238 | 1.0850 | 1.1088 | | 0.5282 | | 0.8400 | 1.1123 | 1.9523 | | 0.9643 |
| | 0.0182 | 2.3969 | 2.4151 | | 0.7781 | | 0.8300 | 2.4382 | 3.2682 | | 0.3516 |
| | 0.0210 | 0.7752 | 0.7962 | | 0.8408 | | 0.8300 | 0.8158 | 1.6458 | | 1.2708 |
| | 0.0154 | 2.1011 | 2.1165 | | 0.4795 | | 0.8200 | 2.1146 | 2.9346 | | 0.0180 |
| | | | | Av. | 0.0200 | | | | | A. | 0.5170 |
| **Period 3. Diet 6 grms Calc. Lact. 5 grms $Na_2HPO_4$ 10 cc. Cod-Liver Oil.** | | | | | | | | | | | |
| 1.6370 | 0.0210 | 1.0049 | 1.0259 | | 0.6111 | 2.9156 | 0.8300 | 1.0556 | 1.8856 | | 1.0310 |
| | 0.0168 | 1.5278 | 1.5446 | | 0.0924 | | 0.8000 | 1.5606 | 2.3606 | | 0.5560 |
| | 0.0168 | 1.8578 | 1.8746 | | 0.2376 | 2.8866 | 0.7800 | 1.9338 | 2.7138 | | 0.1728 |
| | 0.0168 | 1.5300 | 1.5468 | | 0.0902 | | 0.8800 | 1.5842 | 2.4642 | | 0.4224 |
| 1.6034 | 0.0182 | 0.6408 | 0.6590 | | 0.9444 | | 0.9200 | 0.6759 | 1.5959 | | 1.2907 |
| | 0.0169 | 1.7642 | 1.7811 | | 0.1777 | | 0.9240 | 1.8082 | 2.7322 | | 0.1544 |
| | 0.0210 | 1.8033 | 1.8243 | | 0.2209 | | 0.9100 | 1.8845 | 2.7945 | | 0.0921 |
| | | | | Av. | 0.1600 | | | | | | |
| **Period 4. Diet 6grms. Calc. Lact 5 grms $Na_2HPO_4$ 10 grms. Lard (new solution Na HPO)** | | | | | | | | | | | |
| 1.6034 | 0.0896 | 0.7958 | 0.8154 | | 0.7880 | 2.9866 | 0.8800 | 0.8785 | 1.7585 | | 1.2281 |
| | 0.0154 | 0.8503 | 0.8657 | | 0.7377 | | 0.9200 | 0.9598 | 1.8798 | | 1.1068 |
| | 0.0168 | 2.3123 | 2.3291 | | 0.7257 | | 0.8100 | 2.3390 | 3.1490 | | 0.1624 |
| | 0.0154 | 1.3198 | 1.3352 | | 0.2682 | | 0.9700 | 1.4541 | 2.4241 | | 0.5625 |
| | 0.0126 | 0.6284 | 0.6410 | | 0.9624 | | 0.8900 | 0.7480 | 1.6380 | | 1.3486 |
| | 0.0154 | 0.8678 | 0.8832 | | 0.7202 | | 0.8900 | 0.9685 | 1.8585 | | 1.1281 |
| | 0.0210 | 1.9890 | 2.0100 | | 0.4066 | | 0.8100 | 2.1883 | 2.9983 | | 0.0117 |
| | | | | Av. | 0.3330 | | | | | | |
| **Period 5. No Calcium or Phosphorus.** | | | | | | | | | | | |
| 0.5786 | 0.0182 | 0.6284 | 0.6466 | | 0.0498 | 1.8566 | 0.6700 | 0.7152 | 1.3852 | | 0.4714 |
| | 0.0185 | 0.4091 | 0.4276 | | 0.1695 | | 0.6930 | 0.6448 | 1.3378 | | 0.5188 |
| | 0.0210 | 0.1478 | 0.1688 | | 0.4098 | | 0.8200 | 0.5788 | 1.3988 | | 0.4578 |
| | | | | Av. | 0.1800 | | | | | | |

## APPENDIX III.

Many points of interest have emerged during this investigation, and reference to one in conclusion may not be out of place.

A long series of faecal determination on a single animal bring strikingly to one's notice the constant composition of the faeces. A consideration of the question shows that the production of a perfectly-formed faecal mass is a physiological problem essential to health and complicated to a degree. The regulation of the contents of a muscular tube open at both ends must be still more difficult than the regulation of the blood circulating in a closed system.

The constancy of the proportion of Ca and P, for instance, and the difficulty of altering the P percentage composition of the faeces by administration of large doses of P salts suggests an intimate regulation by some controlling mechanism. The problem of the negative Ca balance has always been difficult. Here is a substance, necessary to the bodily economy, not merely being withheld, but ever abstracted from, tissues

as retentive as epiphyseal cartilage. This cartilage as proved by Findlay (65) has not lost its Ca affinity. It is obvious that calcium can only be withdrawn to meet some greater need.

Considering the analogy between the hollow muscle of the gut and of the heart a possible explanation is suggested. The rhythmical contractions of the heart are greatly dependent upon the presence and proportion of mineral substances in the blood, and the slower though equally vital contractions of the gut must be influenced also by this factor. Regarding the thirty-six feet of human intestine as a great "heart" perfused by a Ringer's solution, the question of a mineral withdrawal even from the skeletal stricture of the body, to maintain an altered osmotic pressure, or ionisation, would appear in another light. It should not be forgotten that the osteoclastic mechanism is preserved far into adult life and that the "fixed alkali reserve" is not perhaps so fixed as anatomy would indicate.

Graphs A & B have been drawn to show the ~~abstraction~~ alteration in the channel of excretion of either Ca or P produced by a modification of the mineral content of the food in respect to one constituent only, calcium being constant in one and phosphorus in the other.

GRAPH A

# EXCRETION OF CaO AND $P_2O_5$.
## $P_2O_5$ CONSTANT AND CaO VARIABLE.

GRAPH B

# EXCRETION OF CaO AND $P_2O_5$
## CaO CONSTANT AND $P_2O_5$ VARIABLE.