THE INVESTIGATION OF POISONS IN PULSES AND CEREALS.

A Thesis submitted in accordance with the regulations of the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

by

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VOLUME I.

C O N T E N T S .

INTRODUCTION.

The food of today may be the poison of tomorrow.

In many respects the truth of this has been borne out in a remarkable way as man's knowledge has progressed concerning his "daily bread".

Cereals have always formed the major part of man's diet and have generally been regarded as entirely wholesome. Yet such diseases as pellagra and beri-beri have been associated with the over-consumption of two of these cereals, maize and rice, while scurvy and rickets are now regarded as definite dietary disturbances.

Modern methods of milling and preparing food-stuffs are no doubt responsible for many changes, but increasing knowledge of the constituents of food-stuffs has resulted in the rejection or alteration of many substances which were at one time established as staple articles of diet.

In a series of researches carried out during a period of more than 20 years, Stockman[1] has studied the effects of feeding leguminous seeds and cereals. He concluded that lathyrus poisoning in animals and lathyrism in man was due to a poison present in the peas and was not attributable to any "deficiency" disease.

In a study of the causes of pellagra he[2] concluded from a large series of experiments on animals that the disease was caused by an acid or acids which were not only poisonous

in themselves, but which had a secondary effect of gradually or suddenly withdrawing alkalies, especially calcium, from the blood and tissues. He produced evidence that there was no question of deficiency or avitaminosis. Further, he suggested the possibility of cereals and diets other than maize contributing to this condition. While pellagra exists as a maize disease, he suggested the possibility of other cereals as causative factors.

Holst[3] found that acid extracts of oats and other cereals when neutralised and fed to rats were capable of producing rickets, and that the substance was precipitated by alcohol.

By injecting animals with acid extracts of oatmeal Mirvish[4] produced a drop in blood calcium of over 30% after 24-48 hours, which returned to normal in 48-72 hours. He suggested a relationship with parathyroid disfunction.

On diets of wheat and wheat embryo Hart-Miller and McCollum[5] produced in pigs signs not unlike beri-beri. They suggested a toxic substance in the fat of wheat embryo.

Mellanby[6], investigating the harmful effects of some food-stuffs, found that cereals had a special property of interfering with the calcification of bones. He stated that if oats were steeped for two days, germinated for 1-6 days, and heated to 100°C for 18 hours, their power to prevent calcification was reduced. He postulated the presence of toxic substances which he termed Toxamins.

In some later work[7] he pointed out the aetiological significance of a deficient intake of vitamin A and carotene in such toxic degenerations as those associated with convulsive-ergotism, pellagra and lathyrism. He concluded that, apart from a deficiency of vitamin A and carotene and excess of cereal, some other nutritional defects aid in the production of degenerative lesions in the nervous system.

In continuance of Stockman's investigations the present research was undertaken primarily as a systematic study of the cereals, rice and barley, with a view to determining the presence of chemical poisons in these cereals, and secondly, in an attempt to correlate these findings with the occurrence of beri-beri and other nutritional disturbances.

Cereals were called "frumenta" by Pliny[8] to distinguish them from the "legumina". "Frumenta" indicated "all those kinds of corns from which bread was prepared by the Ancients."

The cereals may be defined as the edible fruits of the Gramineae. The grasses are divided into thirteen tribes of which at least nine furnish important grains for human and animal consumption.

The present research was made on Oryza sativa and Hordeum distichon, members of the two tribes Oryzeae and Hordeae.

STUDIES IN ORYZA SATIVA.

Rice is the grain of a cultivated grass which according to Roxburgh[9] has originated from a wild plant called in India, "Newaree" or "Nivara." Its cultivation was practised in China and Japan as early as 3000 B.C.

Theophrastus[10] described it as similar in appearance to darnel, but that for most of its time of growth " τὸν πολὺν χρόνον ἐν ὕδατι ". The Gauls according to Pliny[11] made a porridge from it which they called "du riz", and he described it as the "most favoured food of all in India where a 'ptisan' is made from it."[12] Its astringent effect was noted by Galen[13].

With changing conditions of soil and artificial irrigation many varieties of rice have resulted and it is now extensively cultivated in India, China, West Indies, Central America, U.S.A. and some of the southern countries of Europe.

It furnishes daily food for more human beings than any other cereal.

The rice plant, Oryza sativa, which belongs to the tribe Oryzeae of the natural order Gramineae is an annual grass which grows from two to ten feet in height.

Its leaves are long and slender; the spikelets are one-flowered, with two small empty glumes, a flowering glume which may or may not have an awn and a smaller palet. The panicles, which droop as the fruit ripens, measure about twelve inches in length (fig.1). The fruit or kernel which is a

caryopsis is enclosed in but does not adhere to the palets.

The cultivation of the plant varies with the type of soil. Where grown in low and irrigated land it is termed swamp rice - "arroz de sementera" (fig.2). There are other varieties which develop in temperate climates, high above irrigation level, which are called mountain rice - "arroz de secano." The more important varieties require repeated irrigation and are semi-aquatic.

Despite the diverse conditions of cultivation, analyses[14] show a remarkable accordance in the chemical constituents of the varieties.

Microscopic Structure.

Sections of the grain for microscopic examination were prepared and stained as follows. The grains were moistened in water, generally overnight. They were then embedded in dextrin paste and cut by freezing microtome. Since the sections were cut from dextrin it was essential first to place them in water. Two methods of staining and mounting were used.

(I) The sections were transferred directly to aqueous iodine solution and stained purple. They were then mounted in Farrant's Medium. The aleurone layer was coloured brownish-yellow while the starch of the endosperm was stained purple.

(II) The sections were left only a short time in the water, then transferred to 70% alcohol and then to absolute alcohol. From this they were transferred to an alcoholic solution of iodine. The starch stained a brownish-purple and the aleurone layer yellow. The sections after staining were passed through Xylol before mounting in Canada Balsam.

The second method was found more satisfactory as the staining faded more rapidly, and very lightly stained sections are most suitable for photographic purposes.

Figs. 3 and 4 show a cross-section of the fruit from which the palets have been detached in cutting. It consists of

(1) Husk or siliceous hull which is composed of -

(a) an outer epidermis of square cells
(b) sclerenchymatous fibres
(c) spongy parenchyma
(d) inner epidermis of polygonal cells.

(2) Pericarp which consists of epicarp, mesocarp, cross-cells and tube cells.

(3) Spermoderm of transversely elongated cells (yellow with chlorzinc iodine).

(4) Perisperm of transversely elongated cells (blue with chlorzinc iodine).

(5) Endosperm consisting of (a) aleurone layer one or two cells deep, (b) starch cells.

During the process of milling various layers of the grain are removed. A study of the milling methods and a microscopic examination of the products give some interesting information.

The fruit, when it has been separated from the rest of the plant and the empty glumes, is termed unhusked or paddy (padi) rice.

Methods of Milling Rice.

1. Native or hand milling.

2. European or machine milling.

1. The Native method of milling may be (a) Direct or (b) Parboiling.

(a) Direct Method - The paddy is pounded in a wooden mortar with a long wooden pestle (fig.5) until the hulls have become detached from the grain. This process is long and tiresome, and results not only in the removal of the hull and a varying proportion of the pericarp, but also in the breaking of a large number of grains.

Winnowing in the open air (fig.7) by means of a cloth or hide serves to separate the detached parts of the grain. The product may be further treated by lightly pounding, to remove a further amount of the pericarp. A cross-section of the product (fig.8) shows the partial removal of the pericarp, but the aleurone layer is still adherent.

A modification of the above method is used by some tribes in India - the Tamils, and by some in West Africa. This consists in (b) Parboiling or "Curing" the rice, previous to the milling process. Braddon[15] describes several methods used by different tribes. The following is the one generally used.

Paddy rice is steeped in an equal quantity of water for twenty four to forty eight hours. It is boiled for about ten minutes and spread out to dry in the sun.

By this process many of the hulls are detached by the swelling of the grain. When the grain is later milled, the hulls are readily detached, and less of the pericarp is removed. The grain is semi-translucent, and being tougher, is less liable to be broken.

2. European or Machine Milling is a more advanced but less desirable modification of the native methods.

In the Direct method, the paddy is passed between two hulling "stones" which crack the husk and after fanning, the grain is left with its adherent pericarp. The pericarp may vary in colour from red to brown or even yellow. The grain is

then passed through the "huller" or scourer, which removes most of the pericarp and the embryo. A further treatment by polishing with sheepskin or pigskin results in the production of White Rice or so-called "unpolished" rice. A glazing process with glucose and talc produces a highly glazed grain which is called "polished rice". This severe treatment results in the production of a grain which is very highly milled and consists solely of the starch cells of the endosperm (figs. 9 and 10).

The method of Parboiling is precisely that used by the natives, but in place of boiling, steam is passed for ten minutes through cylinders containing the soaked paddy. The resultant milling produces a less highly milled grain (figs. 11 and 12) in which particles of the aleurone layer are apparent.

Effect of Milling. By the various stages of milling, the weight of the grain is materially decreased and important chemical constituents are reduced in quantity. Comparison of the chemical analyses (fig.13) shows that native milling removed a smaller proportion of ash, crude fibre and oil than did the modern mill (fig.13.A).

By an analysis (fig.14) of polished and parboiled rice, Fraser and Stanton[16] have pointed out that parboiled rice contains 51% more fat, 39% more P_2O_5 and has an ash 30% higher. The importance of this observation will be considered later.

Chemical Constituents.

Protein. The bulk of the protein consists of a glutelin oryzenin. Three globulins have been isolated by Jones and Gersdorff[17] and by Kondo and Ito[18].

Fat. From an ether extract of bran a yield of 9% was obtained. The oil undergoes rapid deterioration, which, according to Browne[19], is due to an enzyme lipase. The chief fatty acids are oleic acid and palmitic acid, while Jamieson[20] found also the glycerides of linolic, stearic, myristic, arachidic and lignoceric acids.

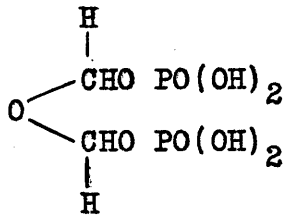
Carbohydrates. Starch constitutes the main bulk of carbohydrate, though Frapps[21] detected some reducing sugars, disaccharides and pentosans, in small amount.

Mineral Constituents.

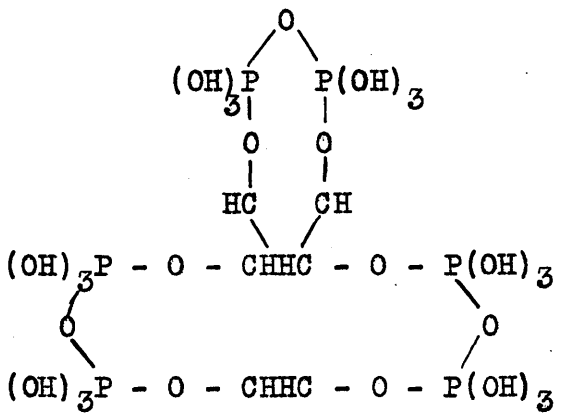
Frapps[22] by an analysis of the mineral constituents of the ash (fig.15) showed that in polished rice there was a marked decrease in the amount of phosphorus.

Phosphorus. It was to the phosphorus content of rice that my attention was directed and attempts were made to ascertain the nature of the phosphorus in the grain.

In 1903 Posternak[23] described the isolation from seeds, of a compound containing 19-20% phosphorus in organic combination, and to it he assigned the empirical formula $C_2H_8O_9P_2$. He called it phytin and described it as anhydro-oxymethylene-diphosphoric acid, with a constitution



Neuberg[24] later showed that it was an inosite-phosphoric acid with a constitution



His work was confirmed by Winterstein[25].

In 1907 Suzuki, Yoshimura and Takaishi[26] found that the enzyme phytase hydrolised the phytin to inorganic phosphoric acid and a series of intermediate compounds.

Patten and Hart[27] and Anderson[28] working on cotton seed found no inosite-hexaphosphoric acid, but isolated inosite-mono-, di- and tri-phosphoric acids. Using 0.2% hydrochloric acid to inhibit enzyme action Anderson[29] later isolated from wheat bran phytic acid and concluded that it is inositol hexaphosphoric acid $C_6H_{18}O_{24}P_6$ or $C_6H_6(OH_2PO_3)_6$. Rather[30] prepared silver and strychnine salts and adopted the formula $C_6H_6(OH)(OH_2PO_3)_5$ or $C_6H_{17}O_{21}P_5$ - inosite penta-phosphoric acid.

Thompson[31] by extraction with hydrochloric acid and precipitation with alcohol, isolated 8.22% phytin from rice bran, but could not obtain any from polished rice.

Enzymes.

In 1907 Susuki, Yoshimura and Takaishi[32] described a method for isolating from defatted rice bran an enzyme which they called phytase. This enzyme hydrolised phytin, producing lower esters of inosite phosphoric acid and inorganic phosphoric acid. Dox and Golden[33] found a similar enzyme present in some fungi, while McCollum and Hart[34] observed similar properties in an extract of animal tissues.

The activity of phytase was originally determined by the estimation of the inorganic phosphate liberated by hydrolysis of phytin. More recently Collatz and Bailey[35] detected the changes produced by the enzyme in the specific conductivity of an aqueous extract of phytin. Its optimum temperature is 55°C and its optimum reaction is at pH 5.4-5.5.

INVESTIGATION OF THE ENZYME.

To determine the extent of enzyme action in the hydrolysis of the organic phosphorus compounds, an extract was made by crushing 200 gm. of paddy rice in a hand mill and macerating it with 100 mils. of cold water at room temperature for four hours. The marc was then percolated with cold water till 500 mils. extract had been collected. To determine the amount of inorganic phosphorus 50 mil. portions of this extract were

used and for total phosphorus assay 5 mil. portions were used.

The Principle of the Phosphorus Assay. The principle of the volumetric molybdate method as used by Richards and Godden[36] and by Cameron and Dow[37] is the precipitation of the phosphate as ammonium phospho-molybdate in the presence of nitric acid and ammonium nitrate; solution of the precipitate in standard alkali, and titration of the excess of alkali by standard acid. The equation evolved is

$$2(NH_4)_3PO_4 \cdot 12MoO_3 + 46NaOH = 2(NH_4)_2HPO_4 + (NH_4)_2MoO_4 + 23Na_2MoO_4 + 22H_2O$$

Each atom of phosphorus requires 23 molecules of sodium hydroxide so 1 mil $^N/_5$ NaOH $\equiv$ 0·6168 mg. P_2O_5.

'Molybdate Reagent' - consisted of the nitric acid solution of ammonium molybdate prescribed for gravimetric work in Statutory Rules and Orders 1932 No.658 made under the Fertilisers and Feeding Stuffs Act 1926. This reagent was kept for seven days in a warm place before use as during that time a cream coloured precipitate was often deposited.

Method of Estimating Inorganic Phosphorus - 50 mils. of extract ($\equiv$ 20 gm. of grain) were diluted with 50 mils. of water, 15 gm. of ammonium nitrate and 5 mils. of nitric acid added. The mixture was heated in a water bath at 65°C with 35 mils. molybdate reagent for 30 minutes. The mixture was allowed to stand for 15 minutes and was then filtered. The precipitate was

washed with 5% nitric acid, dissolved in 5 mils. ammonium hydroxide and boiled with 50 mils. water for 15 minutes. 10 gm. ammonium nitrate were added and the solution was filtered. The filtrate was heated on a water bath to 65°C with 5 mils. nitric acid and to the solution was added in a thin stream 35 mils. of molybdate reagent. This was allowed to stand at a temperature of 65°C for 30 minutes and then set aside to cool for 15 minutes. The supernatant liquid was filtered off through an asbestos filter in a Gooch crucible under slight suction. The precipitate was dissolved in 35 mils. N/5 NaOH, and 50 mils. distilled water and the solution back-titrated with N/5 HCl using phenolphthalein as indicator. 35 mils. N/5 HCl were then added, and the solution heated on a water bath at 90°C for 15 minutes. After cooling rapidly the solution was back-titrated with N/5 NaOH using phenolphthalein as indicator.

The amount of N/5 NaOH was multiplied by the factor derived for the phospho-molybdate precipitate, and the percentage phosphorus was expressed in grammes of phosphorus pentoxide.

Method of Estimating Total Phosphorus.

5 mils. extract (≡ 2 gm. grain) with 0.5 gm. light magnesium oxide in an ignition crucible were dried in an oven at 100°C. The mass was ignited by bunsen flame till the organic matter was oxidised. The mass was dissolved in 5 mils. nitric acid and 50 mils. water and boiled. 10 gm. ammonium

nitrate were added and the filtered solution was heated on a water bath at 65°C with 35 mils. molybdate reagent for 30 minutes. After cooling for 15 minutes it was filtered through a Gooch crucible. The estimation was continued as described under Inorganic Phosphorus.

Estimations were made in triplicate and the mean value expressed as shewn in fig.16.

The effect of controlling hydrolysis by means of an acid solvent was investigated, and an extract was made using 1% HCl as the extracting medium.

An attempt was made to stabilise the grain by heating it with absolute alcohol in an autoclave at 1½ atmospheres for 2 hours. The grain was then dried to constant weight, crushed, extracted with cold water and assayed as above. An extract was also made using 1% HCl as the extracting medium. The results of these determinations are expressed in fig.17. These results show that

(a) a larger proportion of the phosphorus compounds is hydrolysed when water is used as extracting medium.

(b) 1% HCl inhibits enzyme action.

(c) 1% HCl extracts more phosphorus from the grain.

(d) Autoclaving the grain with alcohol serves to stabilise the grain, and prevents hydrolysis of the organic phosphorus.

The zone of the enzyme action was then investigated. If the phosphorus compounds were catabolised in the pericarp and the area, external to the endosperm, a portion of the grain

in which that area was absent should show no evidence of phosphorus hydrolysis. Accordingly extracts of Siam polished rice were prepared and assayed as described above. In fig.18 the results of the analysis indicate that there is no inorganic phosphorus in polished rice and that there is evidence of hydrolysis to a slight degree of organic phosphorus after extraction with water. This suggests the absence of enzymes capable of phosphoric hydrolysis.

As was demonstrated in the microscopic examination of polished rice, there was a variable minute amount of aleurone layer adherent to the endosperm. The evidence thus supports the contention that the enzyme is located in the zone external to the endosperm.

The influence of enzyme action in the parboiling of rice was considered. Paddy rice was steeped in cold water for 24 hours and boiled for 10 minutes when the husks appeared to become detached from the grain. The mass was transferred to a canvas tray and dried in a current of warm air. Thereafter drying was continued to constant weight. 200 gm. were crushed, extracted with 1% HCl, and assayed as described above.

Paddy rice which had been previously autoclaved with alcohol was similarly parboiled and assayed.

From the analyses fig.19, it is seen that in the process of parboiling there is a loss in the total phosphorus content and an increase in the amount of inorganic phosphorus. The hydrolysis of the phosphorus compound takes place during the

steeping of the grain in water. It was also noted that there was a gradual increase in the acidity of the water during the steeping process.

Further analyses were made of extracts of parboiled rice which had been highly milled. These samples of rice were known in commerce as parboiled rice.

In fig. 20 it is shown that during the parboiling of rice about 25% of the organic phosphorus is hydrolised, and that during the subsequent milling process a considerable percentage of the phosphorus is removed but that the ratio of inorganic and total phosphorus is unaltered. It is also seen that there is no evidence of further hydrolysis. This suggests the absence of phospholysing enzymes in milled parboiled rice.

Isolation of the Enzyme.

Attempts were made to isolate the enzyme by two different methods. In the first method suggested by the work of Susuki et alia[38] rice bran was used.

1000 gm. rice bran were refluxed with ether in a Soxlet apparatus for 4 hours. The defatted bran was dried in air, digested with water for 4-5 hours and filtered through a Gooch funnel. The filtrate was neutralised with barium hydroxide and a solution of barium chloride was added, till there was no further precipitate. The precipitate was filtered and the filtrate was slowly added with stirring to a mixture of 85% alcohol and ether. The white precipitate was separated, dissolved

in water and barium chloride solution added. The small precipitate was filtered and the filtrate was gradually poured into a mixture of alcohol and ether. The precipitate, which was allowed to settle overnight, was filtered, washed with alcohol-ether mixture, and dried in vacuo over H_2SO_4. The precipitate weighed 19.45 gm. representing a yield of 1.95%. The white powder was readily soluble in water.

The second method was an attempt to isolate the enzyme during a period of activation. It was considered that if rice was germinated for a period of say 14 days, the organic phosphorus compounds would be hydrolised during this period, in the course of catabolism, to supply energy and food for the young plant. The enzyme would be in an active state, and might be more readily isolated.

Accordingly 500 gm. paddy rice were spread in layers of moist cotton wool and maintained in a dark moist atmosphere for 10 days. The germinating seeds were then crushed in a hydraulic press and the extracted liquor was separated. The crushed seeds were digested with 25% alcohol and then percolated with 1 litre 25% alcohol. The percolate was concentrated in vacuo, and the concentrate was poured into 96% alcohol. The precipitate, after settling, was filtered, washed with 96% alcohol, then with ether and dried in vacuo over H_2SO_4. The precipitate weighed 4.5 gm. representing a yield of 0.9%. The white powder was readily soluble in water.

These preparations were not investigated chemically, but were prepared for animal experiments.

CHEMICAL INVESTIGATION.

The chemical investigation was undertaken with a view to isolating the toxic substance or substances which were believed to be the cause of beri-beri.

Stockman and Johnston[39] described the action of an aqueous extract of polished rice on a monkey and attributed it to an acid, similar to that in maize which caused pellagra. It was thought that the rôle of the organic phosphorus compounds was worthy of investigation and the method used in isolating these compounds was as follows.

The grain was crushed in a mill to a coarse powder. It was macerated with 1% HCl for eight hours at a temperature not exceeding 35°C and then percolated for 24 hours. The percolate was mixed with sodium acetate in the proportion of 29.1 gm. per litre of percolate, and filtered. A concentrated solution (30%) of lead acetate was added to the filtrate till there was no further precipitate. It was filtered. The precipitate was known as the "Lead Precipitate". To the filtrate was added a 30% solution of mercuric acetate till there was no further precipitate. It was filtered. This precipitate was known as the "Mercury Precipitate". The filtrate was rejected.

Method of Isolating the Toxic Substances from the "Lead and Mercury Precipitates."

The precipitate was washed with cold water till the washings gave no reaction to litmus paper. The precipitate was suspended in cold water and hydrogen sulphide was passed in a

slow stream with stirring till all the metal was precipitated as sulphide. It was filtered. The sulphide was discarded. The filtrate was concentrated in vacuo at 35°C to a thin syrup and filtered. The filtrate was poured into absolute alcohol in a thin stream with constant stirring. The precipitate was washed with absolute alcohol and dried in vacuo over calcium chloride. It was known as the "Alcohol Insoluble Precipitate". The filtrate was concentrated in vacuo to a thin syrup, neutalised with sodium bicarbonate, filtered and the filtrate set aside to crystallise.

The method of extraction described above served to separate the esters of phytic acid in a fairly pure state, and minimised the hydrolysis of esters to inorganic phosphorus and lower esters.

(I) The Alcohol Insoluble lead precipitate (AIPb) was separated and dried as a pure white powder. It was very soluble in water and was acid to litmus. It gave reactions for calcium, magnesium and phosphorus.

When heated with dilute H_2SO_4 in an autoclave at 150°C for 6 hours and baryta water was added, the filtrate, when concentrated in vacuo and poured into alcohol, formed a white precipitate, which on drying yielded the reactions of Inositol. With the acetates of lead, copper and mercury it formed dense precipitates. An aqueous solution neutralised with NaOH formed a white precipitate which when filtered and dried gave reactions for Ca and Mg. It was a neutral Ca Mg salt of phytic acid,

insoluble in water, but soluble in acid solution. On adding alcohol to the filtrate, neutral sodium phytate was precipitated.

The free acid was generated by dissolving the AlPb precipitate in water, adding lead acetate solution and decomposing the lead precipitate with hydrogen sulphide. The filtrate was concentrated in vacuo to a thin clear syrup. It formed acid and neutral salts with alkalies which were very soluble in water, while with Ca.,Mg., Ba and the heavy metals it formed two series of salts - acid salts, soluble in water, and double salts insoluble in water but soluble in dilute acid solution.

In fig. 21 the percentage yields are shown. These quantities were obtained from the extraction of 500 gm. of the grain.

The material prepared for animal experiments was isolated from 8 kgm. and 12 kgm. lots. These extractions were carried out in large wooden barrels and subsequently precipitation was done in large porcelain containers.

(II) The Alcohol Soluble Lead Precipitate (ASPb). The crystals which separated were, after further re-crystallisation, analysed and found to be sodium phosphate ($Na_2HPO_4,12H_2O$). The yield was small and variable. In earlier extractions, when cold chloroform water was used as the extracting medium instead of 1% HCl, the yield was quite large, as much as 0.3-0.45% being obtained.

(III) The Mercury Precipitate. - When chloroform water was used for extracting, in earlier investigations, quite a large precipitate was formed when mercuric acetate was added to the filtrate from the lead precipitate. On further isolation the alcohol insoluble portion was found to resemble in appearance and in many reactions those of AIPb. It differed in that no precipitates were yielded with lead or copper acetate and no reactions were obtained for Mg or Ca. In view of the fact that no mercury precipitate was obtained when 1% HCl was used as extractant, further investigation of AIHg was abandoned. It is possible that it represented intermediate degradation products of phytic acid, and probably consisted of lower esters of phosphoric acid with inositol, such as the mono-, di- and tri-phosphoric esters described by Anderson[40].

ACTION ON ANIMALS.

The action of phytates has been investigated by Gilbert and Lippmann[41] who found that whereas in rabbits and guinea pigs the lethal dose by intravenous route was about 50 mg. per kg. b.w., 3 gm. were required hypodermically, while Mendel and Underhill[42] found a corresponding difference in degree of action. The following experiments were made with the Alcohol Insoluble Lead Precipitate (AIPb). A considerable number of experiments were performed, only some of which, for the sake of brevity, are recorded.

Experiment I. (Fig. 22) Frogs.

Hypodermically a dose of 0.1 gm. produced depression in 5 minutes, which was followed by marked paresis and in some cases violent tetanus. The heart stopped in diastole and there was paralysis of the muscles round the site of injection. In doses of 0.02-0.05 gm. there was marked paralysis of the brain and spinal cord which lasted for some hours. It was followed by increased spinal reflexes which lasted for some days.

Experiment II. (Fig.23) Rabbits.

By hypodermic injection a dose of 1.0 gm. caused paralysis of the hind legs. The condition gradually improved in the course of 4 hours. Smaller doses caused depression. Intravenously the action was more rapid and after a dose of 0.3 gm. by the ear vein, a rabbit was markedly depressed and dull in

10 minutes. With larger doses the signs were exaggerated.

Given by mouth the action varies somewhat in the animals. With rabbits a dose of 5 gm. produced drowsiness and lethargy which lasted for a day, but there were no ill effects observed next day. With pigeons (fig.24) a dose of 3.0 gm. proved fatal and the signs produced closely resembled those of polyneuritis gallinarum. There appears to be a limit to the extent of degradation of the esters to inorganic phosphates in the stomach and bowel, and when the undecomposed or partially decomposed esters are absorbed they are toxic and exert a marked action on the brain and spinal cord.

ANIMAL FEEDING EXPERIMENTS.

In 1897 Eijkman[43] published some experiments which he had conducted in Java. He found that a diet of cooked or raw decorticated rice produced in fowls a spontaneous polyneuritis in 3-4 weeks characterised by degeneration of peripheral nerves and atrophy of the ganglion cells in anterior cornu of the spinal cord. He attributed this disease to a toxic substance in rice.

As a result of subsequent experiments it was recognised that a disease was produced in pigeons and fowls which were fed on certain rices. This disease was characterised by paralysis of the legs, followed by paralysis of the wings and retraction, (opisthotonus) or by falling forward (emprosthotonus) of the head (fig.25). The gait of the animal resembled very closely that seen in beri-beri. The peripheral nerves of these animals on histological examination showed typical Wallerian degeneration.

The disease was called polyneuritis gallinarum and from the clinical manifestations and pathological effects it was used as a reaction on which were based the findings of the experiment.

The experiments about to be summarised are described in full in Volume 2, Fig. 26-32. The experiments with pigeons were conducted in pens with concrete walls and floors. Ventilation and light were provided by a wire caging, generally on at least two sides. The pigeons were allowed a dish of water.

Experiment I. (Fig. 26 and 26A.)

To investigate the effect of feeding polyneuritic pigeons with the portion of rice removed in milling, 6 pigeons were fed on Siam polished rice. In 19-21 days they all showed signs of the disease. At the onset of the disease, 2 were fed rice bran (polishings) by the mouth (4 gm.) daily, and one recovered completely in a day, while the other after only a transient recovery died paralysed. Likewise 4 were fed rice bran which had been made into a wash with water and then dried on a steam bath. They all showed a slight improvement, but after prolonged treatment failed to recover their normal condition and died paralysed. While rice bran was successful in restoring polyneuritic pigeons to normal, the curative property was destroyed by heating on a steam bath.

Experiment II. (Fig. 27 and 27A.)

Determinations were made to find if the curative property in rice bran was soluble in alcohol. Accordingly rice bran was extracted with 1% HCl, the extract was neutralised with sodium acetate and filtered. The filtrate was poured into absolute alcohol. The filtrate was neutralised with NaOH and concentrated to a volume such that 1 c.c. ≡ 4 gm. rice bran. Pigeons were fed Siam polished rice till the onset of polyneuritis. After the administration of an equivalent of 20 gm. rice bran there was a slight improvement, which however was only transient. Where the dose was given daily as prophylactic

treatment, there were no indications of polyneuritis after 30 days.

It would thus appear that some protective substance which is extracted by acid and is soluble in alcohol was present in the extract from bran. This substance appeared to prevent but was not able to cure the polyneuritic condition.

Experiment III. (Fig. 28 and 28A.)

The curative treatment of polyneuritis gallinarum was observed using a concentrated preparation of vitamin B_1 obtained from Java.

It was found that where doses of 0.2-0.5 c.c. B_1 concentrate equivalent to 20-30 times the curative day dose of B_1 were given there was a dramatic recovery, but there was no restoration of weight, and the pigeons afterwards died.

This is in agreement with the observations made by O'Brien[44] which I came upon at a later date.

Experiment IV. (Fig. 29 and 29A.)

Siam polished rice was macerated with cold water for 3 days and then percolated till no appreciable residue was obtained on evaporating the percolate. The rice was dried on a water bath and fed to pigeons.

It was found that the pigeons became polyneuritic in from 15-21 days, from which they recovered if given rice bran by mouth. There is a considerable loss in ash value and P_2O_5 content when Siam polished rice is extracted with water; the

ash value 0.1792 and P_2O_5 value 0.1070 compared with 0.4904 and 0.2408 in unextracted rice. The percentage of phosphorus is very low and is not sufficient to preserve a pigeon in good condition.

Experiment V. (Fig. 30 and 30A.)

On a diet of parboiled rice which had been highly milled, pigeons remained quite normal after 147 days. Fraser and Stanton[45] have attributed this to the presence of the sub-pericarpal layer.

From my earlier investigations it will be seen that little or no aleurone layer was left in the parboiled rice used in these experiments. The efficiency of this rice I attribute to the parboiling process which converts at least 25% organic phosphorus to the inorganic state, thereby rendering it non-toxic or capable of assimilation.

Experiment VI. (Figs. 31 and 31A.)

Pigeons fed on unmilled rice remained quite healthy. This is in agreement with the results of all investigators and can be attributed to the presence of the essential substance in the pericarpal and sub-pericarpal layers, which detoxicates and renders the phosphorus capable of being assimilated.

Experiment VII. (Figs. 32, and 32A,B,C.)

Rice bran was mixed into a stiff wash with water and gently dried into cakes and fed to pigeons. After 18-21 days

there were definite signs of polyneuritis which developed to a fatal termination in from 23-30 days. When the bran was mixed with about 20% crushed polished rice and fed in the same way, the pigeons remained in good condition and gained in weight.

This evidence supports the observations of R.R.Williams[46] who found that pigeons on an exclusive diet of rice meal developed polyneuritis, to which he attributed a toxic substance in the outer coats of rice. P. H. Moore[47] found that on a similar diet pigs developed a lameness in the hind legs and dyspnoea on slight exertion. The gait became staggering, and the skin and hair were discoloured. These signs developed after 30-50 days feeding and ceased when the diet was altered to other grain. He suggested a similarity to beri-beri and attributed the disease to a toxic factor in the bran.

When a diet consisting of a high proportion of the inisotol phosphoric esters is administered, even in the presence of the hydrolysing enzymes, the amount of esters may be sufficient to produce on assimilation a toxic condition resulting in the signs manifested.

FEEDING EXPERIMENTS WITH MONKEYS.

Experiment I.

A Rhesus monkey was fed daily on 150 gm. Siam polished rice, cooked by steaming, and fresh fruit. For 40 days there were no perceptible changes, and the animal ate well. On the 41st day it sat huddled in a corner and was reluctant to move (fig.33). There was some paresis of the left arm, which was flexed at the wrist. After 2 days it recovered and appeared quite normal. By the 88th day it was again very quiet and unwilling to move. On exercising it was easily exhausted. It gradually lost weight and its behaviour varied from day to day. Sometimes it was quite active and at other times it was unwilling to move about. After 143 days' feeding there was a recurrence of the wrist drop, which disappeared again. On the 150th day it was unable to move and was scarcely able to support its own weight (fig.34). When it moved it did so laboriously and 4 days later it lay helpless on its side. Its joints were all flexed and the muscles appeared to be very weak and limp. Next day (155th) it was completely paralysed and was chloroformed.

The post-mortem examination showed that the large viscera were normal in appearance and quite healthy.

Experiment II.

A Rhesus monkey was fed daily on 100 gm. Siam polished rice, cooked by steaming, and an apple or orange.

After 109 days' feeding its wrists were flexed but otherwise it was quite active and was able to run and jump. On the 164th day it sat in its cage with its toes and fingers flexed (fig.35) and it walked with a trailing gait. Its legs were flexed at the knees due to extensor muscle weakness; it could only drag its legs slowly. It gradually lost weight and on the 202nd day its diet was altered to 80 gm. Siam polished rice and 20 gm. rice bran. By the 206th day its skin appeared to be very sensitive and itching, it scratched continually and had lost a fair amount of hair. There was a series of coarse tremors in its hands, which gave it the appearance of trembling. It became weaker and sat huddled in its cage. Its fingers were so flexed that it could not use them for climbing on its cage. Its legs were so flexed at the knees that it could only shuffle along (fig.36).

On the 221st day it lay completely paralysed and could not move. 3 c.c. cod-liver oil daily were added to its diet but 2 days later there was no improvement, it lay helpless and was unable to feed itself. It was chloroformed.

There was marked loss of storage fat and wasting of the muscles. The large viscera were much atrophied. In the brain there was gross flattening of the convolutions of the occipital lobes. In the spinal cord there was a gelatinous formation covering the pia-arachnoid membrane, while on section the grey matter appeared definitely pink.

Experiment III.

A Rhesus monkey was fed daily on Siam polished rice, cooked by steaming and mixed with 10 gm. butter and 60 c.c. fresh milk. In addition it was given fresh fruit daily. By the 99th day it had shed some fur on its back and sat with its back bent holding its legs. It could run and jump quite well and was fairly active till the 164th day when there was a gradual change in its appearance. It became drowsy and avoided moving about. If compelled it could run, but stumbled in jumping. There was a marked lack of co-ordination of muscular movement, and any attempt at jumping resulted in it falling on the ground or landing heavily and clumsily. It was given 120 grains marmite in its food on the 169th day, but as this resulted in severe diarrhoea and nausea, its food had to be withdrawn and it was fed for 2 days on milk. On the 174th day its diet was resumed, with in addition 1 gm. marmite daily. There was marked improvement for two days, but muscular weakness again returned, and its limbs were so flexed that it could scarcely climb in its cage. It sat huddled in a corner, with drooping head and its back bent, holding its feet and hands together (fig.37). By the 180th day it was quite helpless and unable to support its own weight. It lay on its side and was found dead next day.

There was a marked absence of body fat and extensive atrophy of muscle. The viscera were healthy and in the lower bowel there was evidence of a little haemorrhage.

Sections of the cord after chromation and treatment with Osmic acid by the Marchi method showed that in all three there was myelin degeneration of the posterior nerve root fibres, both afferent and efferent. In the posterior and antero-lateral area some fibres were involved and in the medulla oblongata there were distinct areas of degeneration round the restiform body at the sensory decussation.

This group of experiments shows that the only role of vitamin-containing foods is that they may delay the onset of the disease, but that they are apparently unable to prevent it. The same signs were elicited in all three experiments, and pathological examination supports this evidence.

Experiment IV.

A Rhesus monkey was fed on 100 gm. Siam polished rice which had previously been extracted with cold water, dried on a water bath, and subsequently cooked by steaming. It was given in addition 0.125 gm. Steenboch's salt mixture daily. After 20 days its skin was very sensitive and it scratched continuously. There was flexion of the wrists, but it was quite active and well till the 49th day when it developed carpo-pedal spasms. It walked on flexed toes and fingers and there was gross flexion of both ankles and wrists, so that its grip was very poor (fig.38). It began to lose weight and became very drowsy. It did not move about much but sat huddled in its cage. After 70 days' feeding it became more active and

and moved with more agility, though there was slight lack of co-ordination evident in finer movements. The right leg was very weak. It trailed it about and did not use it. On the 212th day (5 c.c.) olive oil was added daily to its food, but there was no improvement in its condition, it was unable to climb, and both the left leg and arm were weak and almost completely paralysed. After 224 days cod-liver oil was substituted for olive oil. There was, however, no improvement and on the 245th day it lay on its side completely paralysed and unable to move. It died on the following day.

There was marked muscle wasting, and complete absence of body fat. The viscera though healthy were much atrophied. There was a gelatinous material covering the pia-arachnoid and the brain in section showed numerous punctate vessels in the corpus striatum. Examination of the tissue after treatment with potassium dichromate and Osmic Acid, after the Marchi method, indicated the absence of any degeneration in the Cortex, Corpus Striatum, Midbrain, Medulla or Cord. There were, however, signs of degeneration in the brachial plexus and sciatic nerve, where the myelin sheath was broken up and collected into droplets (fig.39).

Experiment V.

A Rhesus monkey was fed daily on a 140 gm. of a diet consisting of dextrinised rice starch 74, caseinogen 20, salt mixture (Steenboch) 4 cooked by steaming, to which was added 5 c.c. of aqueous extract of Siam polished rice equiva-

lent to 100 gm. Siam polished rice. 2 c.c. cod liver oil were given twice weekly. Fresh fruit was given daily. After 48 days it was quite active and in good condition. The amount of the solution was increased so that the equivalent of 200 gm. rice was given. The salt mixture was discontinued. There was a gradual loss in weight. On the 91st day it suddenly became very weak and could not move. There was some diarrhoea and all its limbs were flexed and it was unable to support its own weight. It lay helpless next day with arms and legs completely paralysed. It was chloroformed.

The muscles were fairly well developed and the viscera were healthy and of normal appearance.

Experiment VI.

A Rhesus monkey was fed daily on 100 gm. of a mixture of dextrinised rice starch 74 and caseinogen 20 cooked by steaming, to which was added 2 gm. dried yeast, 2 drops Haliverol and 10 c.c. aqueous extract of Siam polished rice ≡ 200 gm. Siam rice. It was also given fresh fruit daily. After 28 days' feeding it had shed some fur on its lumbar region, its toes were flexed and on exercising it was quickly exhausted. By the 63rd day there was definite weakness of the right leg which was flexed at the knee. It sat crouched in a corner and when it walked it did so on flexed toes. There was a gradual paralysis of the right leg and arm and on the 98th day it stumbled on climbing and attempts at movement resulted in it falling on its right side. There was complete paralysis of

the right side which developed so that in three days it was completely paralysed and was unable to move or control any movements. It died next day.

The viscera were quite healthy and normal in appearance. In the cord there was a little gelatinous material covering the pia-arachnoid. After treatment by the Marchi method for myelin degeneration, sections of the lumbar cord showed degeneration of Fasiculus gracilis and an involvement of the postero-lateral region. At the region of the cervical enlargement the columns of Goll and Burdoch and the postero-lateral region were involved (fig.40).

In the medulla oblongata, at the level of the pyramidal decussation, the posterior columns and their nuclei showed definite degeneration while there was some involvement of sensory fibres of the mesial fillet. The peripheral nerves showed definite myelin degeneration.

Experiment VII.

A Rhesus monkey was fed 70 gm. of a mixture of dextrinised rice starch 74, caseinogen 20, salt mixture (Steenboch) 0.5 cooked by steaming, to which was added 2 gm. dried yeast, 2 drops Haliverol. It was given fresh fruit daily. After 22 days' feeding it unfortunately developed an attack of colitis from which it died a few days later. Post-mortem examination confirmed this.

Owing to difficulty in obtaining monkeys, this experiment, which was intended to act as a control was not repeated. It is

a significant fact that the above has been generally accepted as a normal diet, and in every way satisfactory.

Experiment VIII.

A Rhesus monkey was fed daily 100 gm. unpolished Temne rice, which was cooked by steaming. It was given fresh fruit daily, generally an apple. There was definite weakness of the right wrist, and occasional coarse tremors, after 31 days' feeding. It gradually grew more drowsy and was not inclined to move unless disturbed. Its right arm became progressively weaker, until by the 73rd day its right leg was also involved. It scratched frequently and its skin seemed to be very sensitive. After 145 days it had shed almost all its fur and walked on flexed toes. It ate well, but there was a gradual decrease in its weight until by the 231st day it began to eat only 70 gm. daily. The right arm was so paralysed that there was scarcely any hand-grip, nor was it able to climb. Flexion of the right knee joint (fig.43) was so marked that it walked with a hobbling gait, which later resulted in any attempt at walking terminating in a fall to the right side. Owing to the extreme weakness of its right arm, it was unable to assist its leg in maintaining an even balance and it could only move on the left side. Complete paralysis of the right side resulted in it lying on the left side unable to stand on its feet. After a day or two no signs of recovery were elicited and it was chloroformed on the 267th day.

The viscera were quite healthy but showed definite

appearance of atrophy. Individual fibres in the cauda equina showed signs of early myelin changes (fig.41), while at the level of the cervical enlargement there was definite degeneration in the posterior columns (fig.42). In the antero-lateral region there were some degenerating fibres especially of fasiculus gracilis. In the medulla oblongata at the level of the pyramidal decussation, the posterior columns and some lateral fibres showed degeneration, while the intra-olivary tracts and arcuate fibres were also involved.

Experiment IX.

A long-tailed monkey was fed 100 gm. unpolished Sierra Leone rice, which it ate uncooked. In addition it was given fresh fruit daily. After 66 days' feeding it suddenly fell into a spasm of coarse tremors during which it lay on its back, with all the digits of its limbs in tonic contraction. It was given 0.2 c.c. Betaxin (vitamin B_1) subcutaneously, but as there was no improvement the dose was repeated 4 hours later. There was no change, the tremors indeed had increased in frequency. Next morning the animal could sit up and was able to walk rather sluggishly. By the 71st day its movements were slow and it did not move much, unless forced to do so. To maintain its daily intake the rice was occasionally cooked. On the 117th day it suddenly had a recurrence of spasm of coarse tremors during which it fell off its spar. Its digits, knee and elbow joints were flexed and it lay unable to move and completely paralysed. It died in an hour (fig.43.A).

There was no abnormality in the tissues and the large viscera were quite normal in appearance. There were small punctate haemorrhages in the stomach.

Experiment X.

A Rhesus monkey was fed 100 gm. Calcutta parboiled rice cooked by steaming. It was given fresh fruit daily. After 102 days' feeding its arms were flexed at the wrist joint and its grip was poor. It gradually decreased its intake till by the 111th day it ate only 50 gm. daily. Though it was able to jump well, it was easily exhausted and its left arm showed weakness and continuous flexion at the wrist. During the next ten days there was a remarkable improvement in its condition and it became very active and appeared quite normal. Its weight had fallen slightly, but the general condition was such that after 175 days the experiment was concluded. The animal appeared to be perfectly normal.

SUMMARY AND DISCUSSION.

On the subject of beri-beri a large amount of work has been done. To review adequately the extensive literature which has accumulated would entail a considerable amount of space which in this account is not desirable.

Beri-beri is a disease which affects rice-eaters. Where rice is the staple article of diet the incidence is high and where little rice is consumed the incidence is low.

It does not run a course like that of an infection. Its onset is frequently characterised by gastro-intestinal irritation, usually in the form of diarrhoea with nausea, while severe attacks may be accompanied by haemorrhage. The complications of the cardio-vascular system which follow are generally denoted by dyspnoea and palpitation, while oedema is marked in swelling of the feet and legs (fig.44), a fact which was at one time used to distinguish it from "dry beri-beri", in which there is no oedema. There may be hypertrophy of the right side of the heart accompanied by fatty degeneration.

Of the changes in the nervous system the most important are those disturbances of the peripheral nervous system which result in a multiple neuritis which is very variable in extent. There is anaesthesia of the skin, wasting and later a paralysis of the limbs. The myelin sheaths become degenerated and the axis cylinders become irregularly swollen and interrupted. There are degenerative changes in the cells

of the spinal cord and root ganglia.

As to the causation of beri-beri, many theories have been advanced of which the chief are (1) Vitamin deficiency and (2) Rice intoxication.

Vitamin Deficiency. In 1897 Eijkman[48] showed that an aqueous extract of rice bran possessed anti-neuritic properties. Grijns[49] and later Pol[50] prepared a decoction from Katjang idju (Phaseolus radiatus) which they stated had prophylactic and therapeutic properties in beri-beri. Their work was afterwards confirmed by Kiewiet de Tonge[51].

Fraser and Stanton[52] in a series of experiments stated that beri-beri had an intimate relation to diet and attributed the disease to an absence of a substance in rice which was soluble in alcohol. Hopkins and Funk[53] following this work isolated in a crude state a substance which was called "Vitamine". From the pericarpal layer of rice removed in milling, Veddar and Williams[54] and later Chamberlain prepared an extract of tikitiki which was found to possess curative properties in beri-beri. It consisted of an alcoholic extract of the grain concentrated in vacuo to a syrupy fluid which in turn was extracted with alcohol, centrifuged and again concentrated to a Sp.G. of 1.32, centrifuged and bottled. One unit of this extract represented 20 gms. rice polishings.

In 1927 Jansen and Donath[55] published the results of their work on rice polishings, from which they had prepared

by acid extraction and absorption on Fuller's earth, a concentrated preparation of which 1 gm. represented the vitamin from 300 gm. rice polishings.

In 1928 McCarrison[56] distinguished between what he called "polyneuritis columbarum" and "beri-beri columbarum". He considered that in the latter a toxic factor was superimposed on a pure vitamin deficiency and was brought about by a shortage though not a lack of vitamin.

From the work of Plimmer and Rosedale[57], Chick and Rosecoe[58], Aykroyd[59] and others, it was realised that the vitamin B complex seemed to contain 3 factors, namely the anti-neuritic, usually called B_1; the pellagra-preventing, known as B_2, and at least a third factor. Investigating the conditions arising out of feeding pigeons on polished rice, Carter[60] found that even if as much as 30 curative day doses of B_1 from yeast were administered, recovery did not occur, and postulated the importance of protein deficiency which he supplied by means of an extract of wheat germ[61].

J. R. O'Brien[62] found that pigeons fed on polished rice and receiving doses 6 to 12 times the daily dose of B_1, failed to recover weight even if given 2 gm. caseinogen daily. He inferred the absence of a third factor B_3.

In fact as many as 5 factors have been postulated. So Megaw[63] speaking of beri-beri and pellagra, has said with truth "perhaps vitamins have been overdone and have been saddled with an undue share in the responsibility for two very important and intriguing diseases."

Rice Intoxication. The possibility of the existence of a toxic substance in rice has, on the whole, not been explored to any extent. In 1907 Van Dieren[64] drew attention to the fact that the signs and symptoms of beri-beri resemble those of pellagra, convulsive ergotism and lathyrism, and suggested grain-intoxication as the cause. Braddon[65] and Hamilton Wright[66] stressed a toxic factor as the causative agent. Ohmori subsequently made some investigations with a cold alcoholic extract of rice and Acton and Chopra[67] published some work on the alcoholic - and water-soluble toxins of damaged rice. Teru Uchi and his collaborators, as a result of extensive animal experiments, advanced evidence of "oryzatoxin" in rice.

By some it was considered that disturbance of lipoid metabolism was responsible for the symptoms of the disease, while Langen concluded that they were due to the lack of a substance necessary for the synthesis of the phosphatides. The importance of the part played by phosphorus was realised by Veddar and Feliciano[68] who pointed out that this was largely removed in the milling of the grain. They found that beri-beri could be prevented when the rice consumed had a P_2O_5 value of 0.50% provided there was at least 75% external layer of the grain remaining. The Committee on beri-beri in the Philippine Islands in its Report[69] adopted this as one of the chemical standards for beri-beri-preventing rice.

While Fraser and Stanton[70] recognised the phosphorus pentoxide standard as an indicator of the extent to which rice had been milled, they did not suggest that a deficiency of phosphorus in organic combination accounted for the occurrence of beri-beri. In early experiments they had found that when paddy was heated in an autoclave at 120°C for an hour and fed to fowls it caused polyneuritis, but when paddy was steeped in water and subsequently treated in the same fashion the fowls remained healthy. While they suggested that the sterilisation of paddy resulted in the destruction of the physiological activity of the protective substances they made no other comment except that the immersion in water seemed to negative the destructive effect of an atmosphere of steam, an effect which they attributed to the "consideration of other physical conditions". They also observed that the parboiling of rice before milling only served the purpose of so hardening the outer layers of the grain that their removal was less easy.

In 1907 Fletcher[71] commenting on human experiments which he had conducted at Kuala Lumpur, concluded that "white polished rice is a cause of beri-beri, acting either by some poison which it contains or by a starvation due to some defect in the nutritive value of such rice". When he used parboiled rice all the patients remained well but two, both of whom were suffering from the disease before the experiment was started.

To follow p. 47.
(Binder's error)
AW.

STUDIES IN HORDEUM DISTICHON.

I have shown (page17) that the effect of steeping paddy in water and of parboiling rice is to induce hydrolysis of the organic phosphorus, so that the resulting phosphorus in the diet is assimilated more readily and that the organic phosphoric compounds which are toxic, are decomposed and rendered non-toxic.

From the experiments on animals (pages 23 to 29) it is seen that when given parenterally, the organic compounds of phosphorus isolated from rice are toxic, and produce a marked effect on the brain and spinal cord. When administered orally, their action is variable, depending on whether they are decomposed in the alimentary tract. If cleavage results, there is only diarrhoea produced, while if they are absorbed into the blood stream without change, they exert a marked effect on the nervous system.

The question of cleavage of the organic phosphates seems to be the determining factor in the utilisation of the phosphorus of the diet. There is no evidence as to the extent of hydrolysis necessary, as the isolation of the different lower esters of inositol with phosphoric acid, is at the moment not possible.

In preliminary work which I carried out on animals with the crude enzyme preparation I was not successful in demonstrating the effects which I was seeking, nevertheless more recent reports by Contardi[72] and by Belfanti[73] indicate that an enzyme phosphatase which they isolated from rice bran

by a method similar to that for isolating vitamin B_1, had an effect on the organic phosphorus compounds. They stated that all vitamin B substances contain these ferments.

Horiuchi[74] and Arnaudi and Francioli[75] have shown that phosphatase from rice bran and cultures of Aspergillus Niger and A. Oryzae grown on glucose, were able to decompose and to synthesise the hexosephosphates.

To isolate the various esters of phosphoric acid with inisotol and determine their respective properties chemically and pharmacologically is beyond the bounds of my present investigations. I am convinced, however, that the solution to the problem of beri-beri is to be found in the role of phosphorus in the diet. The present practice of preventing beriberi and similar diseases with vitamin preparations is in essence the supplying of a means of metabolising phosphorus. With the evidence that I have produced, it is reasonable to conclude that the organic phosphorus compounds which in themselves are storage products of the rice grain, are capable of being hydrolysed to simpler substances by means of an enzyme in the pericarp of the grain. In the absence of the enzyme, the organic phosphorus cannot be decomposed to any extent and when absorbed is poisonous. Furthermore, the phosphorus does not become available for assimilation, so that a disordered metabolism results.

Where rice forms the staple article of diet, and such conditions prevail the process of nutrition becomes abnormal and a clinical syndrome resembling beri-beri is produced.

Barley is one of the most ancient aliments of man. It was cultivated in Egypt as long ago as 1500 B.C.[76] The Greeks preferred barley to anything else for making polenta and it was called κριθή from the Greek διακριθῆναι (to separate out) because[77] when it was first used, the delicate kernels were separated out.

The name "hordearii" or "barley-fed" was given to the gladiators who ate it. Hippocrates mentions three kinds of barley[78] and Theophrastus[79] states that in Asia, it was used for making "sweet bread and good porridge." The Gauls according to Pliny[80] prepared from it a porridge called "orge monde."

Its uses for brewing were known to the ancient Saxons who called it "bere", while in old High German it is "prior", in Norse "eolo" and in Anglo-Saxon "ale" and "beer".

The cultivated types of barley appear to have arisen from a wild form of barley - Hordeum sponteneum - which is still found growing in some parts of Western Asia. Its cultivation now reaches over a remarkably extended range, for of all the cereals, barley is the most tolerant of climate. It is grown in Norway and in Lapland; in northern Canada and is extensively cultivated throughout Europe, Central Asia and North Africa.

The barley plant Hordeum distichon is a member of the tribe Hordeae of the Natural Order Gramineae. It is an annual plant with several smooth stems, two to three feet in height, and has few leaves. The spikelets which are sessile

and one-flowered, occur in groups of three alternating on opposite sides of the jointed rachis. The types of barley are classified according to the number of fertile spikelets.

In the two-rowed variety - Hordeum distichon, the middle spikelet only is fertile, while in Hordeum hexastichon, the six-rowed variety, all three spikelets are fertile (fig.45).

Each spikelet has two slender glumes, one of which - the flowering glume, is five-ribbed, the central rib being prolonged as a long awn or "beard". The two-keeled palet is closely clasped by the edges of the flowering glume (fig.46) and there is a broad groove which lies beneath the palet. The fruit, a caryopsis, is enclosed in, and adherent to, the paleae. On the dorsal side lies the embryo extending about one third of the distance to the apex.

Microscopic Structure.

Sections of the grain were cut and stained according to the methods already described on page 6. A cross-section of the grain (fig.47) shows the following constituents.

1. The flowering glume which consists of

   (a) an outer epidermis of wavy-walled cells which may be crescent shaped or oval.

   (b) Sclerenchymatous fibres with thick and thin walls.

   (c) A spongy parenchyma of quadrilateral or square cells.

   (d) An inner epidermis of large polygonal cells with hairs and stomata.

2. The pericarp consisting of

(a) an epicarp and hypoderm of polygonal cells;

(b) transversely elongated cross-cells;

(c) an endocarp of tube cells.

3. The spermoderm of two layers of elongated cells which stain yellow with chlor-zinc iodine

4. The perisperm of wavy and thick walled cells which stain blue with chlor-zinc iodine.

5. The endosperm which consists of

(a) an aleurone layer of two to four rows of cubical cells with darkly-staining granular contents.

(b) Starch cells with thin walls and packed with starch grains. Owing to pressure by the starch grains the nuclei of the cells are feeble and ill-defined. The embryo lies at the proximal end of the endosperm, partly embedded in its tissue.

The Milling of Barley.

In most cereals the caryopsis is freed from the glumes or floral leaves by threshing, but in barley this is not the case, except in the case of Hordeum nudum - naked barley - where the glumes are readily separated.

During the milling of the grain various layers are removed and the chemical composition of the grain is accordingly altered.

A diagrammatic sketch of the milling process is shown on fig.48. The various products of milling are shown in fig.48.A.

After the separation of the grain from the rest of the plant by the thresher, the barley is "cleaned" in a series of wire meshes and is freed from all foreign grain such as oats, wheat, cockle, etc..

In the next process - blocking - the flow of barley is directed between a revolving drum and wire mesh, by means of which the husk and a varying proportion of the pericarp are removed. The outflowing grain is termed "Blocked" or "First-run" barley. It is light brown to yellow in appearance and a cross-section (fig.49) indicates that while the husk and the outer layers of the pericarp have been removed the endocarp with the spermoderm and perisperm is still intact.

The product of the reel is termed coarse-dust and is used mainly as fuel.

The blocked barley is "damped" and allowed to lie for not less than 24 hours. The extent to which moisture is added depends on the natural moisture of the grain; the moisture content being raised usually to 18.5%.

Damping toughens the barley and obviates the danger of it being broken in the subsequent stages of milling, which, as explained above, are of necessity severe frictional processes. Further frictional action produces "Second-run" barley. If Scotch grain is used, this is called Pot-barley. Microscopic examination of Second-run barley (fig.50) shows that while fragments of the endocarp are distinguishable, the spermoderm and perisperm have been removed to a variable extent. The

endosperm is complete with all layers of aleurone cells.

The dust from the reel is termed "Fine-dust.

By further "rubbing down", "Third-run" barley is produced. It is in appearance almost white, though there are streaks of yellow material adherent to its surface. In fig.51 it will be seen that there are traces of the perisperm, but for the most part this has been removed; and at some zones the aleurone layer has been disturbed.

Extra-fine dust is obtained from the reel and is used as a component in some baking flours.

To produce Pearl barley, more frictional action is applied, and the grain produced is very much smaller in size and whiter in appearance. The purpose of producing a fine pearl barley seems to be that of procuring a grain which is white in appearance and in which there is no trace of the median raphe apparent. This results in "rubbing down" the barley till only the endosperm is left and as figs 52 and 53 indicate, variable amounts of aleurone cells remain.

Effects of Milling.

Comparison of the chemical analyses of the various products (fig.54) shows that while there is a gradual decrease in ash value, protein and fibre content, there is an increased amount of carbohydrate as the milling proceeds.

Chemical Constituents.

Protein. Following the work of Kreusler[81], Osborne[82] classified the proteins of barley. The chief proteins are hordein, which is soluble in alcohol, and an insoluble protein which has not yet been investigated. Leucosin and edestin have also been isolated and Csonka and Jones[83] have detected an α and β glutelin.

Fat. The chief fatty acids in the separated fat are oleic acid and linolic acid. Taufel and Rusch[84] have reported the presence of stearic, palmitic and linolenic acids in the fats which they examined.

Carbohydrates. While starch is the main constituent, sucrose and raffinose are present to a small extent. The pentosans araban and xylan have been detected by Le Clere and Wahl, and Lintner has also shown the presence of galactoxylan.

Mineral Constituents. An analysis of the ash gave the following values for the dry grain -

Unmilled barley gm. in 100 gm. (moisture free)				
K_2O	CaO	MgO	P_2O_5	S
0.71	0.08	0.21	1.04	0.16

Enzymes.

Many enzymes have been ascribed to barley. In the germinating plant Maestrini[85] was able to detect amylase,

invertase, lipase, protease, catalase, and oxidase. Most of the investigations on enzyme activity have been directed to the study of diastase and its function in the process of malting. Krabbe assigned all diastase secretion to the individual cells of the endosperm, while Hansteen, Purnevitch and Linz stated that the aleurone layer did not secrete diastase. All were agreed that the aleurone layer is made up of highly vital cells, which persist until the starch endosperm has been completely absorbed.

Chemical Investigation.

Preliminary work carried out on extracts of barley indicated that the role of phosphorus resembled that in rice. Accordingly investigations were directed to the isolation of the organic phosphoric compounds from the various milled products of Barley.

The method of extraction and isolation was the same as that used in rice and is described on pages 19 and 20.

The following summary may be useful for immediate reference.

Grain crushed. Macerated with 1% HCl, then percolated.

Percolate measured. $Na\bar{A}$ 29.1 gm./litre added.

Filtrate.
30% $Pb\bar{A}$ added.

Precipitate.
Washed with water. Suspended in water. H_2S passed.

Filtrate.
Conc. in vacuo to thin syrup. Poured slowly into absolute alcohol. Filtered.

Precipitate.
Washed with abs. alcohol.
Dried in vacuo over $CaCl_2$.
Dissolved in water.
Poured into abs. alcohol.
Filtered.

Precipitate.
Washed with abs. alcohol.
Dried in vacuo over $CaCl_2$.
Weighed.
A.I.Pb.

Filtrate.
Alcohol recovered.

Filtrate.
Alcohol recovered.

Precipitate
PbS discarded

The percentage yields are shown in fig.55 and were obtained from the extractions of 1 kg. of grain.

The Alcohol Insoluble Lead Precipitate (A.I.Pb) when dried in vacuo was an amorphous white powder which was readily soluble in water and acid in reaction. It yielded reactionsfor calcium, magnesium and phosphorus. When heated in an autoclave at 150°C in a sealed tube with dilute H_2SO_4 for 6 hours, cooled, treated with baryta and filtered, the filtrate, when concentrated and set aside to crystallise, gave the reactions of inositol.

An aqueous solution of A.I.Pb formed dense precipitates with lead acetate and copper acetate. The neutral sodium salt was obtained by precipitating a solution, neutralised with NaOH, with alcohol. The free acid was obtained by degradation of the lead salt with H_2S, and concentration of the filtrate in vacuo. It was a thin clear syrup and formed two series of salts with the alkaline earth and heavy metals. The acid salts were freely soluble in water, while the double salts were soluble only in an acid medium.

ACTION ON ANIMALS.

The pharmacological action of A.I.Pb was found to be similar to the A.I.Pb of rice.

Parenteral administration to rabbits indicated that the intra-venous route was much more rapid and often reached a fatal termination in a short time. Hypodermic injection produced marked depression of the brain and spinal cord followed by increased reflexes.

In frogs doses of 0.02-0.05 gm. given hypodermically caused depression and often paralysis of the brain and spinal cord for 12-24 hours. Increased reflexes persisted for several days.

When given by mouth the action was variable and depended on the extent to which the esters were decomposed. In doses of 5-8 gm. there was drowsiness and marked depression in rabbits.

ANIMAL FEEDING EXPERIMENTS.

Feeding experiments were made with the various products of barley milling; the experiments with pigeons were conducted under the same conditions as described under rice.

FEEDING EXPERIMENTS WITH PIGEONS.

Experiment I. (Figs. 56 and 56A).

After 175 days, pigeons fed on 1st Run barley remained quite normal and gained weight.

This is in agreement with the results obtained from feeding unmilled rice.

Experiment II. (Figs. 57 and 57A).

On a diet of 2nd Run barley pigeons developed typical signs of polyneuritis after 60-70 days' feeding. In all cases there was an initial rise in weight followed by a gradual decline.

Experiment III. (Figs. 58 and 58A).

Feeding on a diet of 3rd Run barley pigeons showed signs of weakness and ataxia after 20-25 days. This condition gradually progressed and after 10 days' further feeding there was evidence of muscular weakness, and definite paresis. In some cases spasms of uncontrolled movement developed very suddenly and the pigeons were unable to control the direction or the extent of movement. While there was no evidence of head retraction, the falling forward of the head (emprosthotonos) was typical of pigeons fed on polished rice.

Experiment IV. (Figs. 59 and 59A).

On a diet of highly milled (4th Run) barley pigeons developed in from 15-22 days the signs characteristic of

polyneuritis. There was weakness of the legs and the gait was quite unsteady. Marked loss of extensor tone was evidenced in the flexion of the toes, which was a characteristic sign (fig.60). The movements of the pigeons were quite incoordinated and uncontrolled. The head and neck muscles later became involved resulting in either emprosthotonic or opisthotonic spasms (fig.61). Histological examination of the sciatic nerve showed myelin granulation and clumping of the fibres (fig.61.A).

Experiment V. (Fig.62).

The barley dust obtained from the milling of 2nd Run barley was mixed into a mash with water and fed by mouth, to pigeons which had developed the typical signs of polyneuritis as a result of feeding on 3rd Run barley.

After 2 doses of 5 gm. there was an improvement in their general condition. The legs became steadier and flight was restored (figs.63 and 63.A). In two days the pigeons appeared to be quire normal and there was no evidence of any neuritic condition. The pigeons steadily gained weight, which was maintained for as much as 12 days, when the experiment was concluded.

Experiment VI. (Figs. 64 and 64.A).

Pigeons which had become polyneuritic on a diet of 4th Run barley were fed dried yeast per crop as curative treatment. In doses of 1 gm. daily there was no definite improvement

in the condition. When 2 gm. daily were given a gradual improvement in general condition followed and they gained weight. When given 2 gm. daily as a prophylactic treatment, the pigeons remained well and quite normal and gained weight.

Experiment VII. (Figs. 65 and 65.A).

As a source of vitamin B complex, marmite was tried as curative treatment for pigeons which had developed polyneuritis on a diet of 4th Run barley. In doses of 1 gm. by the crop there was a variable response. If given continuously after the first dose, there was a gradual improvement with restoration of weight, but if treatment was discontinued the neuritic condition was manifested in a day. In prophylactic doses of 1 gm. daily the pigeons remained normal and maintained their weight.

Experiment VIII. (Figs. 66 and 66.A,B).

Following upon the feeding experiments with rice, the effect of supplying phosphorus in inorganic form, was studied. Pigeons on a diet of 4th Run barley were given by the crop, in addition, 0.33 gm. sodium phosphate ($Na_2HPO_4.12H_2O$) in 3 c.c. water daily.

There was a prolongation of the normal condition, but the addition of inorganic phosphorus to the diet failed to prevent the onset of polyneuritis, though there were no cases of the head and neck muscles being affected. There was also a gradual decline in weight, so that supplying inorganic

phosphorus does not directly, of itself, influence the diet of highly milled barley.

On a diet of 2nd Run barley with 0.33 gm. sodium phosphate as a supplementary diet, the appearance of the neuritic condition was delayed for at least 128 days. The pigeons remained in good condition till a few days before the onset. The addition of inorganic phosphorus to the diet would seem to indicate that a deficiency of phosphorus was a causative factor in polyneuritis.

DISCUSSION.

The feeding of pigeons on barley has shown that where there has been no removal of the pericarp the pigeons have remained normal and gained weight.

Where there is disturbance of the pericarp and sub-pericarp layers in the grain, the development of polyneuritis has followed the feeding of the milled grain. It is interesting to note that in 2nd Run barley, the aleurone layer is intact, yet the presence of the aleurone cells does not ensure protection, as was suggested by Fraser, in his experiments with rice.

Pigeons fed on 4th Run barley where the aleurone layer has been removed to a variable extent, became polyneuritic in from 15 to 22 days.

The curative effect of administration of the pericarp and sub-pericarp layers has been demonstrated.

The prophylactic value of yeast and marmite in the treatment of polyneuritis of barley and the limited use of these substances in curative dose, has been pointed out.

The addition of inorganic phosphate to the diet delayed but did not eventually prevent the onset of polyneuritis.

It has long been recognised that cereals have a harmful effect upon bone formation. As long ago as 1650 osseous cachexia was known in Norway and was attributed to a faulty diet. The causative factor was attributed to a plant[86] which

was at that time greatly used.

Cantigetana Brissonet[87] still later in 1846 showed that where the soil contained less than 1,500 kg. of phosphoric acid to the hectare, osseous cachexia was almost permanently present. When this ratio of phosphoric acid was raised beyond 2,000 kg. the cachexia finally disappeared.

For more than half a century oats have been condemned as a diet on account of the presence of an acid substance which was capable of causing demineralisation of bone. As a result of investigations on rabbits Weiske attributed the harmful effects of oats to an acid substance, and not to a deficiency of calcium.

From some general experiments Burnett[88] found a very wide variation in the mineral content of bones as a result of altering the composition of the food. Experimenting with diets of wheat on pigeons, Chossat[89] found that salts were gradually withdrawn from the bones which gradually became weak, and were liable to spontaneous fracture.

More recently Sherman and Pappenheimer[90] attributed the changes in bones, occurring in rickets, to a deficiency of phosphorus in the diet, and cured the condition by the administration of potassium phosphate. This conclusion was supported by many other investigators.

Investigating the effects of cereals on bone-calcification, Green and Mellanby[91] found that no ill effects resulted when the diets were rich in vitamin D.

It has been suggested[92] that one of the functions of vitamin D appears to be to increase the absorption of inorganic elements from the alimentary canal. When there is a lack of vitamin D a sufficiency of these elements is not absorbed and presented to the tissues requiring them for development.

FEEDING EXPERIMENTS WITH RABBITS.

Experiments were conducted to study the effects of feeding rabbits on a diet of unmilled barley, and to detect what changes, if any, could be observed in the bones of young growing animals, as a result of the diet.

Each group of animals was kept in a wire cage, and provided with fresh water daily. The animals were allowed to run about on a concrete floor, for a short period every day.

Method of Preparation of Bone for Analysis.

The femur, tibia and fibula from both hind legs of the dead animals were X-ray-photographed, after removal of most of the soft tissue. The bones were allowed to dry for three days, and after all connective tissue had been removed, were broken into fragments in a mortar.

Defatting.

By refluxing in flasks, twice for three hours with absolute alcohol, and twice for three hours with methylated ether, all the fat was removed and the bones were dried to constant weight.

Ashing.

The bones were pulverised in a hand-mill and aliquot portions were weighed into Rose crucibles. Ashing was carried out in a combustion furnace. During the first half-hour the temperature was kept about 70°C, thereafter the heating was

continued at a high temperature till the bone ash was of constant weight. The time required for ashing was generally from four to six hours.

Method of Estimation of Calcium in Bone Ash.

Portions of about 0.12 gm. of bone ash were weighed to the 4th place of decimals. The ash was dissolved in 4 mils. HCl.; the solution was diluted to 50 mils. with distilled water and heated to about 90°C. 10 mils. Oxalic acid reagent and 3 drops methyl red were added. From a burette dilute ammonium hydroxide was added till the solution was yellow. The precipitate was allowed to settle overnight. The supernatent liquid was decanted through a Whatman No.44 filter-paper. The precipitate was washed with 60 mils. portions of distilled water containing a trace of ammonia, till the filtrate yielded no cloudiness with calcium chloride solution. The filter-paper was washed with hot dilute sulphuric acid, and the filtrate served to dissolve the precipitate in the beaker. The resulting solution was heated to 70°C, and titrated with decinormal potassium permanganate. The calcium content was expressed as a percentage of calcium oxide.

Oxalic Acid Reagent.

Oxalic Acid 34.0 gm.

Distilled Water to 1000.0 mils.

Dissolved and filtered.

10 mils. of this solution, equivalent to 0.34 gm. Oxalic Acid were used in each estimation.

Method of Estimation of Phosphorus in Bone Ash.

Aliquot portions of 0.05-0.06 gm. of bone ash were weighed to the 4th decimal place and dissolved in 5 mils HNO_3. 70 mils. distilled water and 10 gm. NH_4NO_3 were added. The solution was heated to 65°C and 35 mils. molybdate Reagent were run in a slow stream, from a burette. The resulting precipitate was set on a water-bath at 65°C for 15 minutes. It was then allowed to cool for 15 minutes and filtered through a Gooch crucible under slight suction. The precipitate was washed and further treated as described in pages 13 and 14.

Bone Analysis of Normal Animals.

From an analysis of the bones of normal animals Alstead[101] has shown that with advancing age there is a gradual increase in the proportion of inorganic matter. Whereas in the first month the bone ash is about 45%, at the age of 12 months and over the value ranges from 55%-60%. There does not appear to be much change in the composition of the ash however, in respect of phosphorus and calcium, and despite the variation in bone ash value, the phosphorus content is about 45% P_2O_5 while that of calcium is approximately 50-55% CaO.

Experiment I (Fig.67).

In Group A, 5 young rabbits of the same litter were fed daily on unmilled barley which had been crushed in a mill. 2 received in addition 1 gm. dried yeast daily.

In group B, 6 young rabbits of the same litter were fed daily on unmilled barley which had previously been autoclaved in alcohol at $1\frac{1}{2}$ atmospheres for 2 hours. 2 received in addition 1 gm. dried yeast daily.

The animals in each group ate well and gained weight. In both groups about a week before death the animals became weak and were not inclined to move about. The hind limbs became stiff and inco-ordinate and later quite paralysed. When placed on their side they were unable to regain their normal position. They died generally 2 days after the onset of paralysis.

Experiment II (Fig.68).

In group A, 3 young rabbits of the same litter were fed daily on crushed, unmilled barley with 1 c.c. fresh lemon juice and 3 drops Haliverol each in addition.

In Group B, 3 young rabbits of the same litter were fed on the same diet only in place of Haliverol, each was given 1 c.c. cod-liver oil.

All the animals ate well but gradually lost weight. After 3-4 weeks' feeding they became weak and showed evidence of inco-ordination in their movements. In some the paralysis first affected the fore limbs (fig.70) which were splayed out

and were unable to support the weight of the body. The hind limbs later became involved and the animals died quite paralysed.

Experiment III (Fig.69).

In group A, 4 young rabbits of the same litter were each fed daily on crushed, unmilled barley, with 1 c.c. fresh lemon juice, 3 drops Haliverol and 0.33 gm. Sodium phosphate ($Na_2HPO_4.12H_2O$).

In group B, 4 young rabbits of the same litter were fed the same diet with 1 gm. Marmite each in addition.

The animals ate well and gained in weight.

In group A, after 9-11 weeks' feeding, weakness and inco-ordination of the hind limbs was followed by paralysis of both front and hind limbs. The animals were quite unable to move and died two days after paralysis was manifested.

In group B the first indications of paralysis were noted after about 8-9 weeks' feeding and the typical sequal of events terminated in death.

DISCUSSION.

Radiographic examination of the bones after 3 weeks' feeding on different diets showed that the shadows were denser in the bones of those animals which received cod-liver oil and Haliverol; while in the case of the animal receiving no supplementary diet there was evidence of restricted ossification. Chemical analysis denoted a variation in the ash value, but the composition of the ash with regard to Ca and P appeared to vary only to a slight degree.

Similar examination of bones after 5,9 and 21 weeks of experiment indicated a variation in ash value, but a remarkable consistency in composition. Radiographic examination did not reveal any gross interference with ossification (figs. 71,72,73 and 74).

Studying the effect of the influence of inorganic phosphate on the rate of alcoholic fermentation, Harden[93] expressed his findings according to the equations -

1. $2C_6H_{12}O_6 + 2PO_4HR_2 = 2C_6H_{11}O_5PO_4R_2 + 2H_2O$

2. $C_6H_{12}O_6 + 2PO_4HR_2 = C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O$.

Equation 1 represents the formation of hexosemonophosphate and 2, the formation of hexosediphosphate. By the action of the phosphatase in yeast these esters are hydrolised to the hexose and phosphoric acid.

$$C_6H_{11}O_5PO_4R_2 + H_2O = C_6H_{12}O_6 + PO_4HR_2$$

$$C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2 .$$

Applying this principle of hydrolysis in a study of bone phosphatase Robison[94] found that glucose - or fructose - monophosphate were hydrolysed by bone phosphatase to glucose or fructose and inorganic phosphate.

Embden[95] and later Pryde and Waters[96] isolated from the muscle juice of rabbits and other animals hexosemonophosphoric acid which, it was suggested, was identical with the glucose-monophosphate obtained from the products of fermentation by yeast. Working along similar lines the Eggletons[97] isolated a derivative of creatine which they termed phosphagen, $PO(OH)_2.NH.C:NH\ N.CH_3.CH.COOH$. Later Meyerhoff[98] studying the relation of phosphagen to lactic acid formation showed that the phosphate liberated from the hydrolysis of phosphagen combines with glycogen or hexoses to form hexosephosphoric esters.

Extending the results of these researches to the study of the formation of bone Robison[99] in some preliminary work found that bone contains a very active phosphatase capable of effecting hydrolysis of hexosephosphates and other phosphoric esters. He concluded that in addition to inorganic phosphate, blood contains a phosphoric ester whose calcium salt is soluble. In the presence of the enzyme phosphatase this ester is

hydrolysed, setting free inorganic phosphate whereby the conc. of PO_4''' ions is increased. The product of the concentrations of PO_4''' and Ca^{++} ions then exceeds the solubility product of calcium phosphate which is deposited in the organic matrix of the tissue. He found[100] that this ester was a very important factor in calcification and that in its presence calcification was obtained even at low levels of calcium and inorganic phosphate.

From my experiments with autoclaved barley it has been shown that a drop in the calcium content of the bone has resulted. When yeast was given, the calcium content was raised. The phosphatase of yeast hydrolysed the phosphoric esters to provide the necessary compounds for calcification. The total phosphorus content of bone does not appear to be affected to any considerable extent.

Supplementing the diet of unmilled barley with cod-liver oil or concentrates of vitamins A and D does not appear to influence the deposition of calcium or of phosphorus, and certainly does not maintain the animals in normal condition.

In view of the nature of the gross changes which would be required to produce marked variations in the composition of bone, it was realised that such analytical methods as were used in these experiments could not present a true picture. Indeed a review of the current literature failed to reveal the existence of any methods which might be used to detect the various stages in ossifying bone. This aspect of my research was accordingly abandoned till a more extensive investigation could be undertaken.

CONCLUSION.

From a systematic study of the structure and composition of the cereals, rice and barley, the effect of milling on these grains has been discussed. The far-reaching results on metabolism of a diet rich in these cereals have been demonstrated by experiments on animals. It has been shown that with such diets a toxic degeneration of the nervous system is produced. The significance of other essential substances in the diet is also discussed. While barley does not monopolise the diet of any European country its dietetic relation to rice has been pointed out.

That the subject is one of practical as well as of scientific interest is illustrated by the fact that at the time of going to press, a movement has originated in the United States of America[102] to encourage the consumption of parboiled rice. The reasons given are that not only is this a more remunerative method of milling rice, but it also results in a more highly nutritious product. It is suggested that this movement might be extended to Great Britain with equally beneficial results.

The significance of a cereal diet and its relationship to the structure and function of the body as a whole and in particular of the nervous system has been clearly demonstrated, and while this research is not in itself conclusive it has

established evidence of the importance of the phosphorus compounds in cereals and of the intimate part which they play in the processes of nutrition.

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THE INVESTIGATION OF POISONS IN PULSES AND CEREALS.

VOLUME II.

Fig. 1. Panicles of rice plant.

Fig. 2. Swamp-rice fields.

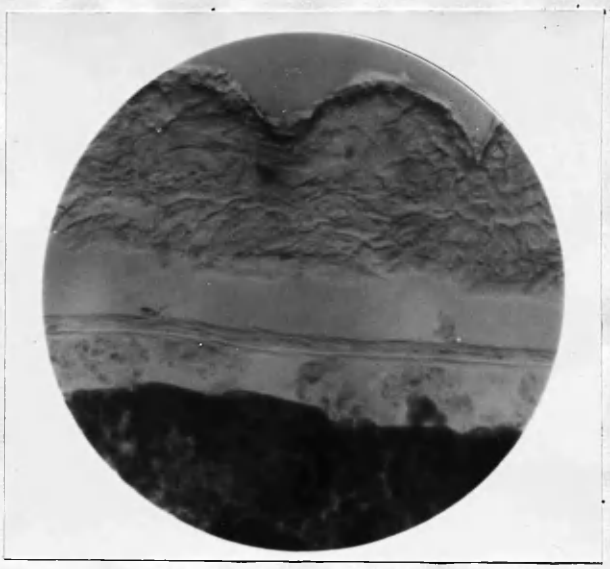


Fig. 3. - transverse section of unmilled rice (paddy).

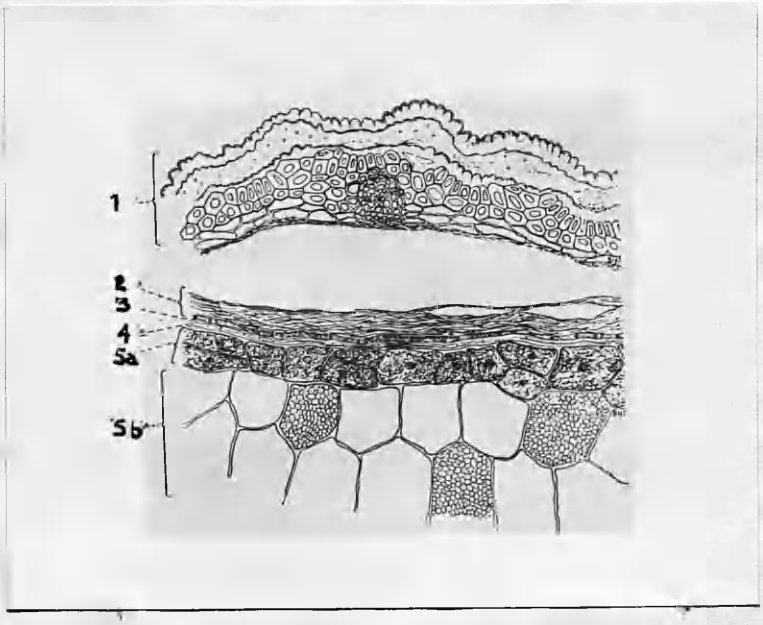


Fig. 4 - transverse section of unmilled rice (paddy) - Diagrammatic.

1. Husk. 2. Pericarp. 3. Spermoderm.
4. Perisperm. 5a. Aleurone layer.
5b. Starch Cells.

Fig. 5 - Native pounding of grain.

Fig. 6 - old type of wooden mortar and pestle.

Fig. 7 - Winnowing grain.

Fig. 8 - Transverse section of native milled grain.

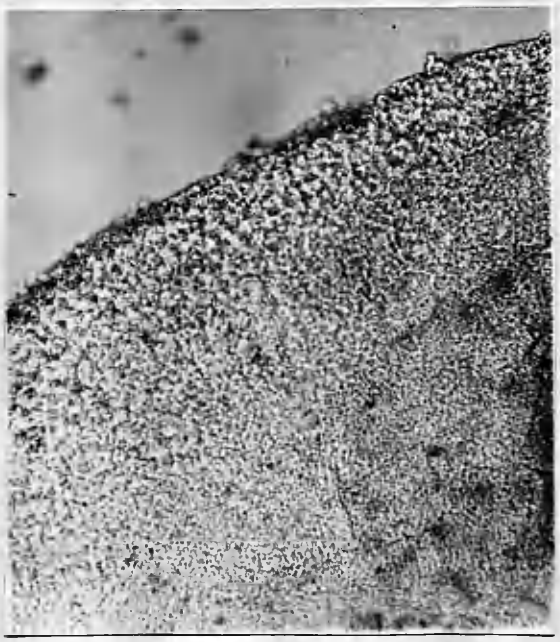


Fig. 9 - Transverse section of highly milled rice (European method).

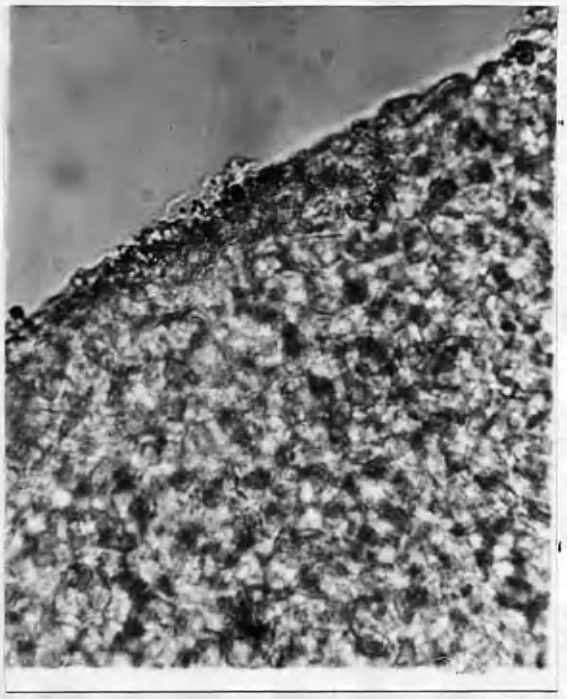


Fig.10 - Transverse section of highly milled rice (European method).

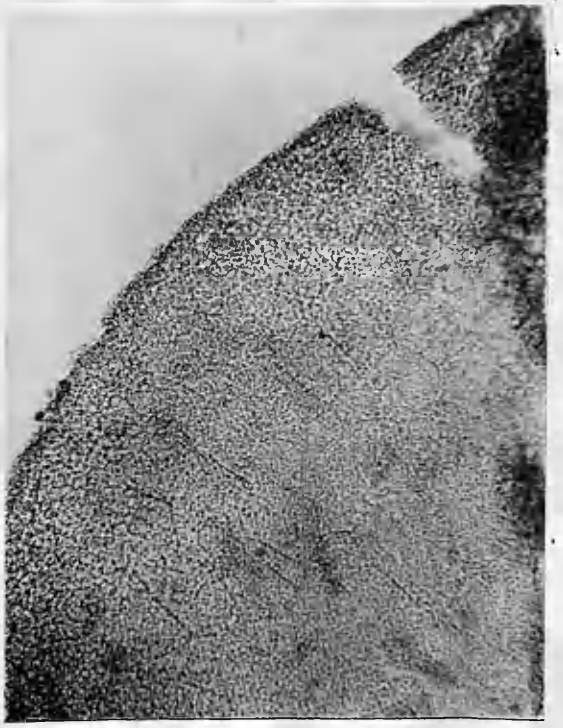


Fig.11 - Transverse section Calcutta parboiled rice.

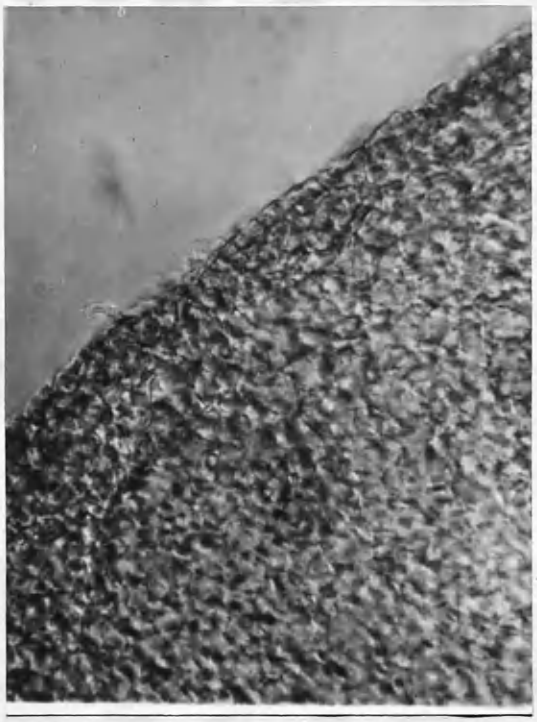
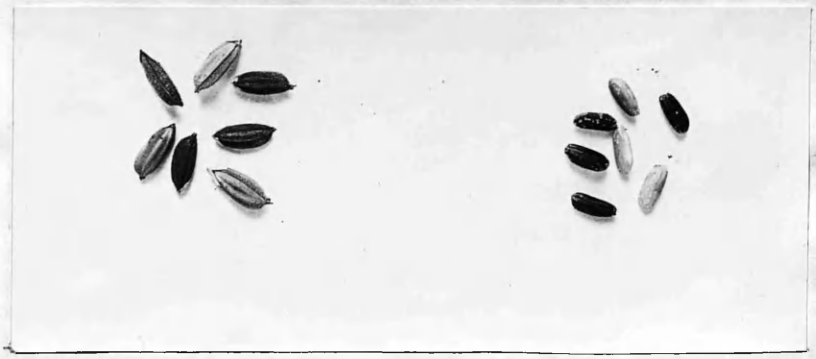


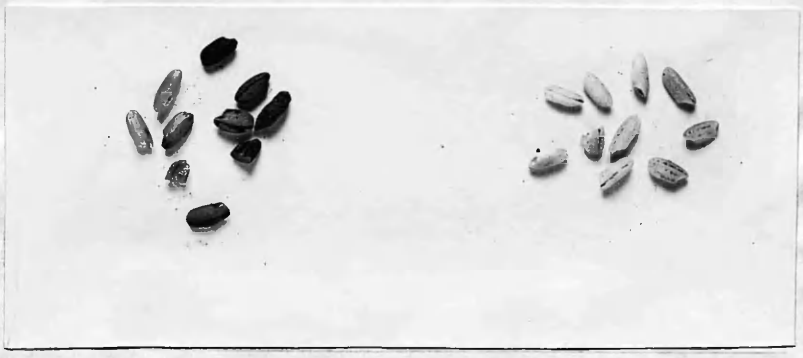
Fig.12 - Transverse section Calcutta parboiled rice.

	Constituents per cent. moisture free			
Sample analysed	Ash	Crude Fibre	Oil	Protein
Paddy Rice	6.08	9.79	1.78	8.43
Native 1st pounded	2.72	2.91	2.85	9.29
" 2nd "	0.94	0.83	1.20	8.87
European "huller product"	0.61	0.44	0.46	8.91
" "polisher product"	0.41	0.34	0.28	9.15

Fig.13 - Analyses of Native and European milled rice.



Paddy. Native Milled.



Native Parboiled Milled. European Milled.

European Parboiled Milled. Siam polished.

Fig.13.A. Naked Eye Appearance of Milled Rice.

gm. (per cent.)					
Sample analysed	Protein	Fat	Carbohydrate	Ash	P_2O_5
Unpolished rice.	10.3	1.89	86.58	1.23	0.64
Polished rice.	10.0	0.25	89.05	0.70	0.30
Parboiled rice.	8.7	0.51	89.79	1.00	0.49

Fig.14. Analysis of rice (Fraser & Stanton).

Sample analysed	K_2O	CaO	MgO	P_2O_5	Ash
Unpolished rice	0.25	0.09	0.14	0.63	0.16
Polished rice	0.09	0.05	0.08	0.27	0.08

Fig.15. Analysis of ash of rice (Frapps).

Grain	Extracting Medium.	Mgm.P_2O_5% Inorganic	Total	% Inorg./ Total.
Paddy rice	Cold Water	50.20	95.94	52.32

Fig.16. Relation of inorganic to total phosphorus in paddy rice.

Grain	Extracting Medium.	Mgm.P_2O_5% Inorganic	Total	% Inorg./ Total.
Untreated paddy rice.	Cold Water	50.20	95.94	52.32
Untreated " "	1% HCl	30.57	250.0	12.23
Autoclaved " "	Cold Water	0.0	100.3	0.0
Autoclaved " "	1% HCl	4.91	252.0	1.95

Fig.17. The relation between enzyme action and inorganic/total phosphorus in paddy rice.

Grain	Extracting Medium.	Mgm. P_2O_5% Inorganic	Total	% Inorg./ Total.
Siam polished rice	Cold Water	1.75	32.70	5.35
Siam polished autocl.rice.	Cold Water	0.0	37.55	0.0
Siam polished rice.	1% HCl	0.0	81.43	0.0
Siam polished autocl.rice	1% HCl	0.0	63.56	0.0

Fig.18. The relation between enzyme action and inorganic/total phosphorus in Siam polished rice.

Grain	Extracting Medium	Mgm. P_2O_5% Inorganic	Total	% Inorg./ Total
Untreated Parboiled Paddy	1% HCl	48.05	174.90	27.48
Autoclaved Parboiled Paddy	1% HCl	18.42	190.90	9.65
Untreated Paddy	1% HCl	30.57	250.0	12.23

Fig.19. The effect of parboiling on the distribution of phosphorus in paddy rice.

Grain	Extracting Medium.	Mgm. P_2O_5% Inorganic	Total	% Inorg./ Total.
Rangoon Parboiled	Water	8.64	31.30	27.61
Rangoon Parboiled	1% HCl	8.89	34.61	25.68

Fig.20. The distribution of phosphorus in highly milled parboiled rice.

Grain	Amount of Grain	Wt. in gm. A.I.Pb pptn.	Yield gm.%
Paddy	500 gm.	0.27	0.054
Unpolished Temne	500 gm.	0.60	0.120
Unpolished Walah	500 gm.	0.53	0.106
Polished Siam	500 gm.	0.25	0.050
Polished Temne	500 gm.	0.15	0.030
Polished Walah	500 gm.	0.165	0.033
Polished Madras	500 gm.	0.076	0.015
Parboiled Unpolished Sierra Leone.	500 gm.	0.32	0.064
Parboiled Polished Rangoon.	500 gm.	0.09	0.018

Fig.21. Yields of A.I.Pb isolated as described Vol.1 page20.

ANIMAL INOCULATIONS OF A.I.Pb RICE.

Experiment I. Frogs. Fig.22.

A. 0.10 gm. A.I.Pb dissolved in 0.5 c.c. water injected subcutaneously into dorsal lymph sac.

Time.	Observations.
10.00 a.m.	Frog active and jumps well. Dose in solution administered.
10.15	Very sluggish. Recovers from dorsal position with difficulty. Does not respond to pinching of toes.
10.25	Does not respond to pinching of toes. Lies flat on abdomen and when placed on back makes no attempt to move.
10.30	Completely paralysed.
5.00 p.m.	About the same. Killed and faradic current applied to dorsal muscles. No response elicited.

B. 0.10 gm. A.I.Pb dissolved in 0.5 c.c. water injected subcutaneously into dorsal lymph sac.

Time.	Observations.
10.20 a.m.	Frog active and jumps well. Dose administered.
10.30	Paretic: very little response to external stimuli.
10.40	The same.
11.00	No change.
11.15	Moribund.
12.00	Dead. No response to faradic current in dorsal muscles. Slight response in abdominal muscles. Heart stopped in diastole.

C. 0.05 gm. A.I.Pb dissolved in 0.5 c.c. water injected below skin of abdomen.

Time.	Observations.
12.00	Frog active and jumping well. Dose administered.
12.15	Movement inco-ordinate, fibrillary twitchings in feet. Jumps fairly well.
12.30	Twitchings becoming generalised, reflexes increased.
12.45	Tetanic spasms. Paralysis of right leg.
2.10 p.m.	Reflexes increased. Generalised tremors.
4.00	Reflexes increasing.
Next day.	Hind limbs inco-ordinate and sluggish. Reflexes increased.
2 days later.	Jumps moderately well and no local paresis. Later recovery apparently complete.

D. 0.03 gm. A.I.Pb dissolved in 0.5 c.c. water injected subcutaneously into abdomen.

Time.	Observations.
3.00 p.m.	Frog active and jumping well. Dose administered.
3.20	Sluggish and moves with difficulty.
3.30	Some muscular twitching.
4.00	About the same.
5.00	Muscular power increasing.
6.00	Reflexes increased. Moves more easily.
Next day.	Apparently normal.

E. 0.01 gm. A.I.Pb dissolved in 0.5 c.c. water injected subcutaneously abdomen.

Time.	Observations.
10.00 a.m.	Frog active and jumping well. Dose administered.
10.30	Slightly depressed but jumps well.
11.00	Sluggish but jumps well when stimulated.
11.30	Responds slowly to pinching and jumps heavily.
12.30	About the same.
2.00 p.m.	Reflexes increased, jumps quite well.
4.00	About the same.
6.00	Reflexes increased. Jumps well.
Next day.	Appeared quite normal.

Experiment II - Rabbits. Fig.23

A. 1.0 gm. A.I.Pb dissolved in 2 c.c. water and injected hypodermically in flank.

Time.	Observations.
10.15 a.m.	Rabbit (1275 gm.) active and quite normal. Dose administered.
10.30	No change.
10.45	Sluggish and not inclined to move.
11.15	Hind legs outstretched and has difficulty in moving.
12.00	Can move front legs but hind legs very weak. When placed on side has difficulty in righting itself.
12.30	About the same.
1.30 p.m.	Hind legs still weak, and unable to regain upright position when placed on side.
3.00	Improvement in general condition but still depressed.
6.00	Can move about quite well.

B. 0.3 gm. A.I.Pb dissolved in 1 c.c. water and injected into ear vein.

Time.	Observations.
10.30 a.m.	Rabbit (1900 gm.) active and normal. Dose administered.
10.50	Depressed and dull, not inclined to move.
11.05	About the same.
5.00 p.m.	Depressed but able to move about quite well.
Next day.	Recovered completely.

C. 0.7 gm. A.I.Pb dissolved in 2 c.c. water and injected into ear vein.

Time.	Observations.
12.30 p.m.	Rabbit (1575 gm.) active and normal. Dose administered.
12.35	Very depressed and lies flat on table.
12.45	Not inclined to move and lies in corner. Can be roused.
3.15	About the same.
6.00	Still depressed and dull but can move about when stimulated.
Next day.	Moves freely but rather dull. Later apparently recovered.

ORAL ADMINISTRATION OF A.I.Pb RICE.

Experiment III - Pigeons. Fig. 24.

A. Pigeon 320 gm.

1st day.

2.0 p.m.	Given 3.0 gm. A.I.Pb suspended in 5 c.c. water per crop.
4.0	Dull and depressed but able to fly.
5.0	Given 15 g. rice bran to eat.

2nd day.

10.0 a.m.	Sluggish and heavy but able to fly and walk. Dose repeated.
3.0 p.m.	About the same.
5.0	Rice bran as before.

3rd day.

10 a.m.	More depressed but still able to fly and walk. Dose repeated.
4.0 p.m.	Gait rather unsteady.
5.0	Rice bran repeated.

4th day.

10.0 a.m.	Still dull but able to fly and walk. Dose repeated.
11.0	Has vomited several times.
12.0	Gait unsteady and not able to walk or stand upright.
5.0 p.m.	Rice bran repeated.

5th day.

Still dull and heavy but can fly.
Experiment concluded.

B. Pigeon 286 gm.

10.30 a.m.	Given 3.0 gm. A.I.Pb suspended in 5 c.c. water by stomach tube into its crop.
11.50	Lies on its side, with only spasmodic attempts at movement.
12.30 p.m.	Lies on breast with toes flexed.
2.00	Legs are paralysed and unable to move.
3.30	Neck is stiff and retracted and resembles very closely "polyneuritic pigeon".
3.45	Given 1.0 g. rice bran suspended in water per crop.
5.00	There is no improvement. The pigeon was found dead in the morning.

C. Pigeon 270 gm.

2.00 p.m.	Given 3.0 gm. A.I.Pb suspended in 5 c.c. water by stomach tube into its crop.
3.5	Unable to fly and very sluggish.
4.00	Lies on its breast, its toes are flexed and it can only move by extending its wings.
6.30	Paretic and unable to move. Its toes are flexed and its head is retracted. Later in the evening was found dead.

Fig.25A. Opisthotonus in Pigeon.

Fig.25B. Emprosthotonus in Pigeon.

PIGEON FEEDING EXPERIMENTS.

Experiment I. Fig.26.

Basal Diet - Siam polished rice.

Curative Treatment - Rice bran mixed with water to mash and dried on steam bath then made into mash with water and fed by mouth.

P.R.I. 20th day. Inco-ordinate flight and gait. Toes flexed. 23rd day - typical head retraction and quite paretic. Was given 4 grammes rice bran as above. There was slight improvement. Gait was very unsteady and flight short and weak. Treatment was repeated but improvement was only slight. The supply diet was given daily and there was gradual improvement in its condition. 117th day - became weaker; later developed head retraction, paralysis of legs and died.

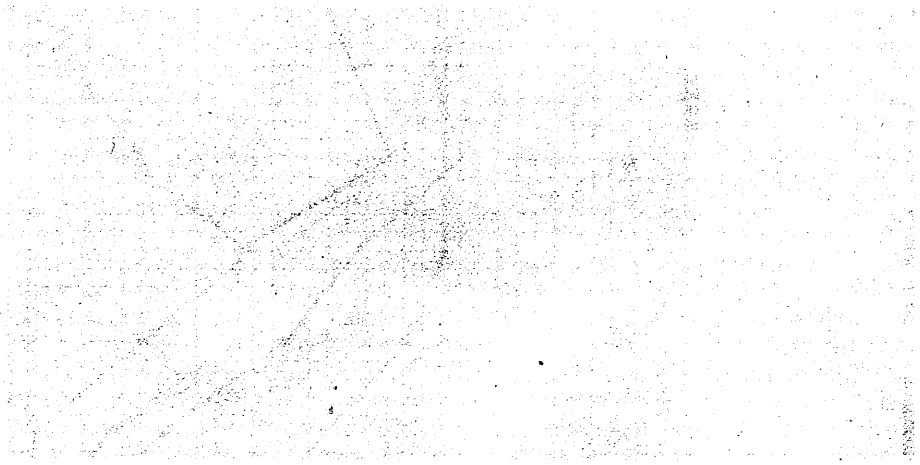
P.R.2. 19th day. Paretic with unsteady gait and unable to fly. Was given 4 grammes rice bran as above. There was a gradual and slight improvement but it remained weak, though movement was more extensive. 36th day - unsteady gait but no head retraction. Fell forward. Treatment was repeated with only a slight improvement. 39th day - gait very unsteady. Fell forward. Daily treatment commenced with gradual improvement which later became less apparent. The flight became poor. 81st day - gait became very unsteady. No head retraction. Later became paretic and died.

P.R.3. 14th day - gait very unsteady. Head retraction. Given 4 grammes rice bran as above. There was slight improvement which was maintained. After 8 days was flying well and was quite active. 36th day - very weak, unsteady gait and flexed toes. Treatment was given daily and improvement resulted. This was maintained for 84 days. 120th day - became very weak and was killed by coal gas on 135th day.

P.R.5. 20th day - became paretic with typical head retraction and flexed toes. Was given 4 grammes rice bran as above. A slight improvement resulted which was maintained with daily treatment. After 117 days its gait was steady and its flight quite strong. 118th day - became much weaker in flight and ten days later developed head retraction and later, leg paralysis. It died on the 127th day.

P.R.O. 20th day - gait was unsteady and flight short. 22nd day - was given 5 grammes rice bran by mouth (mash). In 4 hours was much improved, able to fly and gait steady though weak. Remained quite normal for 12 days. 34th day - weaker, with unsteady gait. 36th day - was very paretic and fell forward with toes flexed. Was given 4 grammes rice bran daily. Slight, transient improvement. Later became paretic and died on 45th day.

S. Grey. 34th day - weak and unable to fly or walk. Was given 5 grammes rice bran as above. Slight improvement next day and treatment repeated. Only slight benefit was that time evidenced. 36th day - given untreated bran and great improvement resulted and was normal next day. 38th day - untreated bran given and pigeon was quite normal in every way.



EXPERIMENT I. Fig.26.A.

Experiment II. Fig.27.

Basal Diet - Siam polished rice.

Curative Treatment - Alcoholic solution extract of rice bran.

X. Brown. 31st day - completely paralysed with head retraction. Was given, per crop, solution equivalent to 20 grammes rice bran (photograph taken). A slight improvement resulted after 35 minutes but paralysis and head retraction remained. After an hour the dose was repeated, but in 15 minutes it vomited, and died shortly afterwards.

Ex.White. Prophylactic treatment - 8 grammes of rice bran daily. After 22 days was flying well and gait was quite normal. It was killed by $CHCl_3$, owing to an accidental injury.

Ex.Yellow. Prophylactic treatment - 8 grammes of rice bran daily. After 30 days was flying well and gait was quite normal. It escaped and was not recovered.

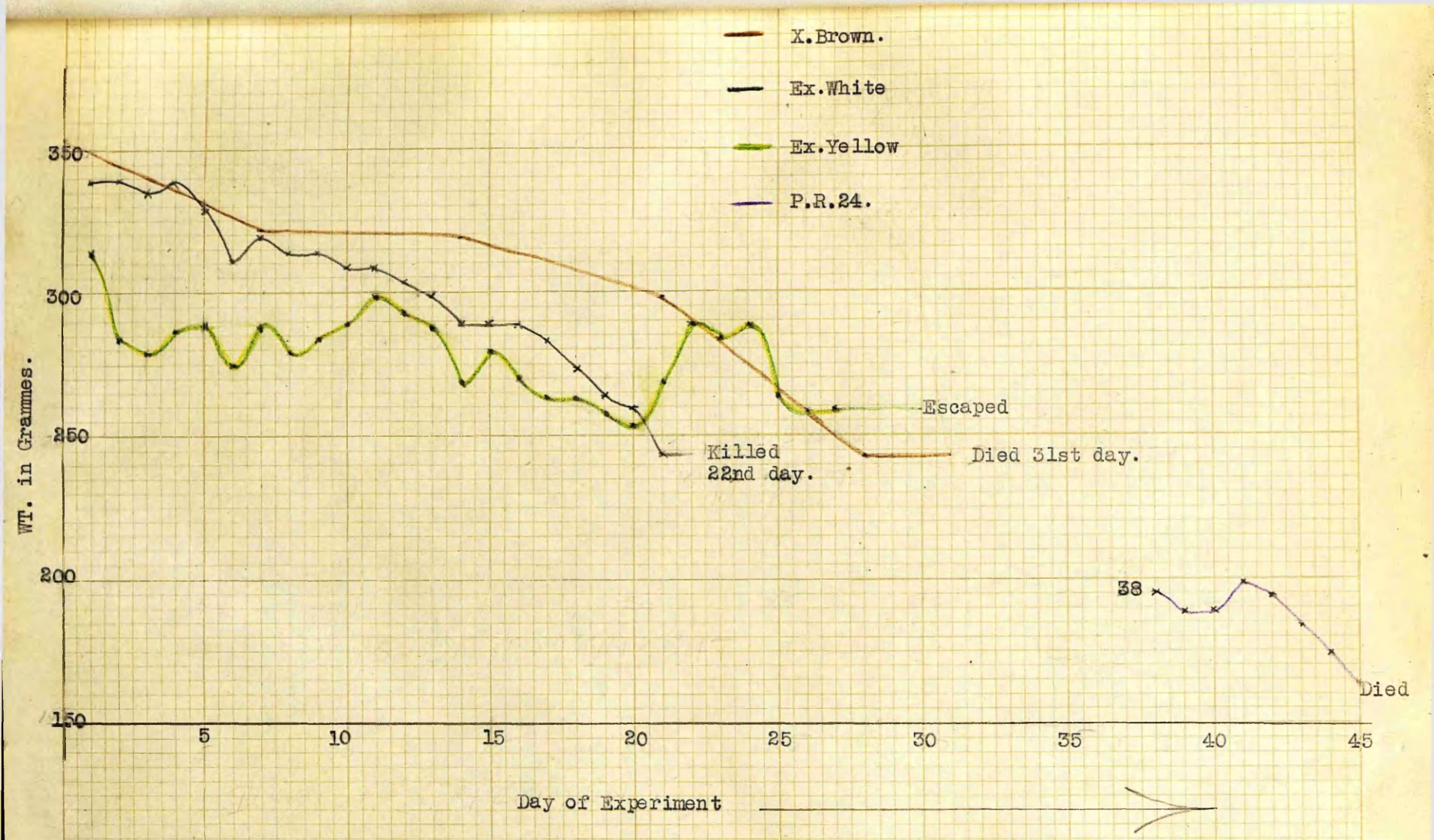
P.R.24. 23rd day - flight weak, gait unsteady and on 26th day unable to fly. It was given daily thereafter alcoholic extract equivalent to 4 grammes rice bran. 37th day - slight improvement, gait steadier, but flight weak. 45th day - though the flight was rather weak the gait was fairly steady. It died next day.

Experiment III. Fig.28.

Basal Diet - Siam polished rice.

Curative Treatment - Vitamin B_1 concentrate.

S.Fawn. On the 32nd day it was unable to fly and, with wings drooped, it fell on its breast when attempting to walk. It was given 1 c.c. concentrated B_1 - equivalent to 0.05 milligram B_1 - hypodermically in the breast and after two hours there was no improvement. 35th day - the dose was repeated but no improvement resulted. 37th day - was very paretic and could not fly. The dose was again repeated, but no improvement resulted. It died on the 38th day.



EXPERIMENT II. Fig.27.A.

Experiment III (Contd.)

W. Orange and Red. After 18 days the legs were weak and the flight short. Was starved for 7 days and there was an improvement in its condition. The flight was good and only the toes were flexed so that it stumbled on alighting. (A photograph was taken). Its diet was resumed on the 26th day On the 30th day it was very weak and unable to fly. Next day it was given 0.1 c.c. concentrate B_1 - equivalent to 0.05 milligram B_1 - hypodermically in the breast. There was a slight improvement after 2 hours, but it died after another hour.

W. Red, White and Blue. On the 33rd day the flight was weak and gait unsteady. On 34th day it was given 0.5 c.c. B_1 concentrate - equivalent to 0.25 milligram B_1 - hypodermically in breast. Next day there was a great improvement and it was able to fly. The gait was steady. B.D. was resumed. 44th day - gait was inco-ordinate and flight weak. 48th day - gait quite steady and stronger in flight. Next day it sat with ruffled feathers and drooping head. 50th day - gait very unsteady and could not fly. Later died.

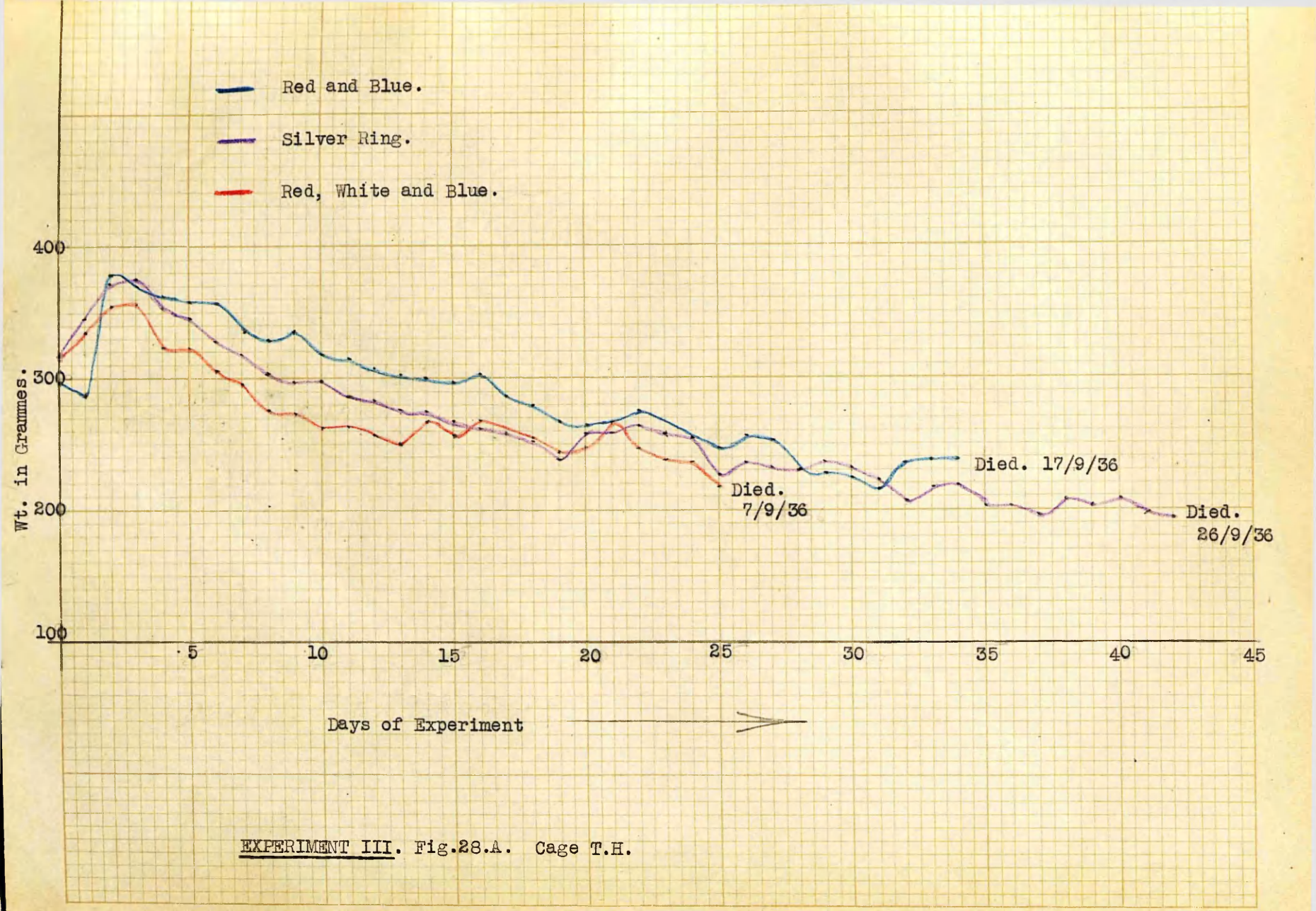
T.H. Silver. 14/9/36 - gait unsteady but no head retraction. 15/9/36 - less unsteady gait and no head retraction. 16/9/36 - gait unsteady and fell forwards. No indication of head retraction. Legs flexed and in spasm. 17/9/36 - condition unchanged. Given at 1.20 p.m. 10 pigeon units Betaxin per breast skin. Not much improved. 18/9/36 - Steadier in gait but unable to fly. Gait unsteady after exercise. 19/9/36 - condition unchanged. 21/9/36 - could fly a little, and gait fairly steady except on exercise. 23/9/36 - condition unchanged. 25/9/36 - unable to fly and unsteady gait. Sat all the time. Given at 2.45 p.m. 10 pigeon units Betaxin per breast skin. 26/9/36 - did not recover. C.N.S. and P.N.S. reserved for histological examination.

Red White and Blue. 5/9/36 - gait unsteady but no head retraction. Given 10 pigeon units Betaxin per breast skin at 8 p.m. 6/9/36 - at 12.30 p.m. gait was steadier but it was unable to fly. Given 10 pigeon units Betaxin per breast skin. More lively but unable to fly. Died at 4 p.m. C.N.S. and P.N.S. reserved for histological examination

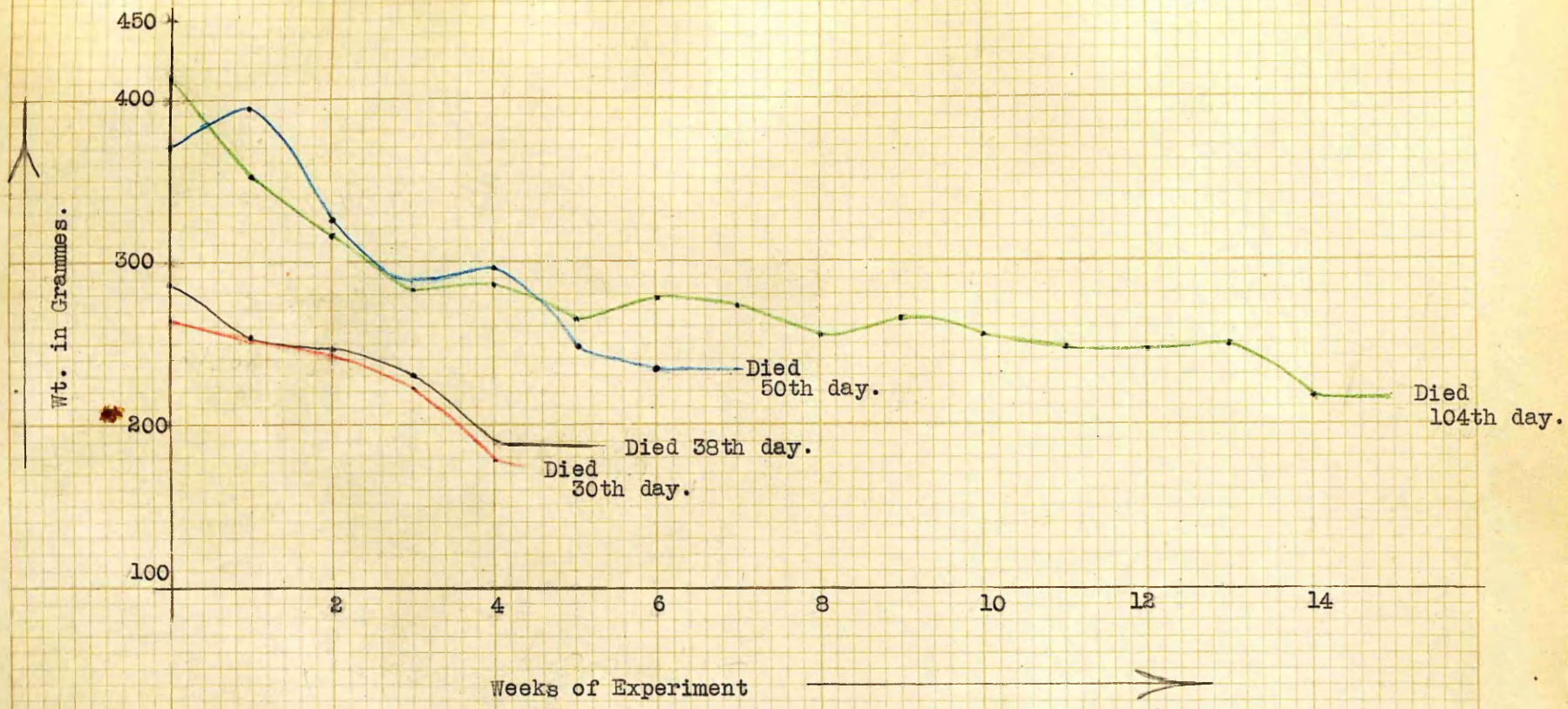
Experiment III (Contd.)

E.S. Green Neck. After 36 days flight was very weak and gait unsteady. It was given 0.2 c.c. B_1 concentrate - equivalent to 0.1 milligram B_1.- Next day the gait was steady and the flight quite strong. The improvement was maintained until 53rd day when gait again became unsteady and flight poor. The dose was repeated. There was a great improvement in the condition and the flight was stronger. 65th day - again weak and gait unsteady. Dose repeated. Improved general condition resulted and this was maintained for 3 days. 73rd day - gait very unsteady. Dose repeated. 74th day - steadier, but not normal. 79th day - again gait very weak and later paretic. Dose repeated. Much improved next day but later very weak and paretic with head retraction. Dose repeated. Weak in legs and could not fly. 87th day - gait very inco-ordinate. Dose repeated. Condition fluctuated for 5 days, with doses every second day, and subsequent improvement. 104th day - had diminished postural tone and made no attempt at movement. Later died.

Red and Blue. 9/9/36 - gait unsteady but no head retraction. 10/9/36 - gait unsteady and head fell forward. Given 10 pigeon units Betaxin per breast skin. 11/9/36 - much improved. Given 10 pigeon units Betaxin per breast skin. Much improved. 13/9/36 - unsteady gait but no head retraction. Unable to fly very well and could not soar. 15/9/36 - condition unchanged. 16/9/36 - weak, with unsteady gait. Sat huddled in corner. Later quite paretic. 17/9/36 - found dead. C.N.S. and P.N.S. reserved for histological examination.



EXPERIMENT III. Fig.28.A. Cage T.H.



EXPERIMENT III. Fig.28.A.

Experiment IV. Fig.29

Basal Diet - Siam polished rice macerated with cold water for 3 days and percolated. The rice was dried on a water bath and fed to the pigeons.

I. Black. 15th day - legs were flexed at joints and right wing drooped. 18th day - flight poor and legs very weak. Was given 1.5 gm. Marmite per crop, but no improvement was apparent. 24th day - legs paralysed and flight very weak. Fed rice bran 3 grammes daily per os. There was gradual improvement and on 43rd day it was quite normal.

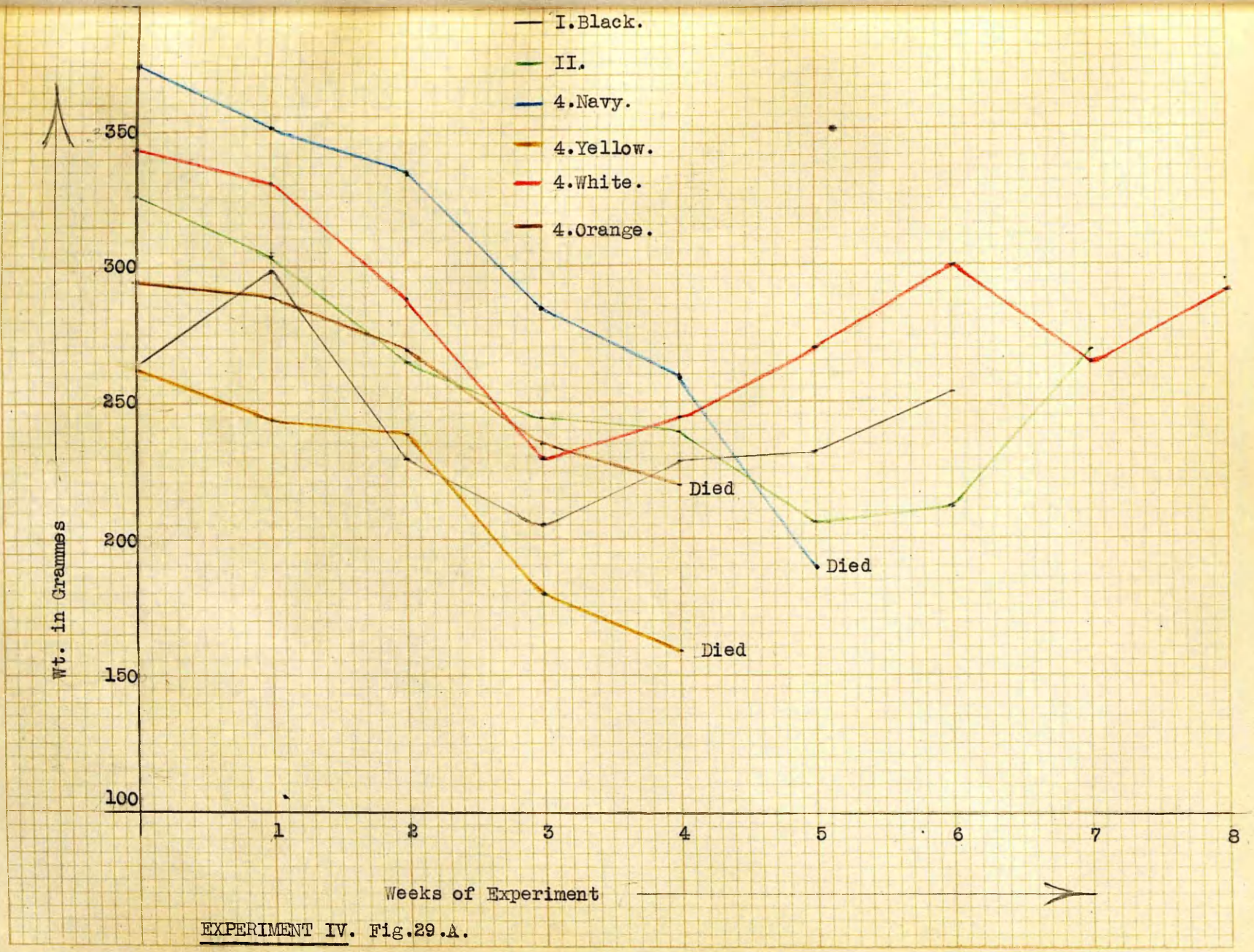
II. 38th day - legs very weak and flight poor. Was fed 3 grammes rice bran daily per os. There was gradual improvement, and on 43rd day was quite normal.

4. Navy. 30th day - flight weak and easily exhausted. 35th day - very paretic. Head retracted. Unable to move at all. Later died.

4. Yellow. 21st day - gait very unsteady and flight very weak. Basal Diet discontinued. Fed groats but on 24th day was very weak and could not walk. Attempts resulted in falling on side. It died next day.

4. White. 21st day - gait very unsteady and joints flexed. Unable to fly. Given diet of rice bran made into mash with water and dried on water bath. Gradually improved and was quite normal on 37th day.

4. Orange. 28th day - flight was weaker and it was easily exhausted. 35th day - very weak and gait unsteady. Was fed groats and rice bran, but died next day.



EXPERIMENT IV. Fig.29.A.

Experiment V. Fig.30.

Basal Diet - Parboiled rice. Calcutta milled.

A. Grey and Black. During 147 days of experiment it was quite normal in flight and gait.

A. Grey. On 37th day it was weak in flight and gait but two days later was quite normal in every way. Remained so until 147th day.

R. Violet. Basal Diet - Rangoon.

After 8th day flight was short and weak but gait quite steady. After 31st day flight was quite strong but gait rather weak. Next day it was much improved, and on 35th day was quite normal, when stopped.

R. Black and White. Report as for above (R.Violet).

Experiment VI. Fig.31.

Basal Diet - Unmilled rice (Temne).

D. Dark. After 57th day it was quite normal and had laid two eggs. Young were hatched on 73rd day. Was quite normal after 100 days.

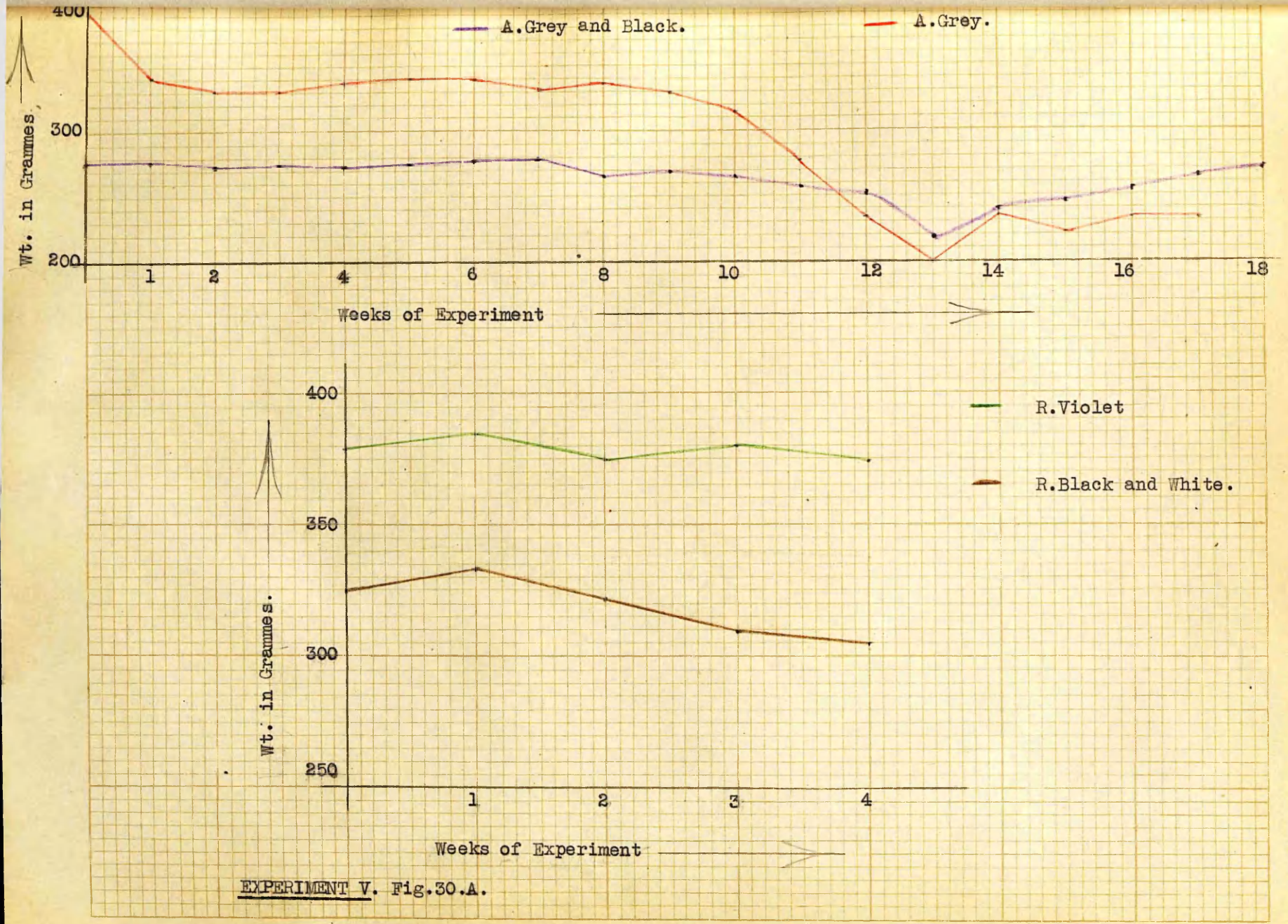
D. Black. Report as above.

D. Young. After 41 days it was quite normal.

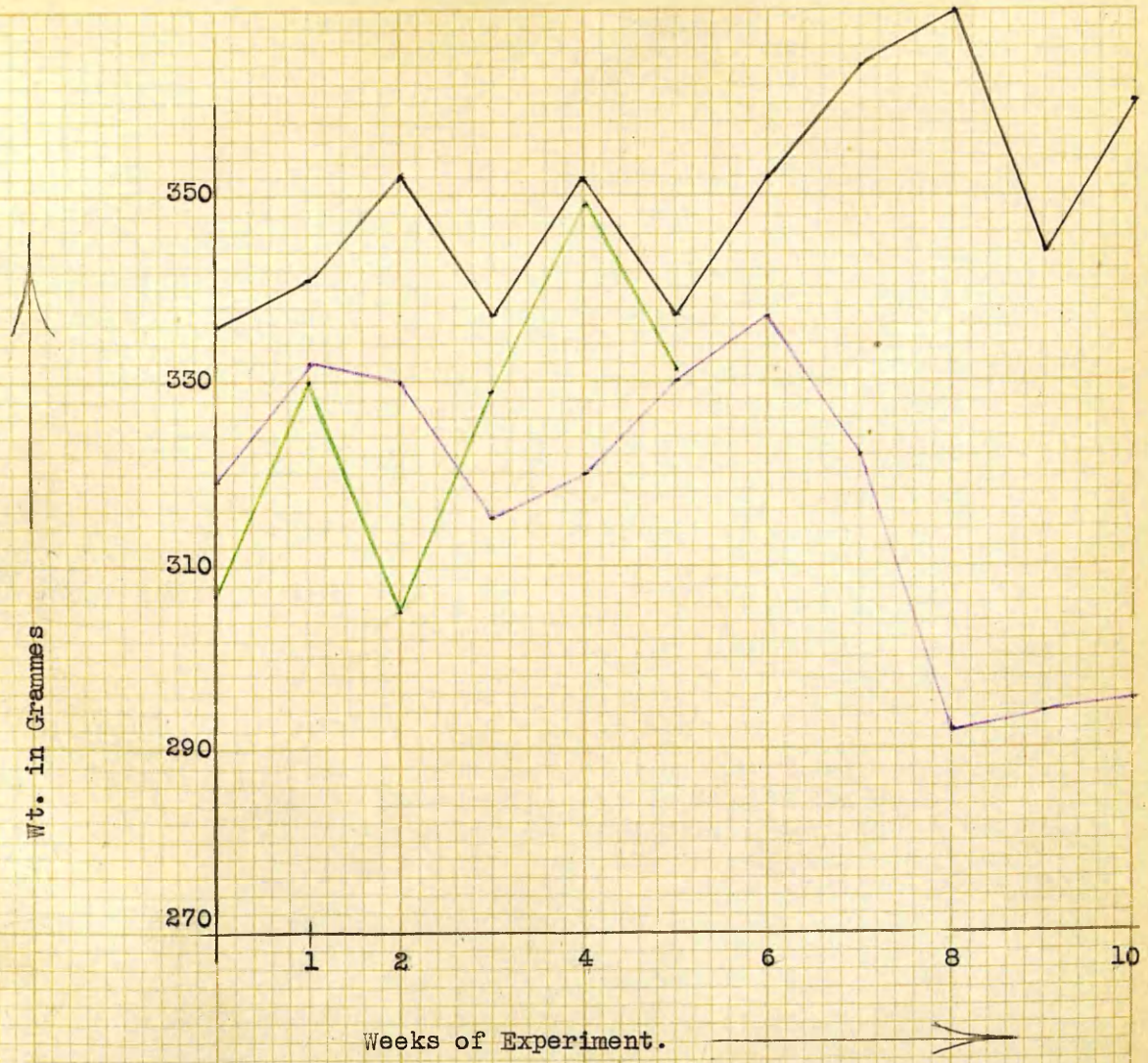
Experiment VII. Fig.32.

Basal Diet - Rice bran mashed with water and dried on water bath.

C. Black. On 9th day flight was weak and short but gait was quite steady. It remained thus until 18th day when its gait was unsteady and wings were drooped. On 19th day it was very weak. The toes were flexed and



EXPERIMENT V. Fig.30.A.



EXPERIMENT VI. Fig.31.A.

Experiment VII (Contd.)

it could only move by spreading out wings. It died on the 20th day.

C. White. On 9th day the flight was weak and short but the gait was steady. It remained thus until 19th day when its gait was very unsteady and it did not move much. On the 22nd day it was quite paralysed, toes flexed, and unable to support itself. It was given 1.0 gm. dried yeast in water per crop, but died quite paralysed two hours later.

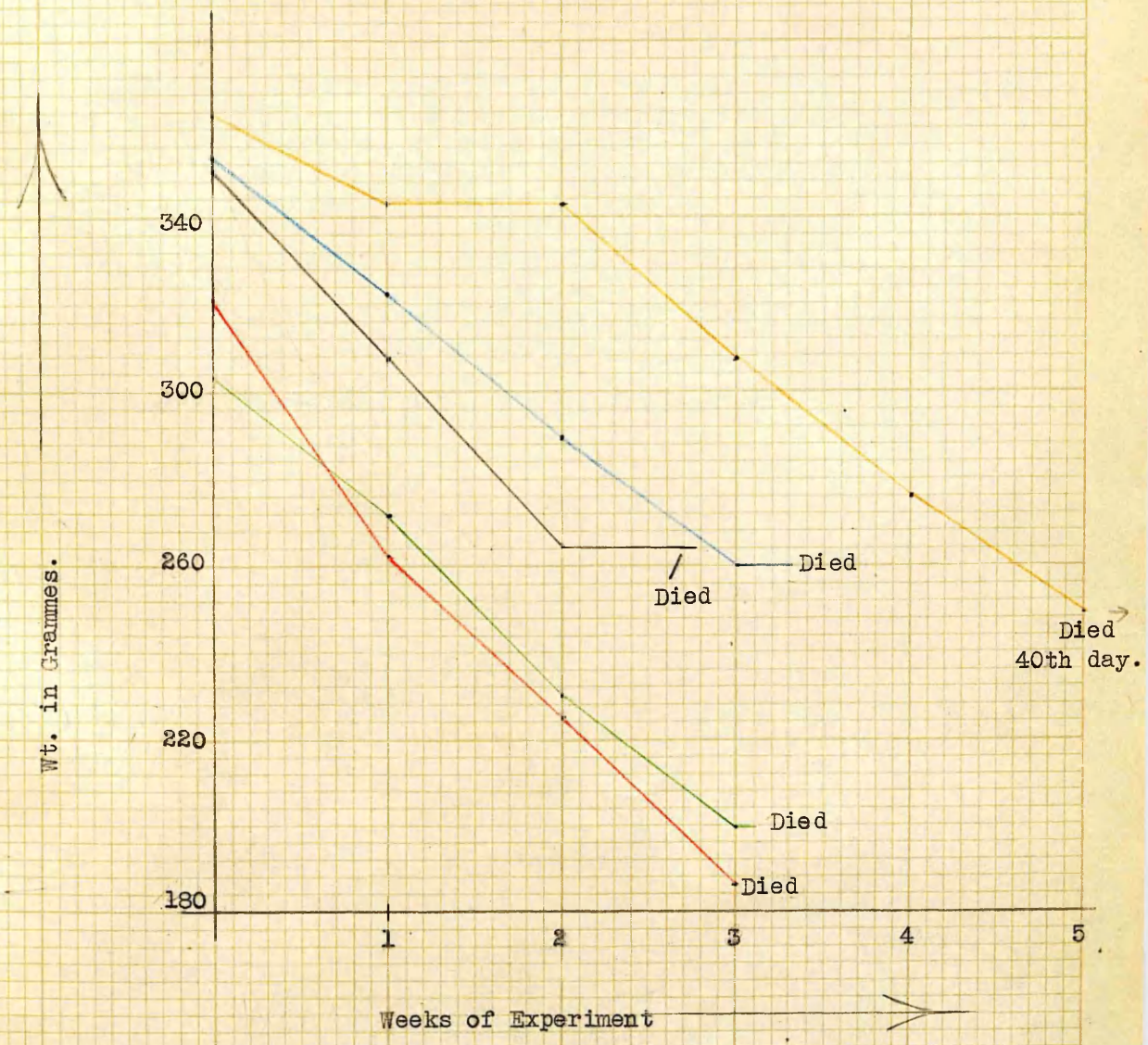
R.I. After 7 days flight was short but gait steady. It remained thus until 23rd day when it became dull and heavy, not inclined to move about. The wings were spread out. It died on the 24th day.

R.II. After 7 days flight was short and weak but gait was steady. It remained so until 23rd day when it could not fly, had a very unsteady gait, was very weak and had its wings outstretched. It was given 0.1 c.c. B_1 concentrate - equivalent to 0.05 milligram B_1 - per breast skin subcutaneously but there was no improvement. It died on the 24th day.

7.I. After 21 days it was easily exhausted and flew only a short distance. Gait was unsteady. After 38 days although still easily exhausted the gait was steadier. This condition was unchanged until 74th day when its gait was quite steady and it remained normal until 118th day, when experiment was concluded.

7.II. After 21 days gait was weak and unsteady. It was easily exhausted, and remained so until the 38th day when its head retracted and its gait was very unsteady. On the 40th day it died, being paralysed and unable to move.

S.R.7. Remained well during 101 days.



EXPERIMENT VII. Fig.32.A.

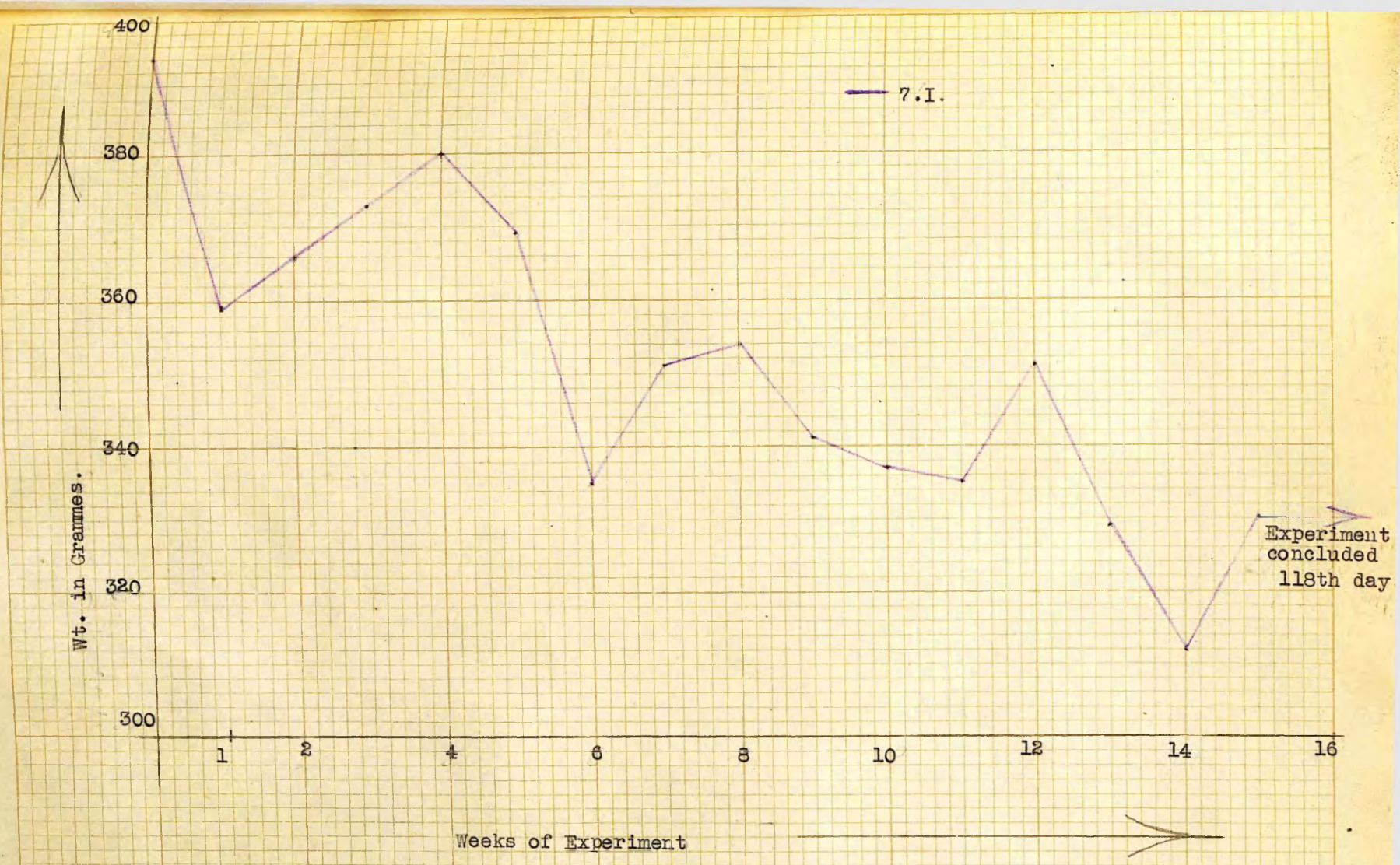
Experiment VII (Contd.)

S.R.8. On 64th day its gait was unsteady but flight was quite strong. It remained thus until 75th day when it gradually became normal again. On 101st day it was flying well and was quite normal.

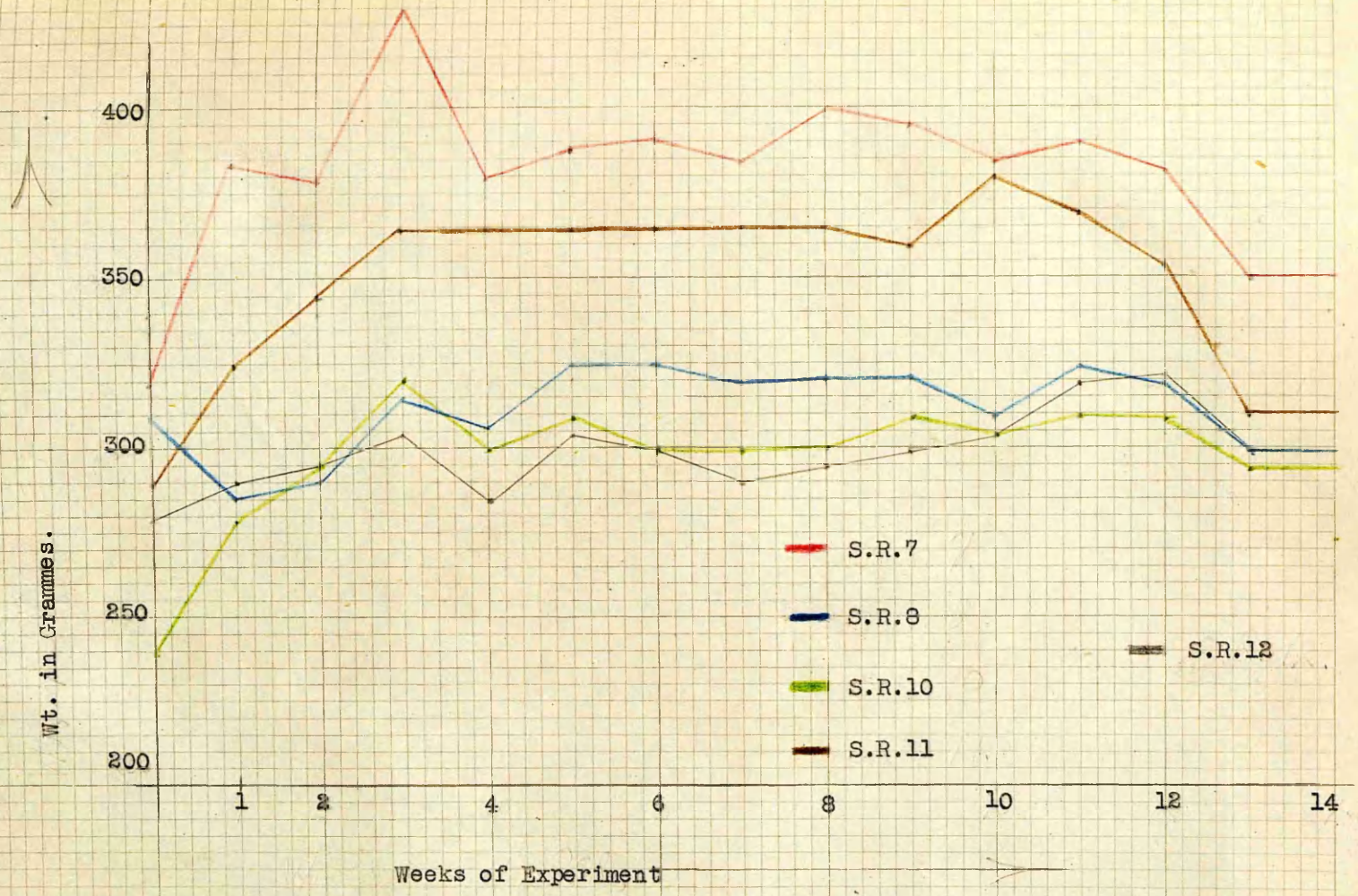
S.R.10. Remained well during 101 days.

S.R.11. Remained well during 101 days. Killed with coal gas.

S.R.12. Remained well during 101 days.



EXPERIMENT VII. Fig.32.B.



EXPERIMENT VII. Fig.32.C.

Fig.33. Monkey after 41 days' feeding Siam polished rice.

Fig.34. Monkey after 150 days' feeding Siam polished rice.

Fig.35. Monkey after 164 days' feeding Siam polished rice.

Fig.36. Monkey after 206 days' feeding Siam polished rice.

Fig.37. Monkey after 176 days' feeding Siam polished rice.

Fig.38. Monkey after 49 days' feeding extracted Siam polished rice.

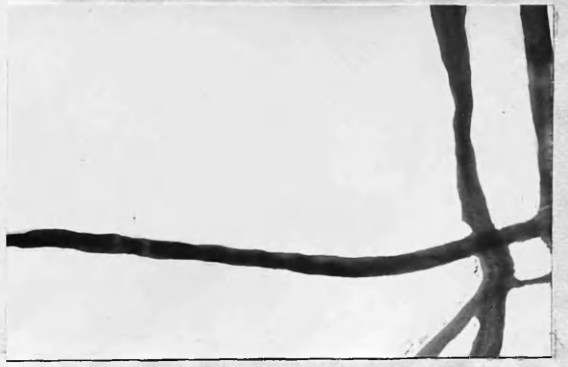


Fig.39. Peripheral Nerve (Brachial Plexus) of monkey fed on Siam polished rice (Marchi stain).

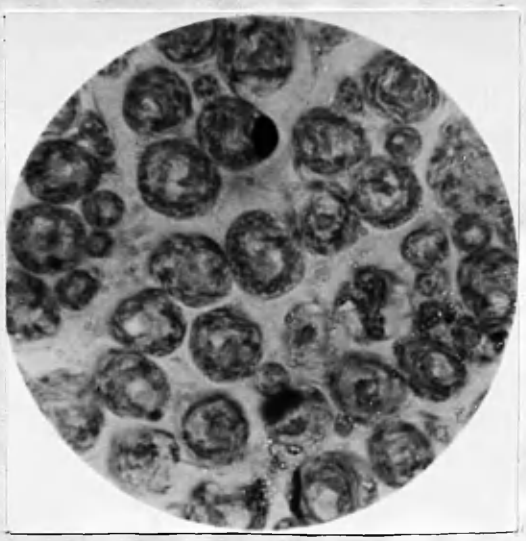


Fig.40. Myelin degen. Cauda Equina.
Rice extract.

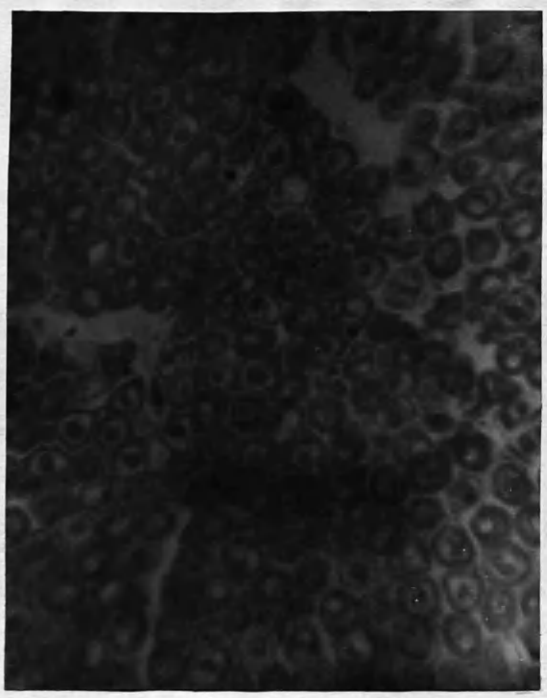


Fig.41. Myelin degen. Cauda Equina.
Unpolished rice.

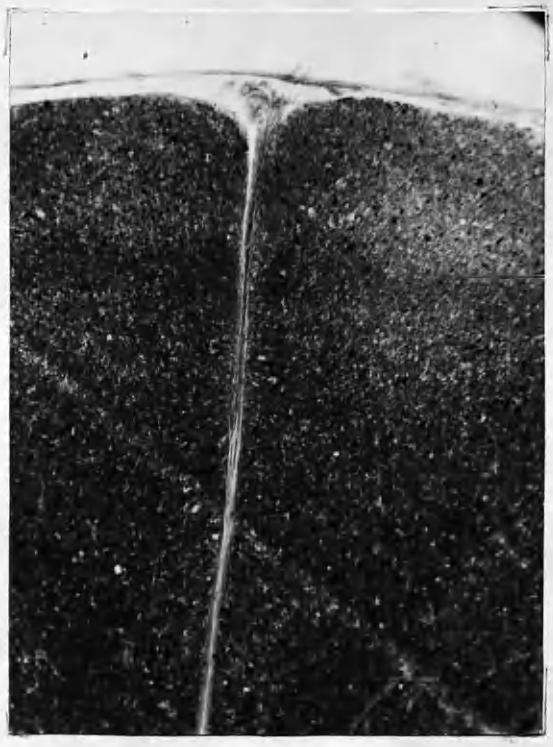


Fig.42. Marchi degen. Posterior Column. Cervical enlargement. Unpolished rice.

Fig.43.A. Monkey after 117 days' feeding Unpolished rice.

Fig.43. Monkey after 231 days' feeding Unpolished rice.

Fig.44. Typical case of "dry" and "wet" beri-beri.

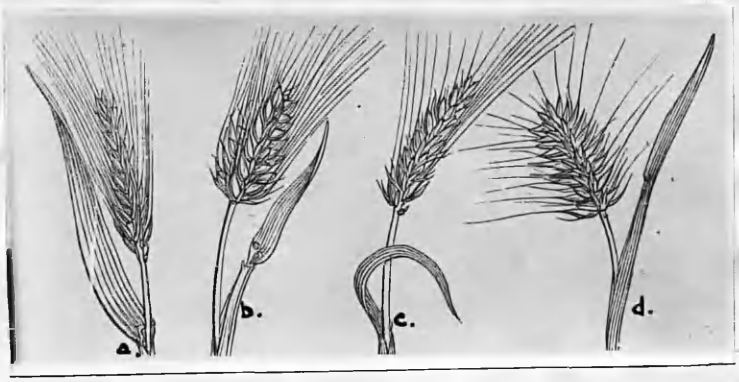
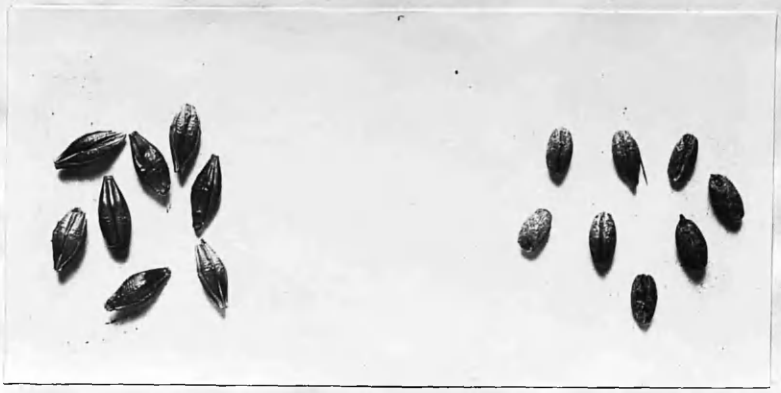


Fig.45. Varieties of Barley.

a. H. vulgare.
b. H. hexastichon.
c. H. distichon.
d. H. zeocitron.



Unmilled Barley.

First Run Barley.

Second Run Barley.

Third Run Barley.

Fourth Run Barley.

Fig.46. Naked Eye Appearance of Milled Barley.

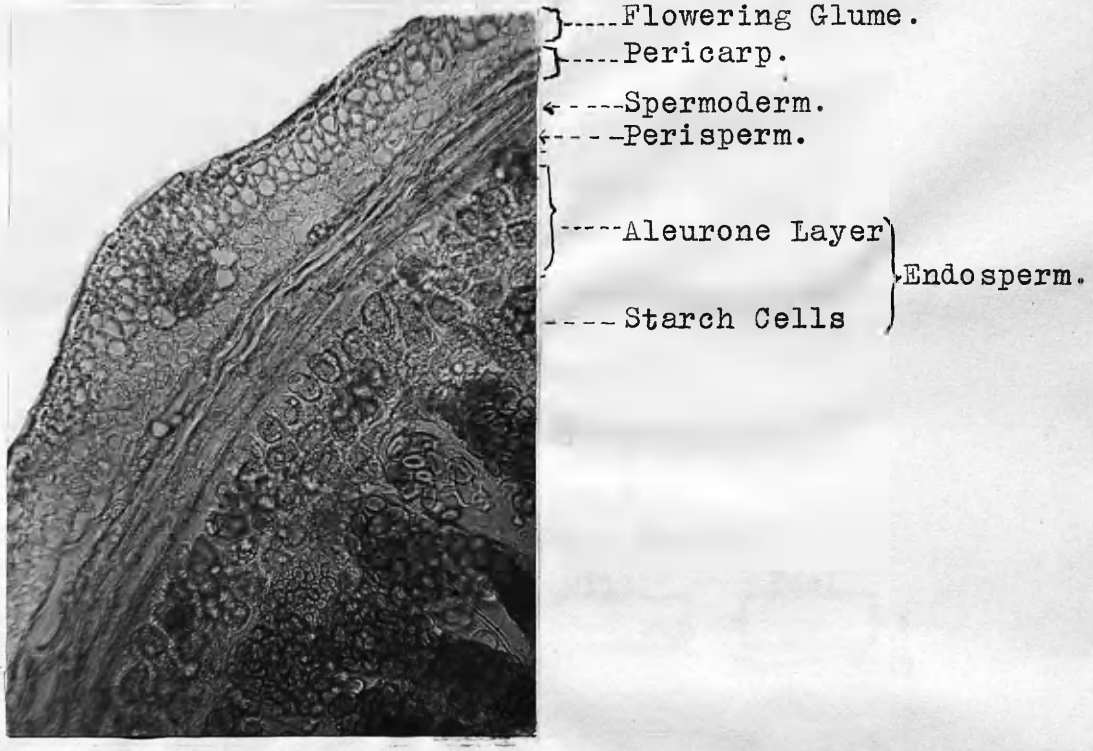


Fig.47. Transverse Section Unmilled barley (X180).

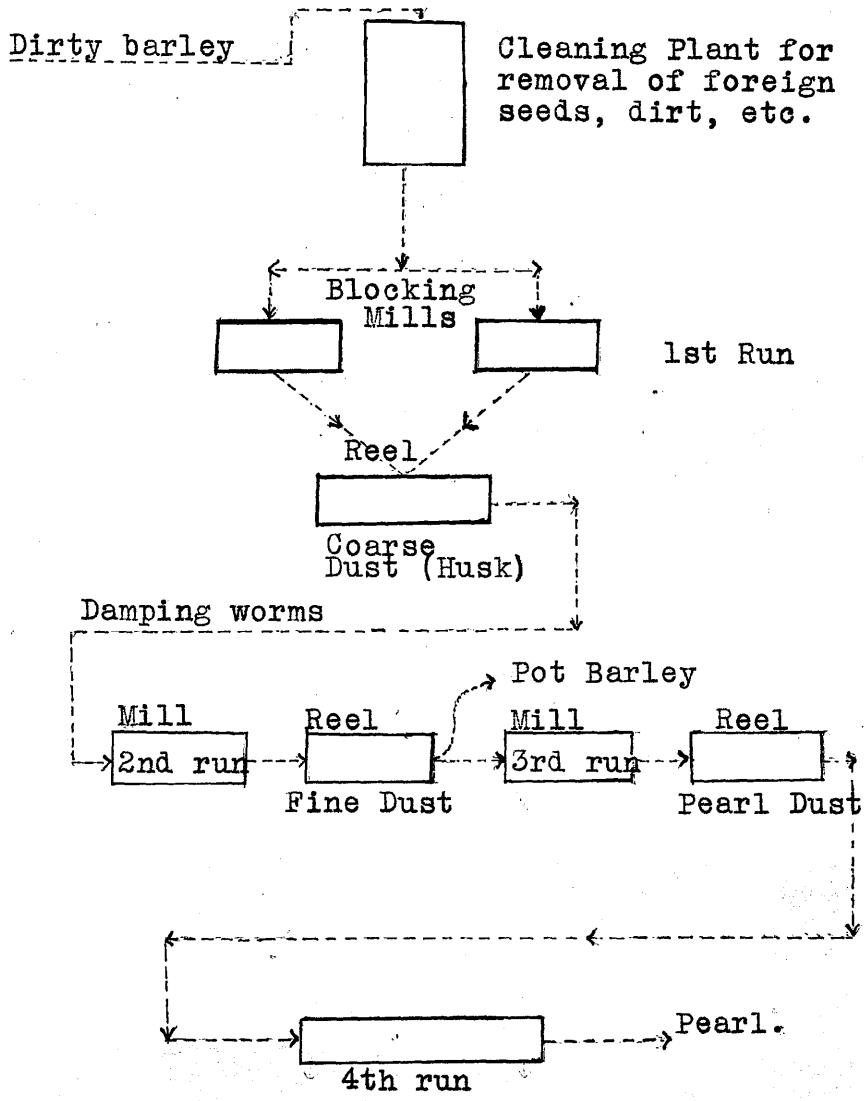


Fig.48. Diagrammatic sketch of Milling of Barley.

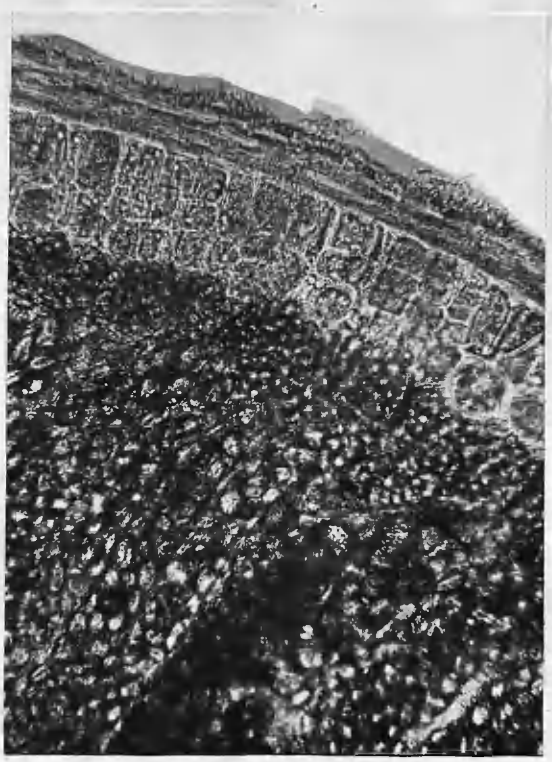


Fig.49. Transverse Section of First Run barley (X150).

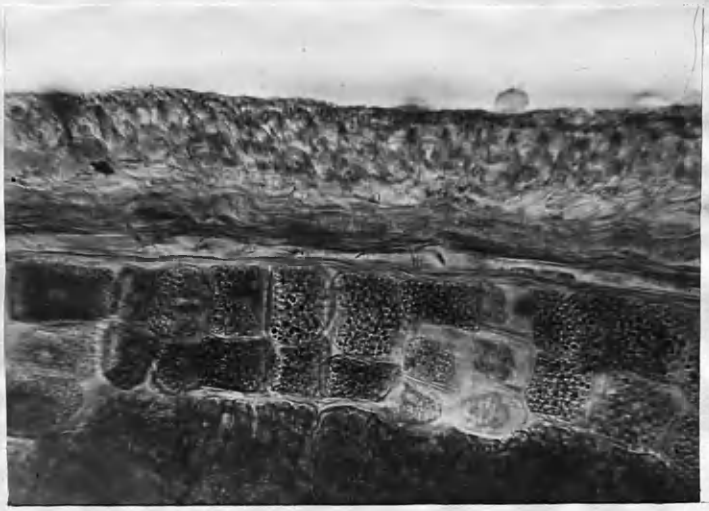


Fig.49a. Transverse Section of First Run barley (X220).

Fig.50. Transverse Section of Second Run barley (X33).

Fig.50a. Transverse Section of Pot barley (X155).

Fig.51. Transverse Section of Third Run barley (X180).

Fig.52. Transverse Section. First Pearl barley (X30).

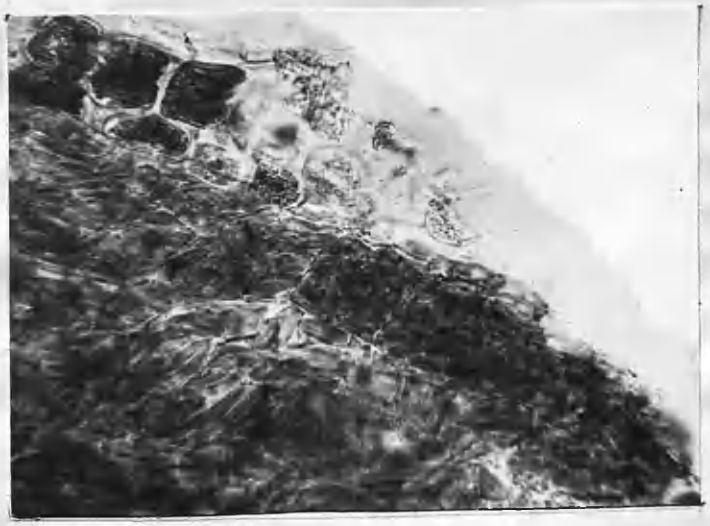


Fig.52.a.

Transverse Section. First Pearl barley (X220).

Fig.53. Transverse Section
Second Pearl barley (X30).

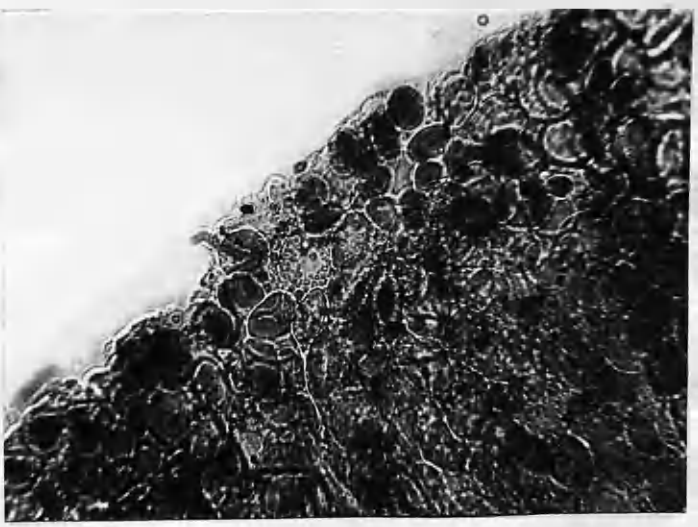


Fig.53a. Transverse Section
Second Pearl barley (X180).

CHEMICAL ANALYSIS OF BARLEY AND MILLED PRODUCTS.

Description	Sample No.	Ash %	Protein (N x 6.25)	Oil %	Fibre %	Carbohydrates %
Rough Barley	326	2.19	8.86	1.67	2.57	69.79
1st Run Barley	325	1.60	8.27	1.40	1.05	72.36
2nd Run Barley	327	1.00	7.34	1.00	0.43	75.71
3rd Run Barley	328	0.79	7.08	0.41	0.35	78.53
4th Run Barley	331	0.60	6.33	0.70	0.28	78.82

Fig.54.

Source	Yield per kg. in gm.	% Yield in gm.
Unmilled Barley	0.96	0.096
1st Run Barley	2.90	0.290
2nd Run Barley	1.30	0.130
3rd Run Barley	0.42	0.042
1st Pearl Barley	0.05	0.005

Fig.55. Yield of A.I.Pb from Barley.

Experiment I. Fig.56.

Basal Diet - 1st Run Barley.

A.B.A.Fawn. Remained normal with good flight and steady gait during 73 days.

A.B.A. Blue and White. Remained well, being normal in flight and gait during 73 days.

A.B.Black. During 175 days remained well; flight strong and gait steady.

A.B.Blue & White. During 175 days remained well; flight strong and gait steady.

Experiment II. Fig.57.

Basal Diet - 2nd Run Barley.

C.B. Red, White & Blue. On 38 days flight was weak and gait unsteady. This condition continued. On 59th day became very weak and died later in the day.

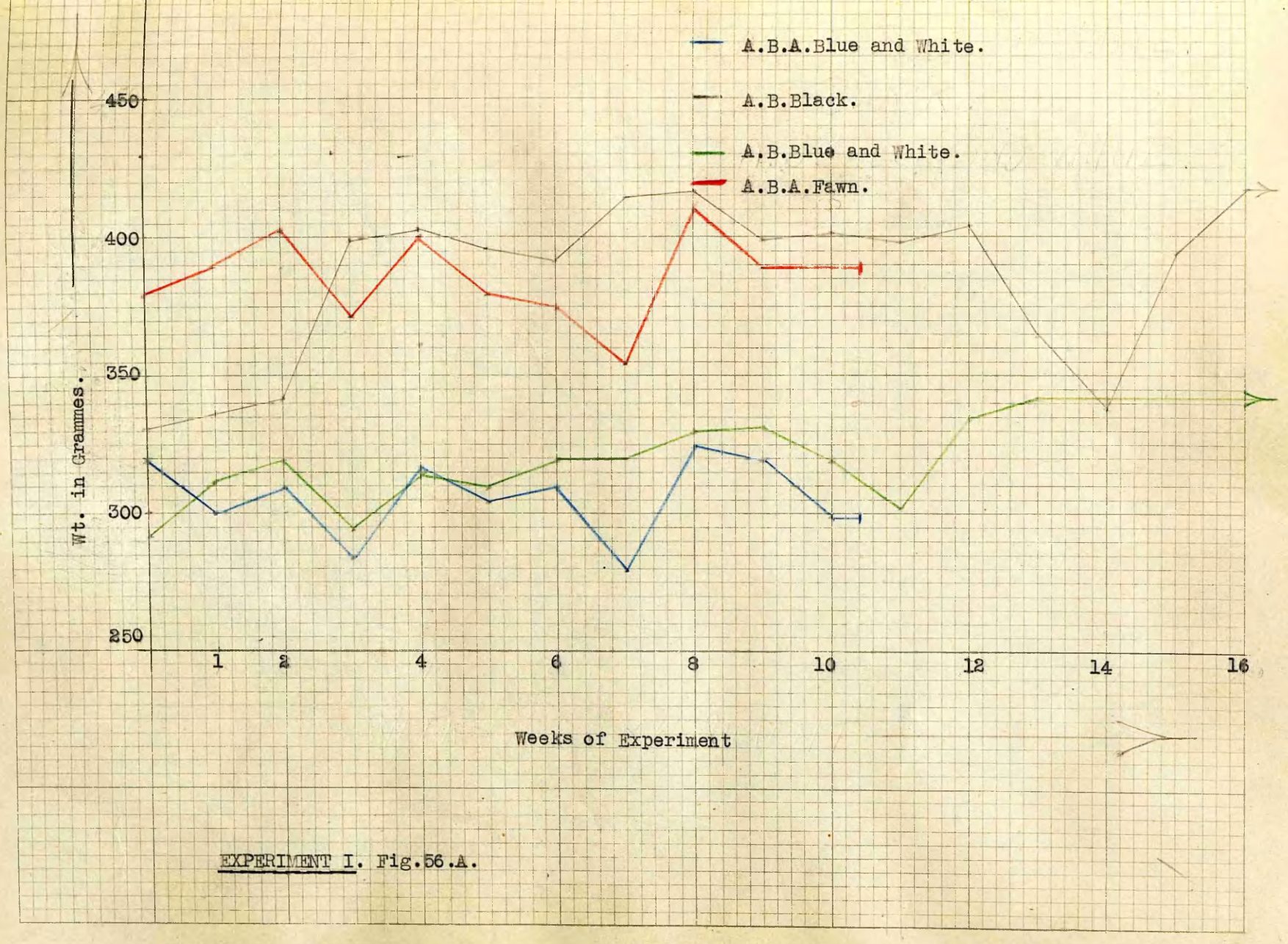
C.B. Blue & Red. Report as above.

C.B. Spot. On 7th day flight weak and gait rather unsteady. By 9th day recovered and flight quite strong and gait steady. On 62nd day gait was very unsteady and could not fly. Very paretic and unable to move.

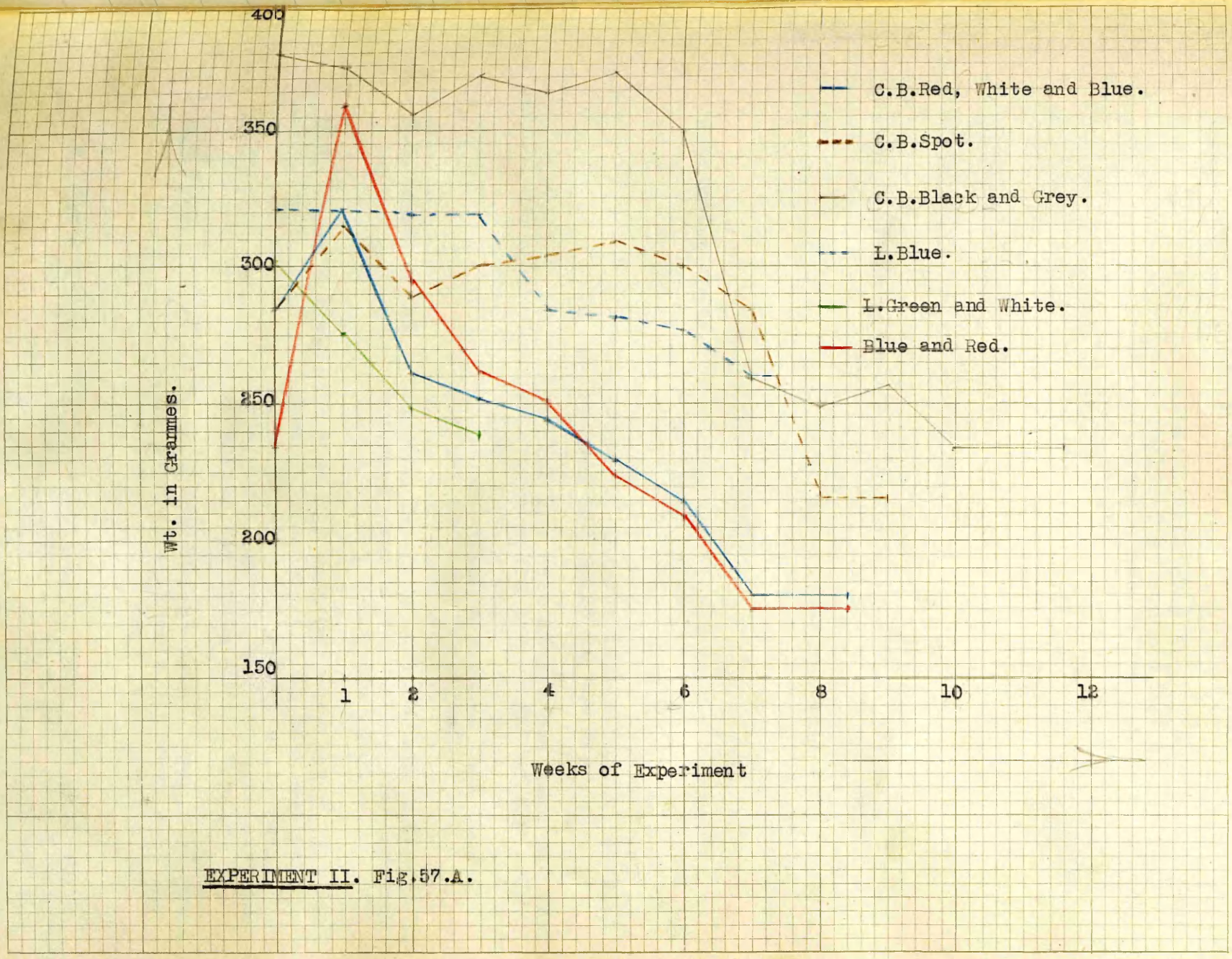
C.B. Black & Grey. On 76th day gait was unsteady and flight was weak. Next day condition worse; attempts at walking resulted in falling forward - emprosthotonus. On 80th day toes were flexed and walked with hobbling gait. Unable to fly. Died next day.

L. Blue. On 44th day flight good but easily exhausted and gait became unsteady after exercising. On 51st day gait very unsteady and legs unable to support body; unable to fly. Later in day, toes were flexed and head retraction followed.

L. Green & White. On 15th day unable to fly; gait was unsteady. Basal diet discontinued and given only water After/



EXPERIMENT I. Fig.56.A.



EXPERIMENT II. Fig.57.A.

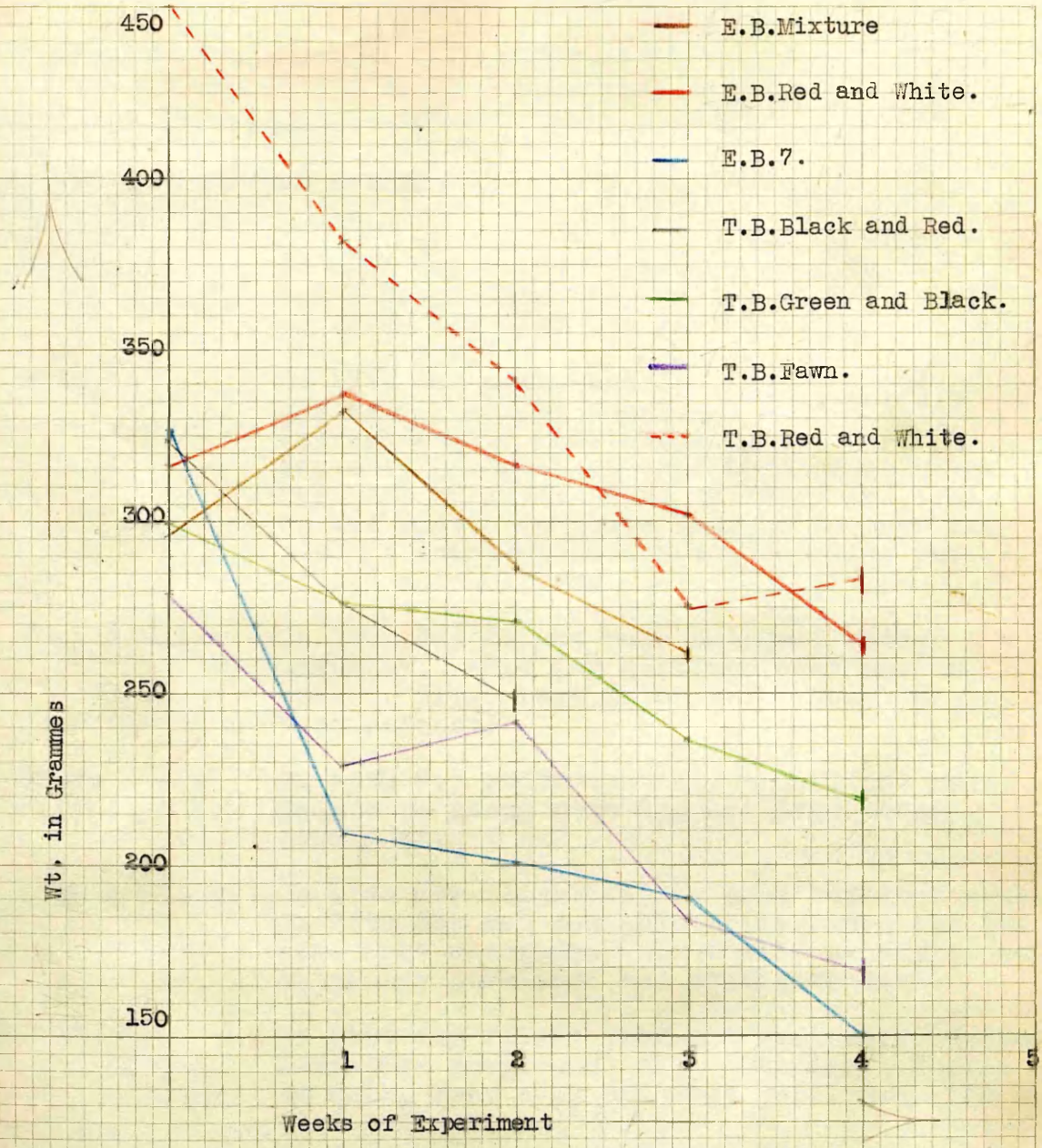
Experiment II (Contd.)

After 2 days able to fly and gait almost normal. Diet resumed but on 22nd day gait very unsteady and lay on side with head retracted.

Experiment III. Fig.58.

Basal Diet - 3rd Run Barley.

E.B. Mixture.	On 18th day flight weak but gait steady. On 23rd day flight short and easily exhausted; legs weak and stumbled. After 2 days, sat in corner; gait very unsteady and staggered with no apparent control of movement. Fell into series of emprosthotonic spasms.
E.B. Red & White.	On 25th day flight weak and stumbled on alighting. Next day easily exhausted and walked with staggering gait, falling forward on its breast. On 29th day lay in spasms of uncontrolled movement, dashing against the walls of the cage.
E.B. Y.	On 31st day gait ataxic and legs not very able to support the weight of the body; flight rather weak. On 33rd day in spasms of uncontrolled movement; head thrown forward and legs paralysed.
T.B. Black & Red.	On 20th day gait unsteady and attempts at movement resulted on falling on one side. Unable to fly and quite paretic.
T.B. Green & Black.	On 21st day gait unsteady and flight weak. Gradual recovery and on 35th day gait quite steady, but flight still weak. Later very paretic and inco-ordinate in movements and quite unable to fly.
T.B. Fawn.	On 21st day flight rather weak, but gradual improvement till 34th day when gait was unsteady. In 2 days legs paretic and unable to fly.
T.B. Red & White.	On 21st day flight poor, but gait quite steady. After 28 days gait rather unsteady but on 29th day flight very poor and was ataxic, with wings spread out on floor.

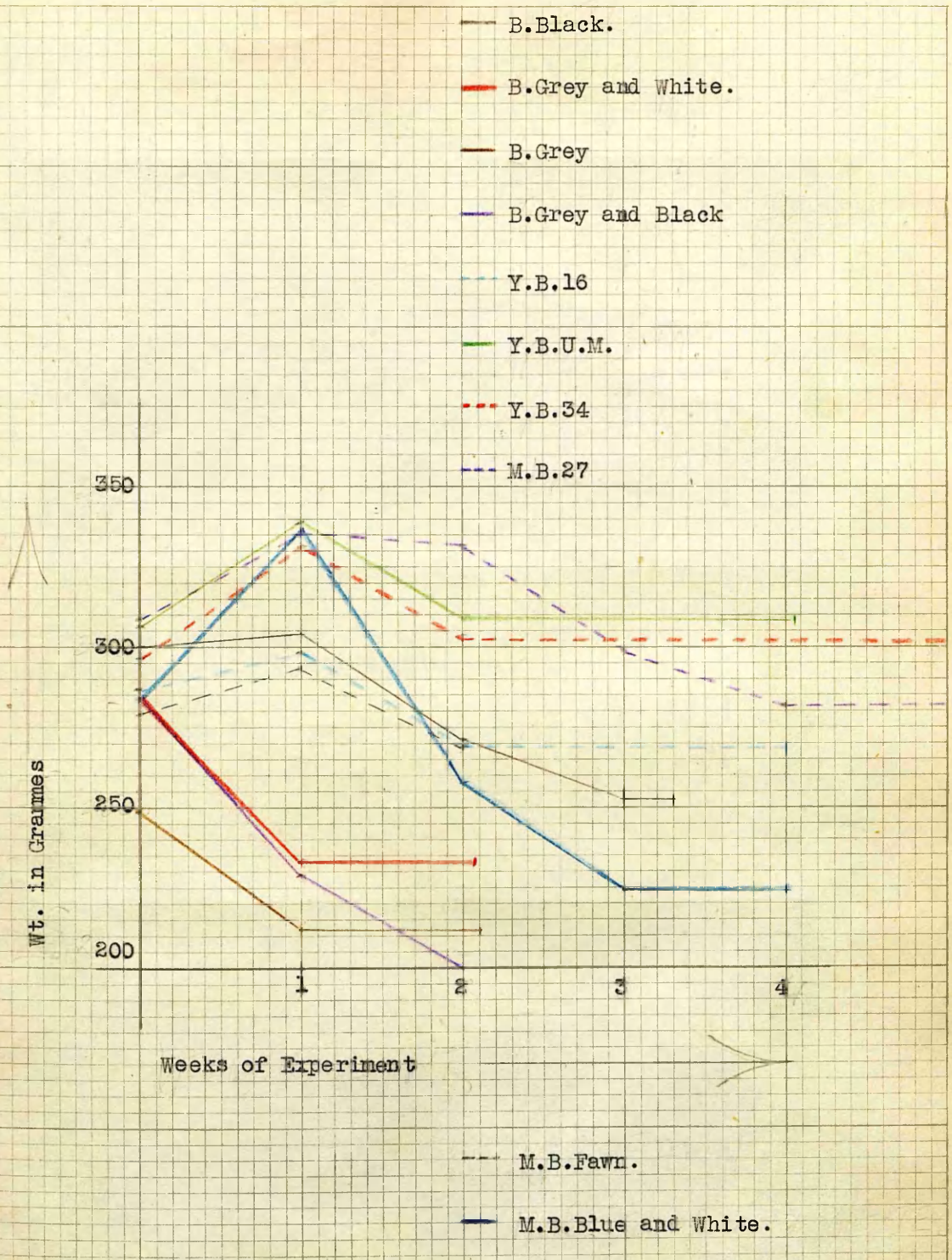


EXPERIMENT III. Fig.58.A.

Experiment IV. Fig.59.

Basal Diet - 4th Run Barley.

B. Black.	On 26th day suddenly became weak in legs and 2 hours later lay paralysed and unable to move. It died the same day.
B. Blue.	On 17th day weak, easily exhausted and gait unsteady. Flight short but 2 days later improvement in gait and flight. On 22nd day sat in corner of cage huddled up with feathers ruffled. Gait unsteady and fell on one side. Complete loss of balance and posture maintenance. Next day quite paralysed and helpless.
B. Grey & White.	On 15th day flight poor and gait rather unsteady. Next day gait unsteady and unable to fly. Legs later paralysed, but no head retraction.
B. Grey.	On 15th day quite unsteady and flight poor. Unable to stand next day and legs later paralysed.
B. Grey & Black.	On 17th day flight fairly good but legs weak. On 26th day was not able to fly much and its legs were unsteady. Was easily exhausted and later lay paralysed and unable to move.
Y.B. 16.	On 21st day flight poor and gait very unsteady. Condition progressed till 28th day. Unable to walk, very weak and legs paralysed. No head retraction.
Y.B. U.M.	On 21st day gait unsteady but flight fairly good. On 30th day gait very unsteady, movements inco-ordinate, later paretic and unable to stand.
Y.B. 34.	On 28th day very weak in flight and quite unsteady. Movements were inco-ordinate. 35th day - gradually less able to move and developed toe flexion and fell forwards.
M.B. 27.	On 28th day gait inco-ordinate, flight without direction. Complete absence of postural tone and of sense of direction. On 35th day paretic with head retraction.



EXPERIMENT IV. Fig.59.A.

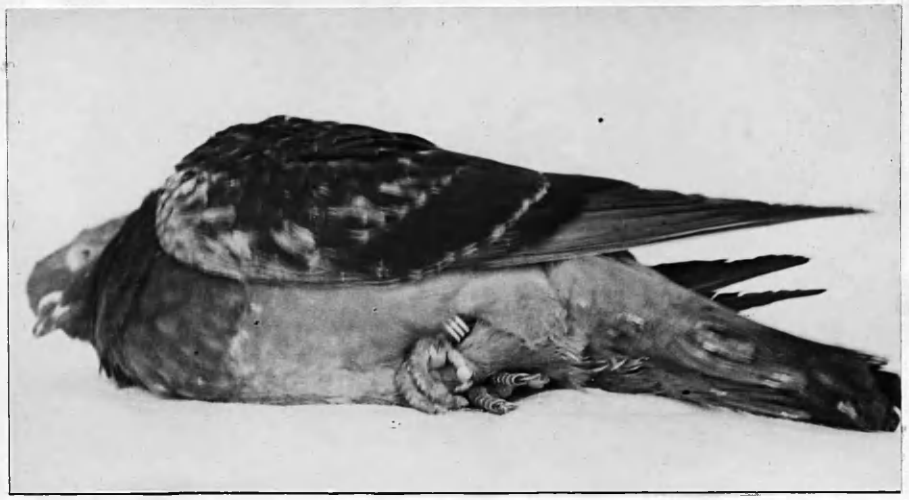
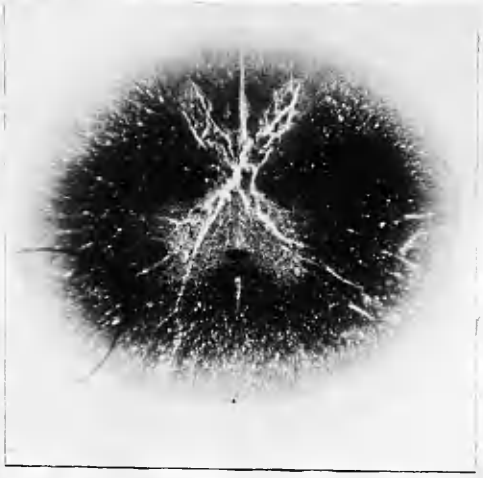


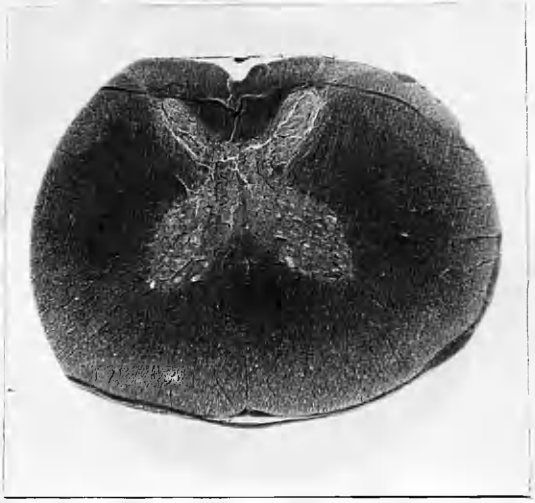
Fig.60. Pigeon after 17 days' feeding Fourth Run barley.

Fig.61. Pigeon after 20 days' feeding Fourth Run barley.

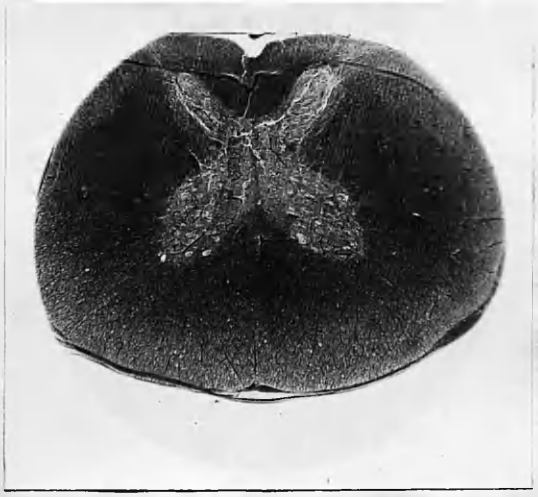
Fig.61.A.



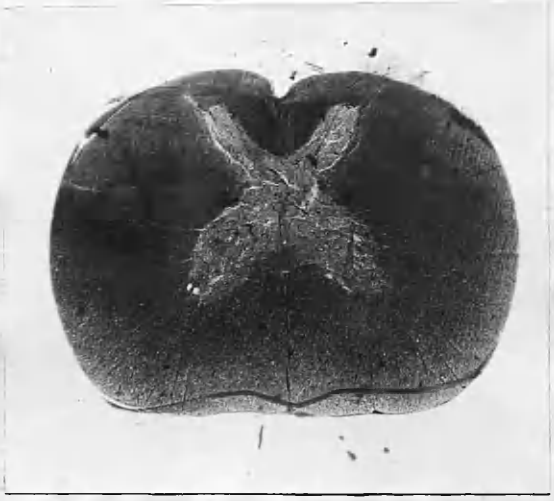
T.S. Spinal Cord Pigeon.
20 days' 4th run barley (Marchi).



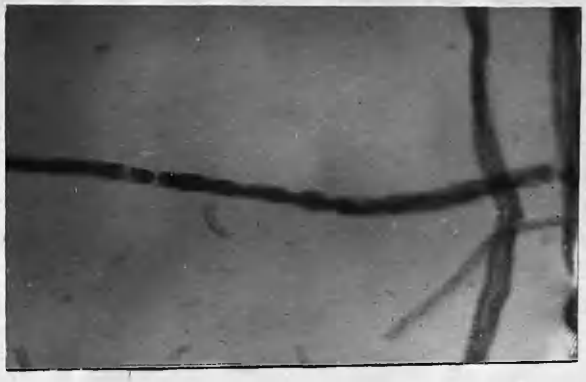
T.S. Spinal Cord Pigeon.
20 days' 4th run barley + 0.33 gm. $Na_2HPO_4.12H_2O$ (Marchi).



T.S. Spinal Cord Pigeon.
39 days' 3rd run barley (Marchi).



T.S. Spinal Cord Pigeon.
4th run barley. Marmite curative dose (Marchi).



Peripheral Nerve Pigeon.
4th run barley (Marchi).

Experiment IV (Contd.)

M.B. Fawn. On 19th day legs weak, gait unsteady but flight quite good. Later general condition weak and on 22nd day sat huddled in corner; flight was short and weak and gait inco-ordinate - attempts at walking terminating on falling to right side. Later head retraction developed.

M.B. Blue & White. On 19th day while flight good, legs were very weak and gait unsteady. By 28th day gait was inco-ordinate and later paralysed, with toes flexed.

Experiment V. Fig.62.

Basal Diet - 3rd Run Barley.

Curative Treatment - Barley dust per os.

E.B. Mixture. On 25th day sat in corner and very weak. Gait very unsteady, staggered without control of direction. 3.30 p.m. - given 10 gm. barley dust in mash by mouth. 4.15 p.m. - very weak and appearance of head retraction. 6.15 p.m. - still no visible change, fell on right side and gait very unsteady. 8 p.m. - still weak but definite improvement. 9.30 p.m. - no head retraction apparent but still weak. On 26th day given 5 gm. barley dust as before. Walked with increasing facility but gait was not quite steady. Next day 5 gm barley dust given but condition still weak and fell forward, attempt at flight unsuccessful. On 28th day much stronger though gait still weak. Able to fly a short distance but easily exhausted. It was found dead next day. Examination of peripheral nerves by Marchi's method showed granular myelin with fragmentation.

E.B. R.W. On 28th day very weak and in series of spasms of uncontrolled movement. Lay on side quite paralysed when not in spasms. 4.0 p.m.-given 5 gm. barley dust per os. In an hour slight improvement but muscular spasms still occurred. Next day very much improved and flew well. Gait/

Fig.63. Pigeon after 26 days' feeding 3rd. Run barley.

Fig.63.A. Pigeon after 2 doses (4 gm.) barley dust.

Experiment V (Contd.)

Gait was quite steady. Remained well and quite normal for 8 days without further treatment. Experiment concluded.

L. Blue. On 26th day unable to fly and gait very unsteady. Later developed head retraction. At 5 p.m. given 5 gm. barley dust per os. 6.45 p.m. - no signs of improvement and unable to stand; head retraction still present. Next day - 9 a.m. - given 5 gm. barley dust as before. 10 a.m. - improvement but unable to sly. 2 p.m. - flight quite strong and gait much steadier. Next day dose repeated. Flight strong and gait quite steady. It remained well and normal for 7 days when experiment was concluded.

T.B. G. & B. On 26th day gait very unsteady and unable to balance. Later quite paretic and unable to stand. Given 5 g.m. barley dust at 4 p.m. Next day greatly improved but still rather unsteady. 27th day - dose repeated and later flew short distance. Improved gradually to normal condition. Improvement maintained for 12 days, when experiment was concluded.

Experiment VI. Fig.64.

Basal Diet - 4th Run Barley.

Curative Treatment - Dried yeast per crop.

C.B. Spot. After 15 days very paretic and unable to stand. Given 1 gm. dried yeast in 5 mil. water per crop. After 5 hours there was no improvement. Dose repeated. Still no improvement. Next day was able to fly a short distance, but gait still rather unsteady. Dose repeated, but no change. On 17th day slight improvement, but gait still unsteady. Dose was repeated daily for 3 days, but there was little improvement as the gait was still unsteady though flight stronger. On 21st day daily dose increased to 2 gm. daily and after 9 days the gait was steady. The legs were much stronger and flight was quite good. After exercising/

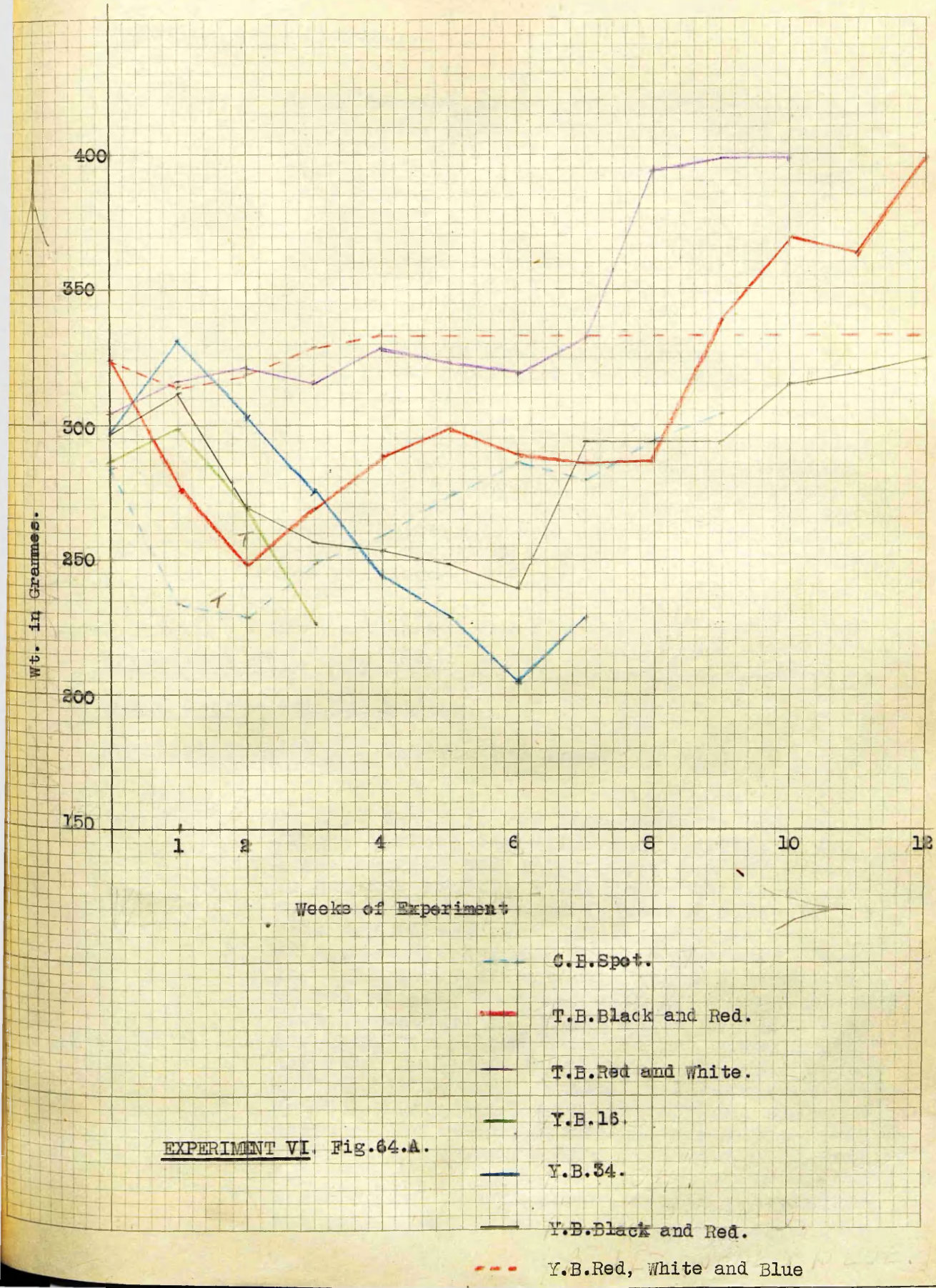
Experiment VI (Contd.)

exercising, its gait was unsteady and it was easily exhausted. After 50 days' curative treatment, the flight and gait were quite normal in every way. Histological examination of peripheral nerves failed to show any disturbance of the myelin sheath.

T.B. B. & R. After 16 days gait very unsteady; staggered from side to side. No control of movement. Given 1 gm. dried yeast per crop - no change. Dose repeated at 4 p.m. 7 p.m. - no change. Next day no improvement. Dose repeated. On 18th day flight stronger and much improved but gait still unsteady. Dose repeated daily for 6 days, but no definite improvement, so daily dose increased to 2 gm. After 3 days still ataxic but flight improved. After 70 days' treatment the flight was strong and quite normal but the gait, while not unsteady, was weak and not normal. Histological examination of sciatic nerves showed some clumping of the fibres.

T.B. R. & W. After 18 days legs weak and ataxic, unable to fly and later unable to stand. Given 1 gm. dried yeast per crop daily but no improvement after 3 days. Dose increased to 2 gm. daily, after 4 days able to fly short distance but gait unsteady. On 37th day there was no definite improvement, and even after 70 days the gait was weak and flight was short and ill-sustained. Histological examination of the sciatic nerves showed some clumping of fibres and some granulation of myelin.

Y.B. 16. After 15 days gait unsteady and flight poor. Later gait was very unsteady and unable to walk. Given 1 gm. dried yeast per crop. Slight improvement next day. But on 17th day unable to walk and weak. Dose repeated but next day unable to fly and quite paretic. On 19th day slight improvement which was only transient. Toes became flexed and was unable to move. It died next day. Histological examination showed granulation of myelin and clumping of fibres.



EXPERIMENT VI. Fig.64.A.

Experiment VI (Contd.).

Y.B. "34" After 16 days gait unsteady and inco-ordinate, flight very poor. Given 1 gm. dried yeast per crop. No improvement. Dose repeated next day but condition worse and developed head retractions. Dose increased to 2 gm. On 18th day slight improvement but still weak and later toes flexed and wings outspread. Dose repeated. Next day no improvement and very paretic. Later died. Histological report was as for "16".

Y.B. B.R. During 86 days with supplementary diet of 1 gm. dried yeast per day, remained quite active and well.

Y.B. R.W.B. With supplementary diet of 1 gm. dried yeast per day. On 51st day stumbled on alighting and gait was inco-ordinate, flight was weaker and some ataxia. Remained so for 12 days and became quite well and normal by 76th day. Remained normal till 163rd day when experiment was concluded.

Experiment VII. Fig. 65.

Basal diet - 4th Run barley.

Curative Treatment - Marmite per crop.

M.B. "27" On 31st day gait was inco-ordinate and weak, flight without direction and ataxic. Given 1 gm. Marmite per crop. Next day improvement in general condition but gait still inco-ordinate and legs weak. Gradually became worse till on 38th day it was very paretic and had retraction of head. Given 2 gm. Marmite per crop. Next day gait quite steady and flight strong. Remained so till 42nd day when gait very unsteady, toes flexed though flight quite strong. Next day head retraction recurred and given 1 gm. Marmite. General improvement, but gait became unsteady next day. Gradually became weak till by 52nd day typical head retraction, spasms of uncontrolled movement and later paresis developed. 2 gm. Marmite were given but died next day.
Histological report showed myelin granulation and some clumping of fibres.

Experiment VII (Contd.).

M.B. B.W. On 19th day legs weak and gait unsteady though flight strong. By 43rd day gait was so unsteady it could not walk or fly. Given 1 gm. Marmite per crop, but no improvement. Next day its toes were flexed and there was emprosthotonus. Dose repeated but no improvement. On 45th day dose repeated but remained weak and paretic. Died later.
Histological report - myelin degeneration of sciatic nerves.

M.B. "F". On 19th day legs very weak and gait unsteady. By 22nd day attempts at walking resulted in falling on side. There was gross inco-ordination. Given 2 gm. Marmite per crop. After 5 hours improvement in general condition - flight stronger and gait steadier. After 3 days emprosthotonus evident and gait unsteady. Next day typical head retraction and paretic. Given 2 gm. dried yeast, but no improvement. Died next day.
Histological report as for B.W.

M.B. R.B. With supplementary diet of 1 gm. Marmite per crop daily, remained quite normal during 163 days and maintained its weight.

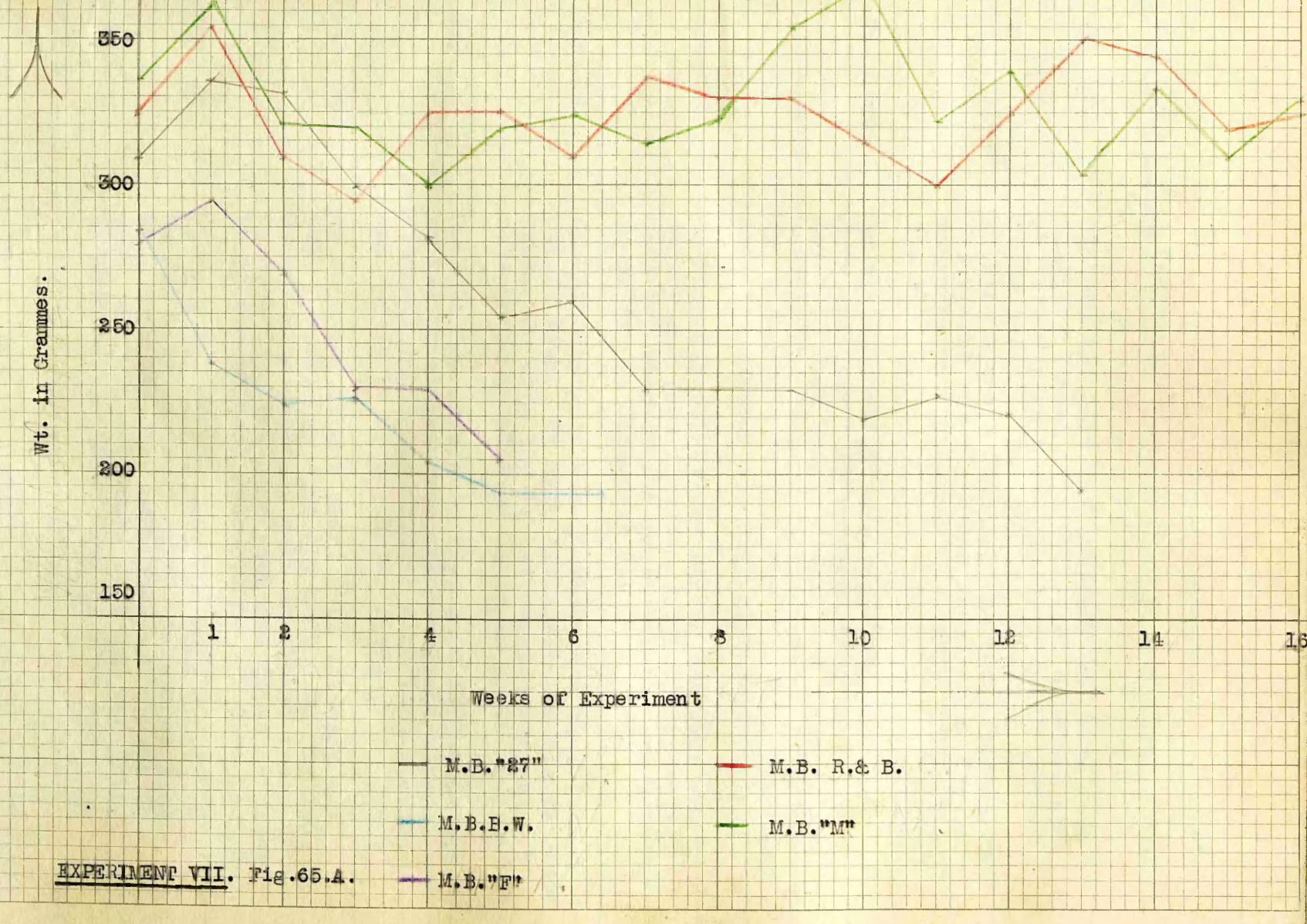
M.B. "M". Report as for R.B.

Experiment VIII. Fig. 66.

Basal Diet - 4th Run barley.

Supplementary Diet - 0.33 gm. Sodium Phosphate per crop daily, given in water.

4B. 7. On 39th day gait was unsteady and next day its toes were flexed and flight was weak. On 46th day it lay with legs extended and toes flexed and was unable to move. Attempts at movement were made with drooped wings. There was no head retraction. It died on the 50th day.



EXPERIMENT VII. Fig.65.A.

Experiment VIII (Contd.).

4B. 10. On 40th day gait was unsteady and toes were slightly flexed. It recovered somewhat and by 51st day gait was steadier and flight quite strong. On 66th day stumbled on walking a short distance and easily exhausted. Its flight was short and weak. Next day it was paretic and unable to stand. It died on the 68th day.

4B. Blue. On 41st day flight was short and weak and gait unsteady. It remained thus till 45th day when toes became flexed and attempts at walking resulted in falling over. By 56th day was unable to fly; lay with legs extended and toes flexed. It died next day.

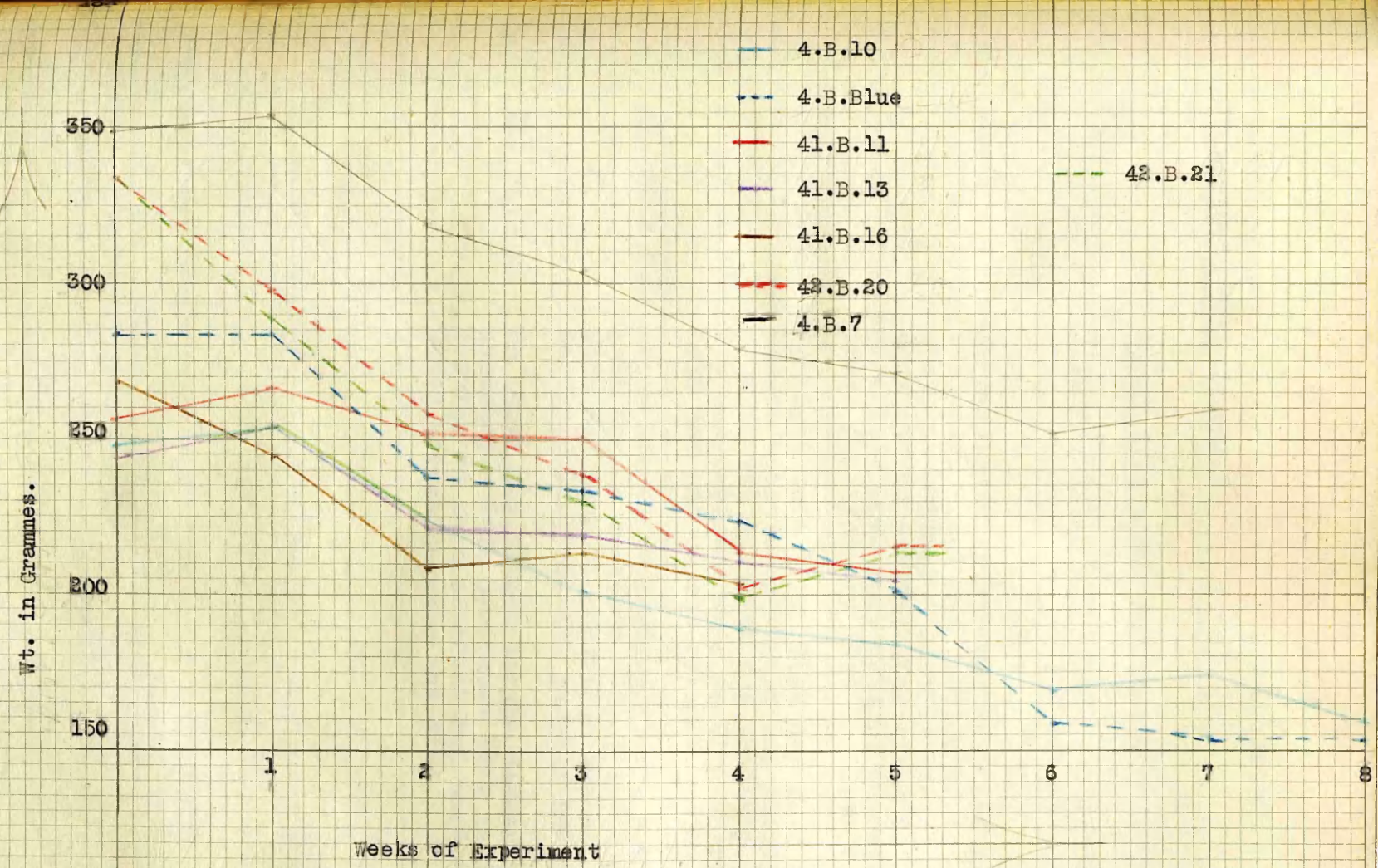
41B. 11. On 24th day flight was strong but gait unsteady and toes flexed. Continued thus till 35th day when it lay quite paretic and unable to move. It died next day.

41B. 13. On 28th day gait was unsteady and toes flexed, flight weak. By 30th day unable to fly or walk. On 35th day it lay paretic and not able to move and died later in day.

41B. 16. On 26th day unable to fly, gait very weak and walked on flexed toes and outspread wings. After 28 days, lay paretic with toes flexed and unable to move. Later died.

42B. 20. By 24th day flight poor, gait unsteady and toes flexed. On 31st day unable to fly and walked with unsteady gait on flexed toes. On 37th day, lay paralysed and quite helpless; there was no head retraction. It died next day.

42B. 21. On 24th day flight weak and toes flexed. Walked with unsteady gait. Continued thus till 39th day, lay paralysed and unable to move, did not eat much food and was thin. No head retraction. Died on 40th day.



EXPERIMENT VIII. Fig.66.A.

Experiment VIII (Contd.).

Basal Diet - 2nd Run barley.

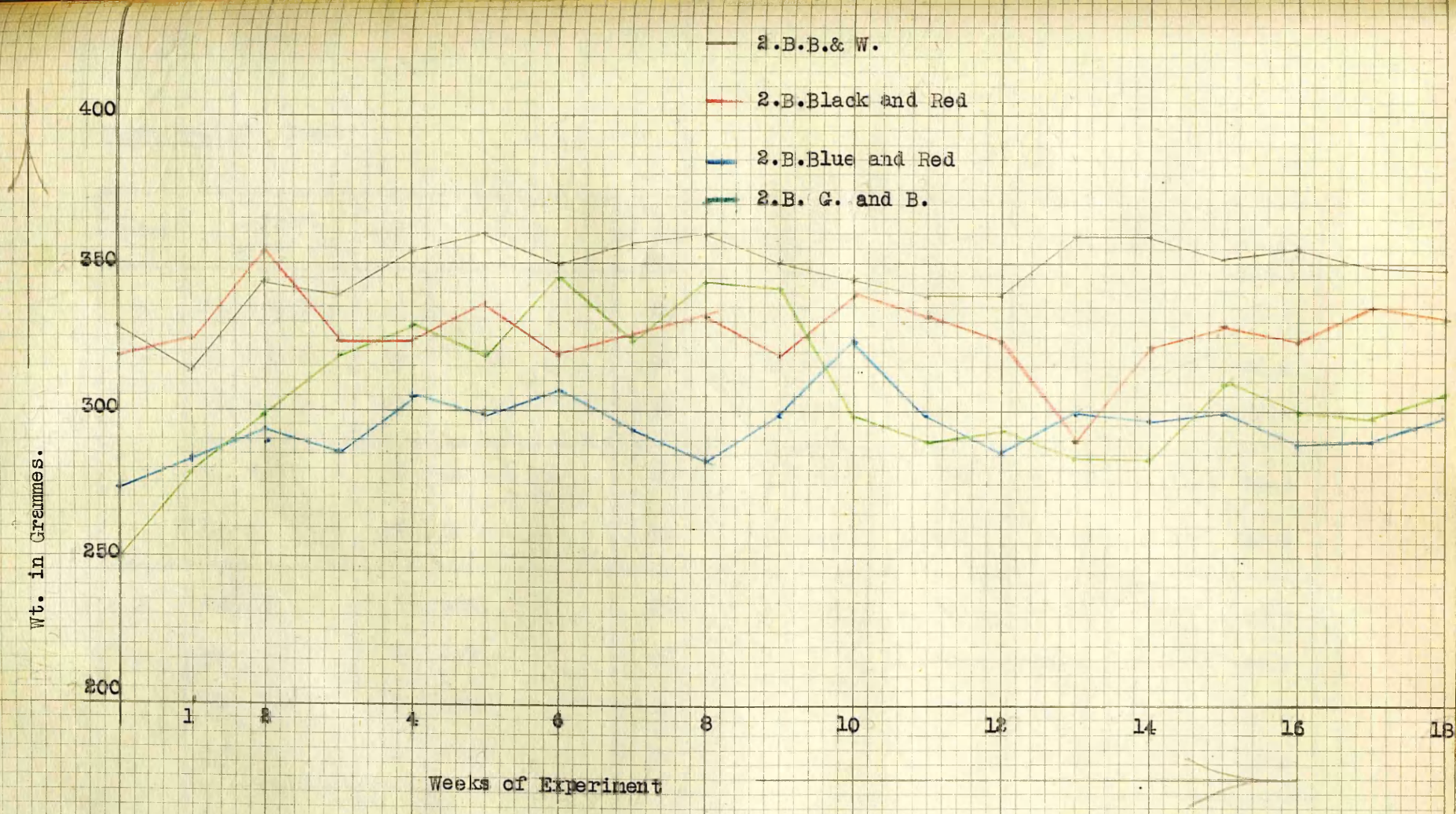
Supplementary Diet - 0.33 gm. Sodium Phosphate per crop daily.

2B. G & B. On 128th day flight was weak and gait became unsteady. Next day flexion of toes and opisthotonus evident. Went in spasms of incoordinate movements. Was given 0.5 gm. Sodium phosphate per crop. Slight improvement, but later relapsed and died on 130th day.

2B. B & W. Remained well till 198th day when its condition became weak and gait was not steady. Next day toes flexed and gait was unsteady. Unable to fly and movement only on flexed toes. Paretic next day and unable to move. Died on 201st day.

2B. Bk.& R. After 131 days' feeding flight was weak and gait became unsteady. Next day flexion of toes and general condition weaker. Unable to fly. On 133rd day paretic and died later.

2B. Be.& R. On 140th day flight became weaker and gait was unsteady. This continued till 149th day when flexion of toes apparent and unable to fly. Later became paretic and died.



EXPERIMENT VIII. Fig.66.B.

Experiment I. Fig.67.

Group	Animal Cage No.	Code No.	Diet	Duration of Expt. Days.	Wt.of Bone gm.	Wt.of Ash gm.	% Ash	% CaO	% P_2O_5
A	RD. G.& B.	13	B.	20	3.1618	1.5348	48.54	56.26	42.9
	RD. W.& F.	14	B.	26	3.7276	1.8542	49.74	54.53	44.3
	RD. G.& R.	15	B.	43	3.9240	2.0084	51.17	55.51	43.9
	RC. W.	16	B. + 1 gm. D.Yeast.	20	2.5004	1.7492	69.95	56.11	43.4
	RC. B.	17	B. + 1 gm. D.Yeast.	24	4.2234	2.2234	53.38	55.61	43.0
B	RAA. B.	1	AuB.	33	1.7480	0.7566	43.28	52.48	44.8
	RAA. B.& R.	2	AuB.	33	2.2184	1.0808	48.75	51.77	44.1
	RAA. G.& V.	3	AuB.	42	2.6346	1.3638	51.76	52.29	45.5
	RAA. R.	4	AuB.	52	2.3476	1.1642	49.58	51.34	46.0
	RAB. F.	5	AuB + 1 gm. D.Yeast.	31	2.5264	1.1830	46.84	55.52	45.1
	RAB. V.	6	AuB + 1 gm. D.Yeast.	45	2.2742	1.1260	49.51	56.14	46.4

Experiment II. Fig.68.

Group	Animal Cage No.	Code No.	Diet	Duration of Expt. Days.	Wt.of Bone gm.	Wt. of Ash gm.	% Ash	% CaO	% P_2O_5
A	H. R.	10	B + 1 c.c. fresh lemon Juice, 3 drops Haliverol.	22	3.5358	1.6918	47.85	57.14	44.3
	H. V.	11		35	3.0762	1.4142	45.96	56.79	42.8
	H. U.	12		56	3.1350	1.5102	48.17	56.98	43.4
B	CLO. R.& B.	7	B + 1 c.c. fresh lemon Juice, 1 c.c. Cod-liver oil.	18	3.7888	1.9792	52.24	53.18	44.3
	CLO. B.& W.	8		26	3.9896	1.9962	50.03	53.78	44.7
	CLO. R.& W.	9		27	3.6934	1.7068	46.20	56.56	44.8

Experiment III. Fig.69.

Group	Animal Cage No.	Code No.	Diet.	Duration of Expt. Days.	Wt.of bone gm.	Wt. of Ash gm.	% Ash	% CaO	% P_2O_5
A	RP. G.& W.	24	B + 1 c.c.fresh lemon juice, 3 drops Haliverol 0.33 gm.Na_2HPO_4, $12H_2O$.	46	0.8928	0.4509	50.51	52.95	45.2
	RP. G.& B.	25		103	3.5164	1.8676	53.14	56.61	42.9
	RP. F.	26		105	3.6190	1.9292	53.30	56.58	42.1
	RP. G.& R.	27		66	4.3472	2.2218	51.12	55.35	44.2
B	RM. G.& W.	21	B + 1 c.c. fresh lemon juice, 3 drops Haliverol, 0.33 gm. Na_2HPO_4, $12H_2O$ 1 gm. Marmite.	32	3.4688	1.6914	48.35	52.76	44.1
	RM. B.	22		61	2.8646	1.2724	44.39	56.82	44.8
	RM. W.	23		63	3.5616	1.7288	48.54	56.40	44.1
	RM. F.& W.	28		152	1.7630	0.8807	49.96	56.69	44.3

Fig.70. Rabbit after 3 weeks' feeding Unmilled barley.

Fig.70.A.

Brachial Plexus
Rabbit fed Unmilled
Autoclaved Barley
(stained Marchi).

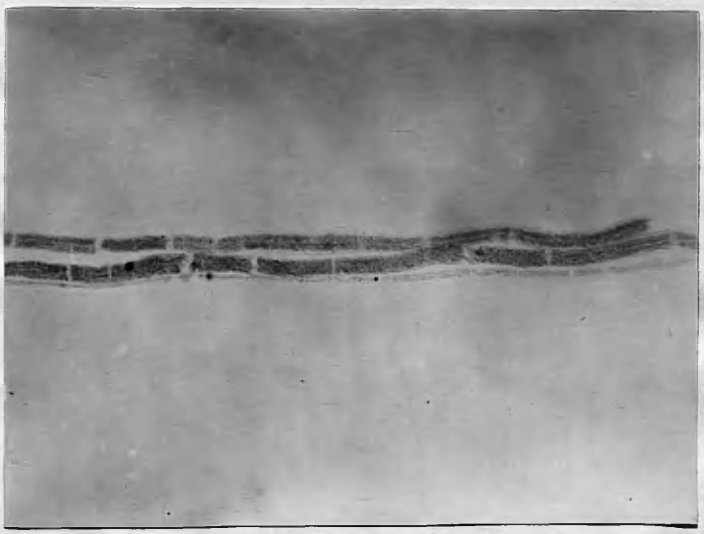


Fig.70.B.

Sciatic nerve Rabbit
fed Unmilled barley
(stained Marchi).

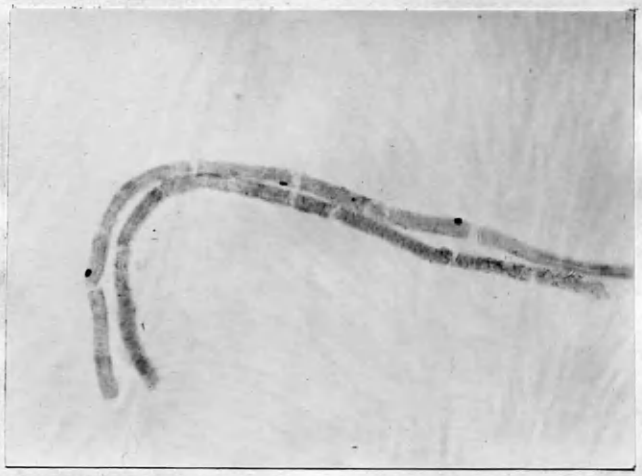
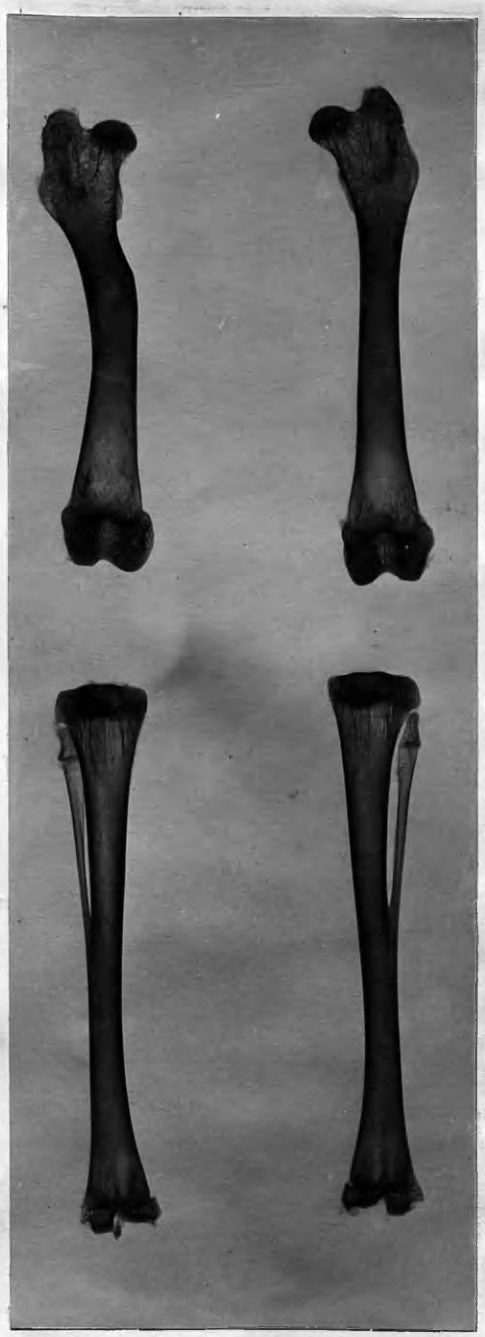
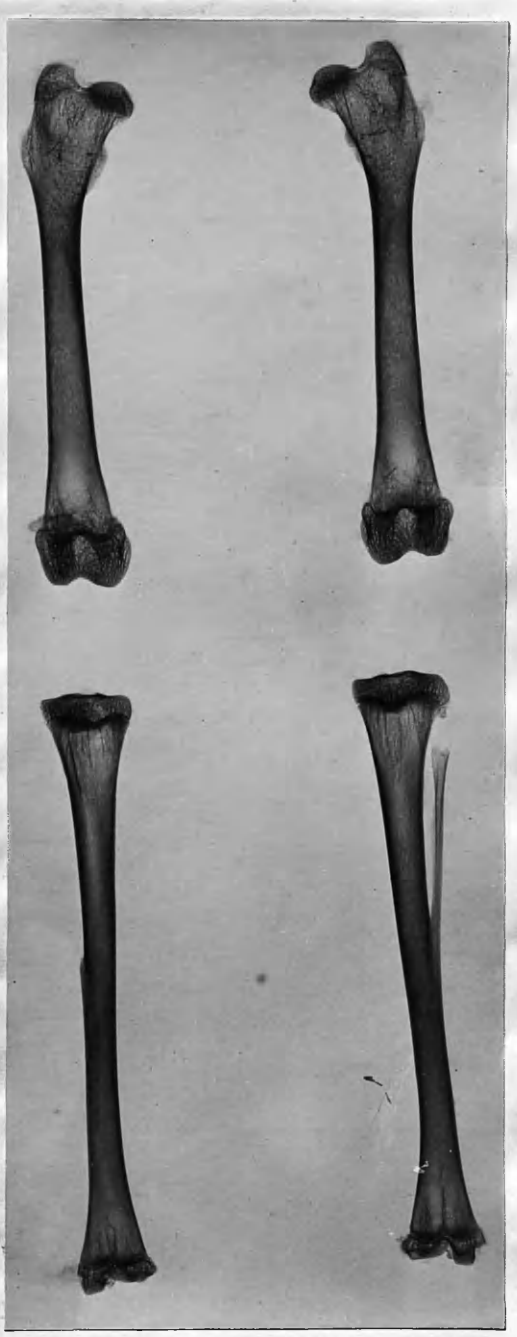


Fig.70.C. Sciatic Nerve Rabbit fed Unmilled Autoclaved barley + 1 gm. dried yeast.

Radiograms - RABBITS - 3 weeks' Experiment. Fig.71.



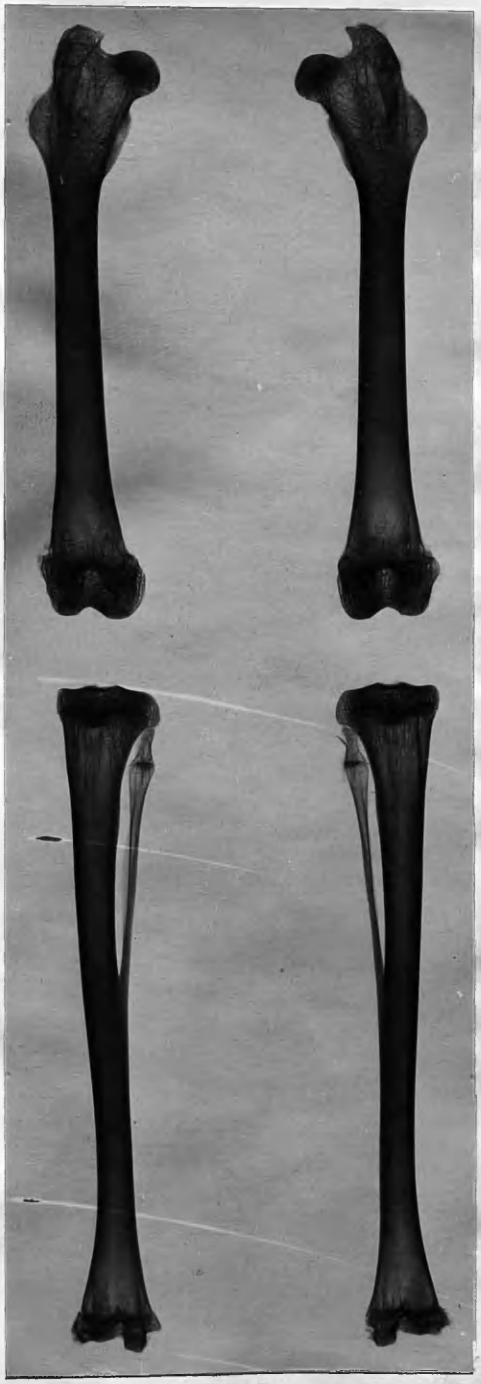
13.



16.

Diet.		Unmilled Barley.	Unmilled Barley + 1 gm. Dried Yeast.
gm.%	Ash	48.54	69.95
	CaO	56.26	56.11
	P O	42.90	43.40

Radiograms - RABBITS - 3 weeks' Experiment. Fig.71.



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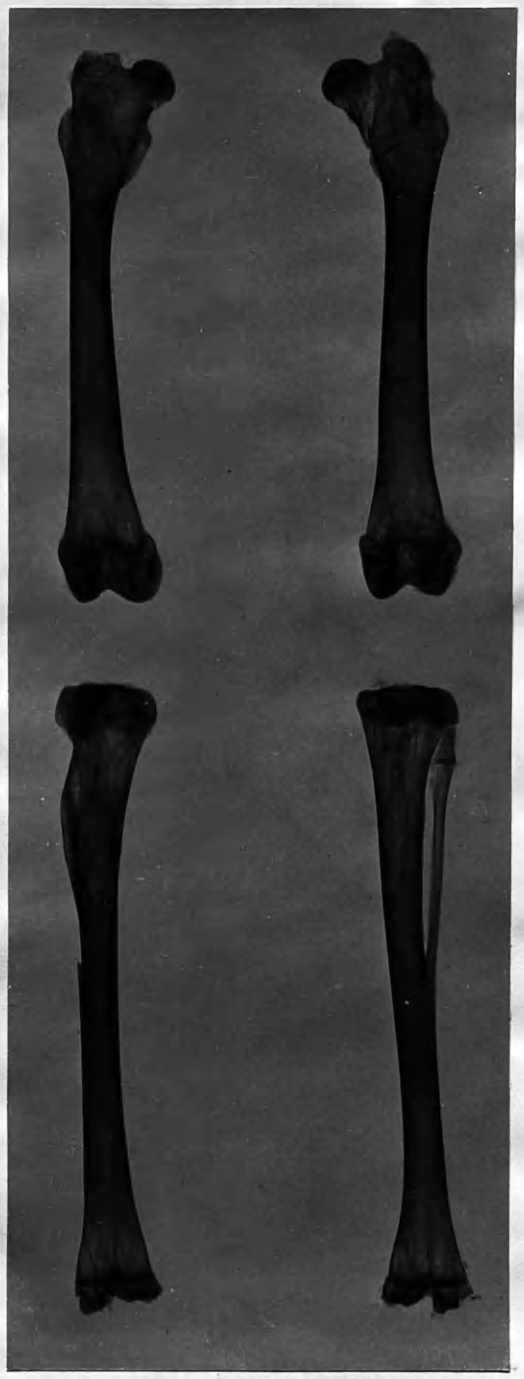
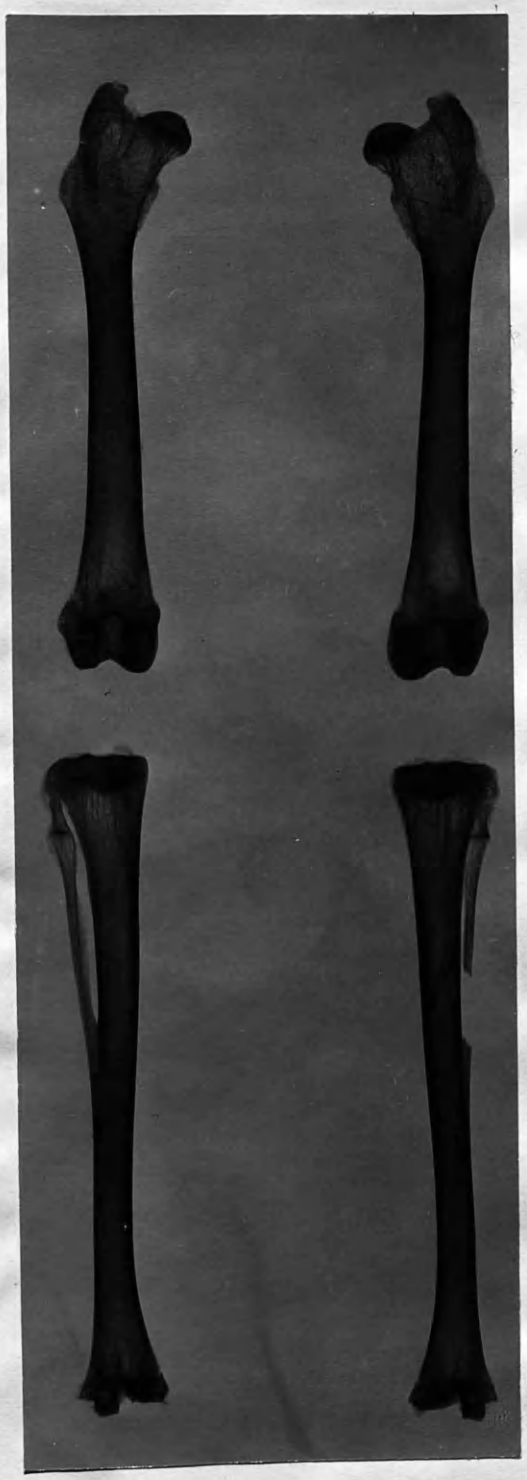


10.

		Unmilled Barley + 1 c.c. fresh lemon juice + 1 c.c. cod-liver oil.	Unmilled Barley + 1 c.c. fresh lemon juice + 3 drops Haliverol.
Diet			
gm.%	Ash	52.24	47.85
	CaO	53.18	57.14
	P_2O_5	44.30	44.30

Radiograms - RABBITS - 5 weeks' Experiment. Fig.72.



15.

Diet. Unmilled Barley

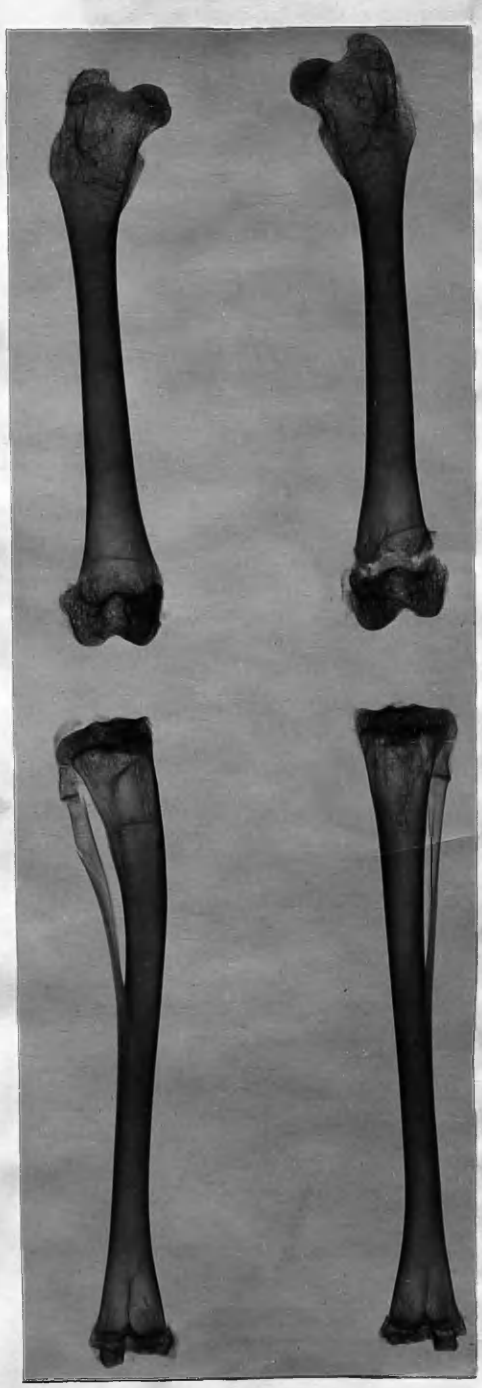
Ash	51.17
CaO	55.51
P_2O_5	43.90

11.

Unmilled Barley + 1 c.c. fresh lemon juice + 3 drops Haliverol.

	45.96
	56.79
	42.80

Radiograms - RABBITS - 5 weeks' Experiment. Fig.72.



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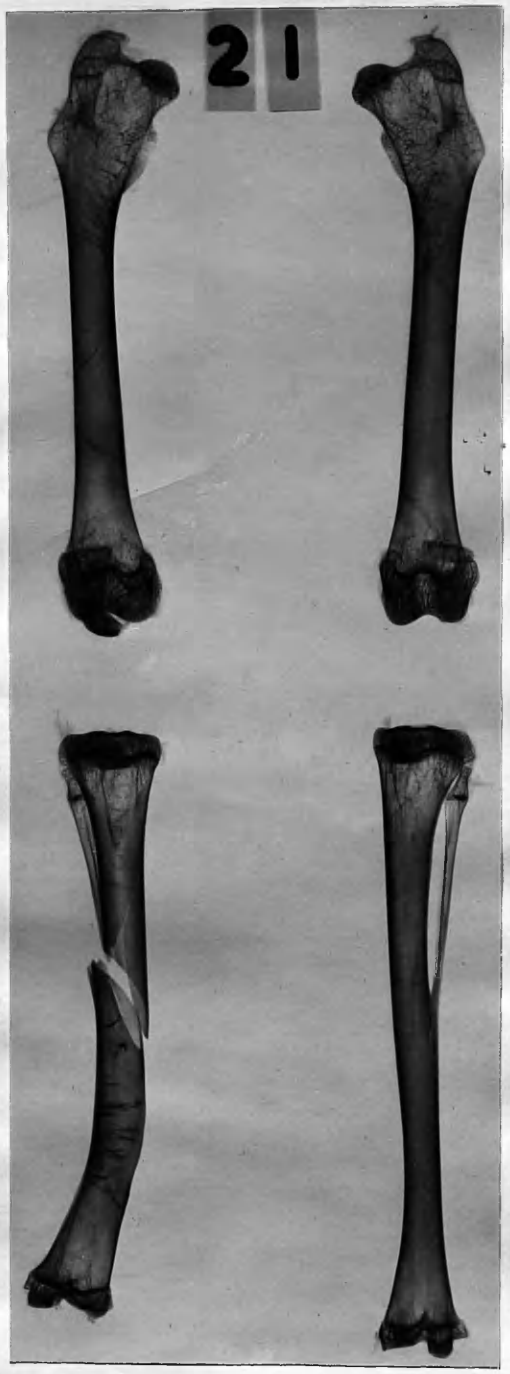
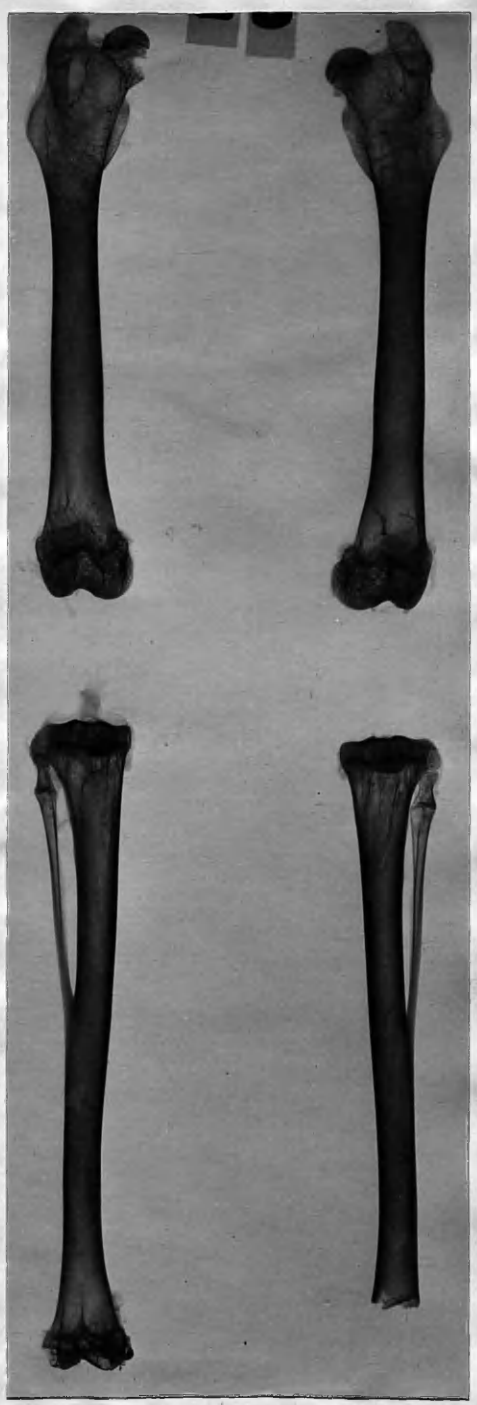


21.

	9.	21.
Diet.	Unmilled Barley + 1 c.c. fresh lemon juice + 1 c.c. cod-liver oil.	Unmilled Barley + 1 c.c. fresh lemon juice + 3 drops Haliverol + 0.33 gm. $Na_2HPO_4.12H_2O$ + 1 gm. Marmite.
Ash	46.20	48.35
CaO	56.56	52.76
P_2O_5	44.80	44.10

Radiograms - RABBITS - 9 weeks' Experiment. Fig.73.



23.

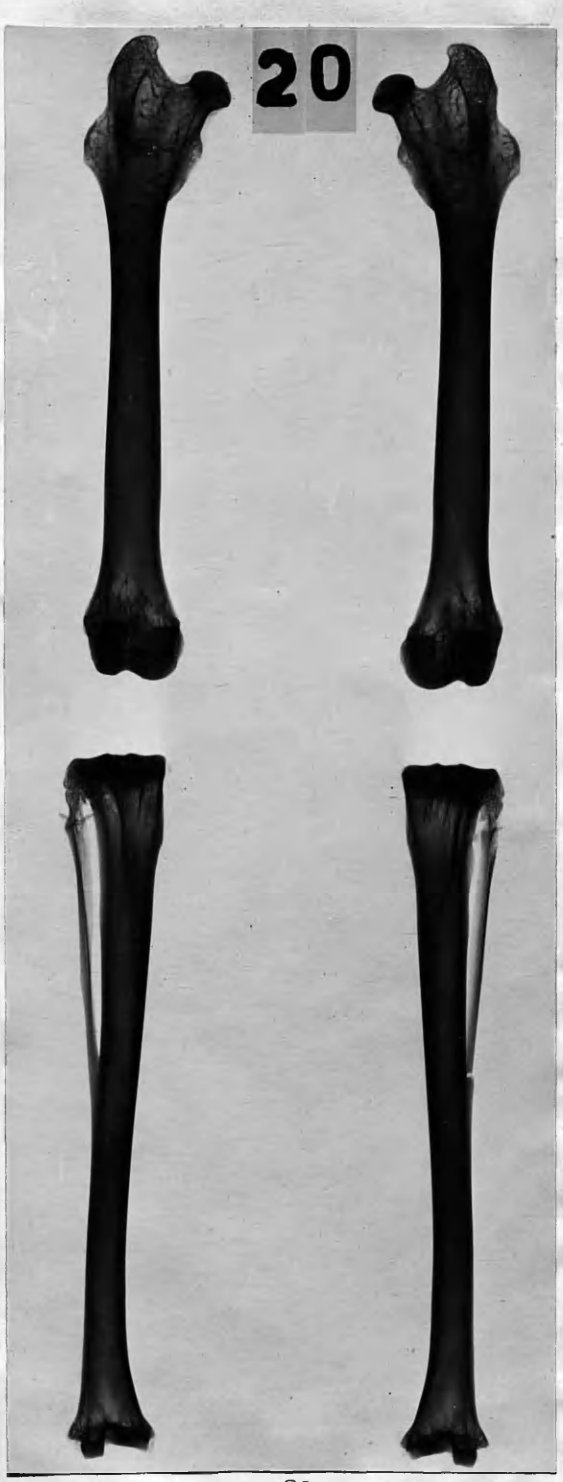
27.

Diet Unmilled Barley + 1 c.c. fresh lemon juice.
+ 3 drops Haliverol
+ 0.33 gm. $Na_2H.PO_4.12H_2O$
+ 1 gm. Marmite.

Same, but no Marmite.

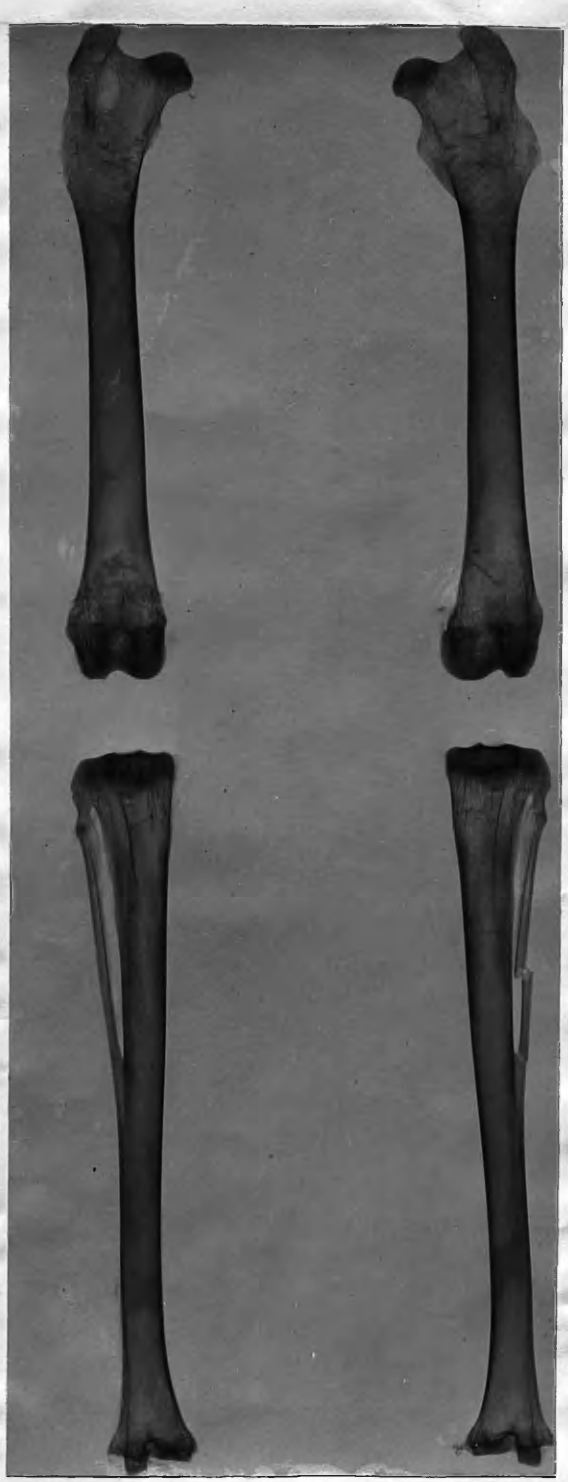
Ash	48.54	51.12
CaO	56.40	55.35
P_2O_5	44.10	44.20

Radiograms - RABBITS - 21 weeks' Experiment. Fig.74.



20.

Diet.	Unmilled Barley + 10 gm. fresh cabbage.
Ash	61.38
CaO	57.20
P_2O_5	42.60



28.

Unmilled Barley + 1 c.c. fresh lemon juice. + 3 drops Haliverol. + 0.33 gm. $Na_2HPO_4.12H_2O$ + 1 gm. Marmite.
49.96
56.69
44.30