by us,* and its actual fixation for any given H^+ concentration, as shown to-day, constitute, as will readily be understood, but different expressions of the same phenomenon, being a consequence the one of the other.

The bearing of these results on the general law of the independence of the optimum temperature of an enzyme of the concentration of the latter, will not have escaped notice. We see in them but instances of the working of this law, which they throw a new light upon, in indicating that in its original statement it is stated too briefly,† and now requires amplification. In consequence, re-stated, it becomes: the optimum temperature of any ferment, or ferment function, occurring in a given enzymic preparation is independent of the concentration of the enzyme, the duration of the action and *the chemical reaction—or* H^+ *concentration—of the medium* being constant.

The question asked at the beginning of the investigation thus finds itself answered; and, if in a manner different from the limited sense in which it was formulated, still the answer is none the less instructive, in the light which it throws on a general aspect of the mechanism of enzyme action.

The Effect of Certain Dietary Deficiencies on the Suprarenal Glands.

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[Plate 1.]

During recent years McCarrison (1919 *a*, *b*, and 1920) has published a series of important papers dealing with the effect of deficient diets on the various organs of the body. One very striking result which he has described was a great enlargement of the suprarenal glands, with pronounced increase in their content of adrenaline, in pigeons fed on polished rice and suffering from the consequent polyneuritis. McCarrison's experience led him to put forward the tentative suggestion, that the increased content of adrenaline might be significant of increased output of adrenaline during the development of the disease, and that this might account for the occurrence of œdema both in experimental polyneuritis produced in pigeons and in the wet form of human

* Arthur Compton, 'Roy. Soc. Proc.,' B, vol. 88, pp. 408–417 (1915).

† Arthur Compton, 'Roy. Soc. Proc.,' B, 87, pp. 245–254 (1914); 'Ann. Inst. Past.,' vol. 28, pp. 866–878 (1914).

beri-beri. In certain later experiments on pigeons fed on polished rice with the addition of butter and onions, he found that the polyneuritis, which was quickly produced on this diet, was only rarely accompanied by œdema.

There were several points in McCarrison's suggestive observations which seemed worthy of further investigation. In the first place it was not clear whether the effect of an exclusive diet of polished rice on the adrenals of pigeons was due to specific deficiency of the accessary factors concerned with the development of polyneuritis, or to the more general deficiency of protein, fat or salts entailed by such a diet. In the second place, assuming the effect to be a specific one, and McCarrison right in suggesting a causal connection between excess of adrenaline and the appearance of œdema, corresponding to the wet form of beri-beri, it seemed that the investigation of this connection might lead to results of more general pathological importance. While the work was in progress McCarrison himself found reason to withdraw the suggestion, which he had put forward at an earlier stage, so that the position to which my own experiments have led me is in substantial agreement with that which McCarrison now holds. At the same time it seems worth while to put on record the experiments made on this aspect of the question.

Finally, McCarrison's observations on the occurrence of similar adrenal changes in inanition, the apparent relation between their incidence and the fall of body weight in pigeons fed on polished rice, and his opinion based on the study of histological material, that the enlargement of the cortex is disproportionately large compared with that of the medulla in the adrenals of these birds, suggested that a partial explanation of the enlargement might be found in the storage in the cortex of the gland of lipoids set free by the breaking down of body tissues. This hypothesis has been tested by observations on the cholesterol content of the blood and adrenals of normal and experimental pigeons. The further possibility that enlargement produced by deposition of lipoids in the cortex might be associated with increased residual content of adrenaline has been tested by feeding rabbits and pigeons on diets containing excess of cholesterol.

Methods.

The pigeons used in these experiments were Blue Rocks, less than one year in age. In order to produce polyneuritis I have used artificial feeding with polished rice, except in one experiment, which will be referred to later. In the birds which were artificially fed, polished rice to an amount of 15–20 grm. was introduced daily into the crop. Great care was taken to prevent the birds so fed from becoming "crop-bound." When too much rice was found

in the crop at the time of feeding, one or two feeds were omitted till the crop was empty.

When other substances were added to the dietary, they were introduced into the crop by means of a graduated syringe with a catheter tube attached. "Marmite" was given in two strengths, 1 grm. in 2 c.c. or 2 grm. in 3 c.c., made up with tap water. Olive oil and cod liver oil were administered in the same way. Fat-free casein was given in the form of pellets, rolled from a thick paste of the protein with water, and dried.

In the first two weeks of feeding the birds retained the rice which was placed in their crops, but later some of them tended to reject a portion, though not all, of the feed. This tendency was only noticed in pigeons fed on polished rice without the addition of "marmite." When "marmite" was given, the rice was never rejected.

The initial and final weights and the cloacal temperature were recorded. The weighings were made with the crop empty. The final weighings were made after death, and when necessary, the crop was opened and emptied.

The birds were killed by a uniform method, the neck being rapidly dislocated. In the earlier experiments a culture was taken from the heart's blood in every case, but as these cultures were invariably sterile, this precaution was discarded in the later experiments. After death the adrenals were quickly dissected out, carefully cleaned from surrounding tissue and dried with filter paper before weighing. A saline extract of the glands was made, which was acidified, boiled, filtered, and made up to a known volume of 3 c.c. to 5 c.c. This solution was always perfectly clear and suitable either for colorimetric or physiological estimation of adrenaline.

For the estimation of adrenaline the routine method used was that of Folin, Cannon and Dennis (1912). It was found convenient in making up the standard uric acid solution to use twice as much 4 per cent. lithium carbonate solution as that recommended by these authors (vide Seidell, 1913).

Folin, Cannon and Dennis compared the results obtained by their method with those given by the physiological estimation devised by Elliott (1912) in a good many different animals, but it was thought advisable, in view of the important part played by uric acid in avian metabolism, to institute a further comparison on the suprarenal glands of normal and experimental birds. The results of such comparison showed clearly that for purposes of this investigation the figures obtained by the colorimetric method indicated the actual content of adrenaline, and that no appreciable error was introduced by the presence of other substances which might be capable of giving the colorimetric reaction. Table I gives some of the results, and I have introduced a typical tracing of an estimation on a pithed spinal cat (fig. 1).

Table I.—Comparison of Methods of Estimating Adrenaline.

Initial weight in grammes.	Weight of adrenals in milli-grammes.	Weight of adrenaline in milligrammes determined physiologically.	Weight of adrenaline in milligrammes determined colorimetrically.	Remarks.
350	21·0	0·028	0·030	Normal.
450	19·0	0·028	0·031	,,
320	31·0	0·095	0·095	,, (some time in the laboratory).
319	16·0	0·045	0·046	,,
319	27·0	0·060	0·056	,,
440	67·0	0·105	0·103	Pigeon with polyneuritis fed on polished rice.
430	71·0	0·086	0·090	,, ,,
420	55·0	0·168	0·159	,, ,,
345	20·0	0·105	0·115	Pigeons without symptoms fed on polished rice + 1 grm. caseine + 1 grm. marmite daily.
340	25·0	0·133 (fig. 1).	0·128	

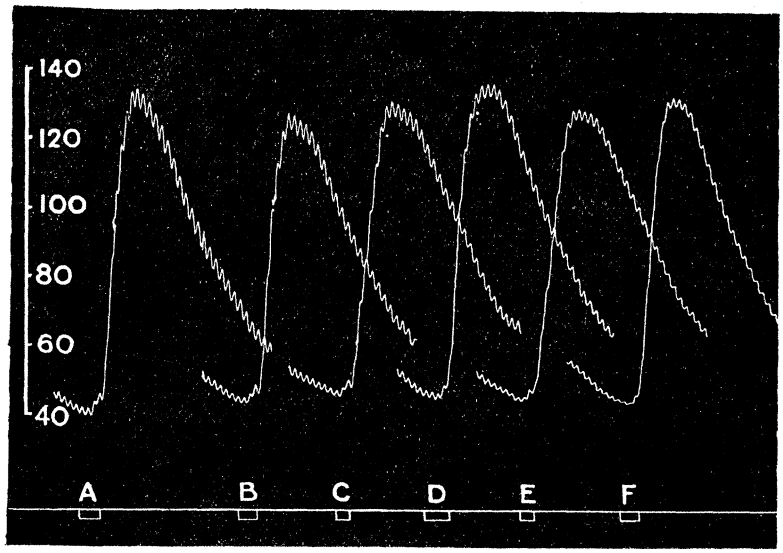

FIG. 1.—Physiological estimation of adrenaline in the suprarenal glands of a pigeon fed on polished rice + 1 grm. marmite and 1 grm. of casein daily. C and E = response to 0·35 c.c. of extract (total volume = 5·1 c.c.). A, D, and F = response to 0·0098 mgrm. B, to 0·0084 mgrm. of adrenaline. Total content of glands = 0·133 mgrm. of base.

For quantities of the order estimated the differences observed are within the limits of experimental error.

In a certain number of birds the adrenals were estimated separately, and in normal birds, even when there was some difference in weight between the

two adrenals, the content of adrenaline appears to be identical in the two organs (*cf.* Elliott (1912)). In experimental polyneuritis, however, there is often a large difference in weight between the two adrenals, and there appears to be some corresponding difference in content of adrenaline.

Observations on Normal Pigeons.

In the early stages of this work I was not aware of the variations which occur in the adrenal-content of pigeons in captivity fed on a normal diet, and in consequence some of my first observations on the effect of diet on the suprarenals were, in this respect, imperfectly controlled.

The data which are presented here do not enable me to state definitely how far lack of exercise and prolonged caging, and how far seasonal variation account for these differences in the amount of adrenaline in the glands of normal pigeons. In Table II the average results obtained from three series of normal birds, kept caged for varying periods, are given. The extreme variations of individual birds are indicated by the maximal and minimal figures placed beneath each average. Birds whose organs showed any gross pathological changes at autopsy were discarded.

Table II.—Pigeons on Normal Diet.

	Number of series.	Period of time during which birds were caged.	Number of birds in series.	Body weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of body weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of body weight in milligrammes.
Average	(1)	March 1	13	*318·4*	*25·2*	*79·1*	*0·051*	*0·160*
Maximal		to		352	34·5	113·5	0·096	0·300
Minimal		March 15		289	16	50·2	0·033	0·112
Average		April 5	4	*304*	*18·5*	*60·9*	*0·077*	*0·225*
Maximal		to		306	22	71·9	0·101	0·330
Minimal		May 28		301	15	49·3	0·035	0·115
Average	(11)	May 5	8	*284*	*24*	*84·5*	*0·096*	*0·338*
Maximal		to		314	32	101·9	0·129	0·411
Minimal		June 30		248	15	60·5	0·064	0·258

The striking difference between the average figures for content of adrenaline per kilogramme of initial body weight in Series 1 and 11, *i.e.*, 0·160 and 0·338 mgrm., makes it clear that in estimating the effect of any diet on the suprarenals the influence of caging and seasonal change must be excluded by comparison with birds on normal diet kept for the same period under identical conditions.

Observations on Pigeons fed with Polished Rice.

As an initial experiment two series, each of twelve birds, were fed, those of one series artificially with polished rice, while those of the other were allowed to feed themselves on polished rice which had been autoclaved for 1½ hours at 130° C. The artificially fed birds of Series 2, with two exceptions, developed polyneuritis in from seventeen to twenty-nine days. In Series 3 six birds showed well-marked symptoms between the sixteenth and twenty-second days; one was found dead on the ninteenth day with multiple liver abscesses and was excluded from the record, and the remaining five were killed on the twenty-fourth and twenty-fifth days without having shown symptoms of polyneuritis. In this experiment the birds were killed and the estimations made as soon as the diagnosis of polyneuritis was certain.

The general *post mortem* findings resembled those described in detail by McCarrison (1919 *a*), except with regard to the incidence of "œdema." Only three pigeons showed any sign of this. In pigeon 1, which was fed artificially for twenty-three days, the pectoral muscles were definitely more moist than normal, and the peritoneum and pericardium contained very small quantities of fluid. Pigeon 7, which was fed in the same way for twenty-three days, showed the same picture at autopsy, and pigeon H, which fed naturally on polished rice for twenty days, had a little fluid in the pericardium at death.

The average results for Series 2 and 3 are given in Table III. In Series 3 the birds have been divided into two groups and the results averaged separately, group (*a*) consisting of birds without symptoms of polyneuritis, and group (*b*) of those which acquired symptoms in from sixteen to twenty-two days.

The birds of this series which, after the first few days, ate very little, lost

Table III.—Pigeons Fed on Polished Rice.

	Number of series.	Number of birds in series.	Number of days on diet.	Initial weight in grm.	Final weight in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilog. initial weight in mgrm.	Weight of adrenaline in mgrm.	Weight of adrenaline per kilog. of initial weight in mgrm.
Average ...	(2)	11	*22·8*	*371·8*	*293·0*	*63·9*	*172·0*	*0·132*	*0·356*
Maximal ...			31	460	361	86	277·4	0·314	0·683
Minimal ...			17	280	200	44·5	117·1	0·058	0·193
Average (a)	(3)	5	*24*	*364*	*229·8*	*38·0*	*104·4*	*0·082*	*0·225*
Average (b)		6	*18·7*	*350*	*256·7*	*39·3*	*112·3*	*0·102*	*0·291*
Average total		11	*21*	*356*	*244*	*38·7*	*108·7*	*0·093*	*0·261*

weight more rapidly than the artificially fed birds of Series 2. As under these conditions they were largely living on their own tissues, they may have derived thence an amount of accessory substance sufficient to delay the appearance of polyneuritis.

In Series 2 the average weight of the adrenals was 172·0 mgrm., and of the adrenaline 0·356 mgrm. per kilogramme of the original body weight. In Series 3 these figures were 108·7 mgrm. and 0·261 mgrm. The corresponding figures for the first group of normal pigeons given in Table II were 79·1 mgrm. and 0·160 mgrm. The results from these normal pigeons cannot, however, be regarded as strictly controlling this experiment so far as the content of adrenaline in the suprarenals is concerned, since the birds which gave them had not been caged for the same length of time as those fed on rice.

This experiment, and all subsequent ones, confirmed McCarrison's findings in regard to adrenal hypertrophy in avian polyneuritis. The figures obtained for the store of adrenaline in this experiment, though nearly double those yielded by normal birds investigated at the same time, were not nearly so high as those obtained later in other normal birds. This, in itself, seemed to indicate that changes in the adrenaline-content of the suprarenals are not necessarily of importance in the development of avian polyneuritis. Further evidence in this direction was supplied by later observations.

A further series of six pigeons which were fed with polished rice, and which were killed for analysis at the same time as Series 11, in Table II showed a large increase in the store of adrenaline when compared with the normal birds, which in this case provided an accurate control.

The results of this series are given in full in Table IV.

Table IV.—Series 12. Pigeons Fed on Polished Rice.

Distinguishing number.	Number of days on diet.	Initial weight in grm.	Final weight in grm.	Weight of adrenals in mgrm.	Weight of adrenals per kilog. of original weight in mgrm.	Weight of adrenaline in mgrm.	Weight of adrenaline per kilog. of original weight in mgrm.
0	32	270	238	45	166·7	0·168	0·622
1	32	310	257	35	112·9	0·126	0·406
2	19	310	247	48	154·8	0·240	0·774
3	32	320	259	41	128·1	0·199	0·622
4	28	300	270	52	173·3	0·167	0·557
5	31	310	265	66	212·9	0·161	0·519
Average ...	—	303	256	48	158·4	0·177	0·584

This experiment was rigidly controlled not only by observations on normal pigeons kept under identical conditions, but also upon birds fed on polished rice with the addition of a daily ration of "marmite," and showed conclusively that there is a definite increase of adrenaline in the suprarenals of birds fed on polished rice with consequent polyneuritis. The significance of this increase will be discussed later.

The Addition of other Substances to the Diet of Polished Rice.

According to Kellner's analyses, quoted by König (1893), polished rice contains 12·55 per cent. of water, 7·88 per cent. nitrogenous substances, 0·53 per cent. of fat, 77·8 per cent. of non-nitrogenous extractives and 0·78 per cent. of ash. A diet of polished rice is therefore deficient in protein, fat and salts as well as in the accessory substances—fat soluble A and water soluble B. The mixed pigeon seed on which my normal birds were fed contained 2·33 per cent. of nitrogen (as against 1·24 per cent. for polished rice), and 2·2 per cent. of ether soluble constituents.

A series of experiments were now made to show the effect on the hypertrophy of the adrenals and their content of adrenaline, of adding some of the deficient constituents to the diet of polished rice.

The first group of experiments lasted from April 6 to May 7. In them the effect of the addition of "marmite" (a commercial extract of yeast), and of a fat deficient in all accessory factors was tried. Chick and Hume (1917) had previously shown that the daily dose of commercial yeast extract required to protect pigeons of 300–400 grm. weight from polyneuritis when fed on polished rice was from 1·0 to 2 grm. Four series of birds were started on April 6—Series 4, 5, 6, and 7. The first were given 1 grm. of "marmite" daily, the second 0·75 grm. of "marmite," the third 1 grm. of "marmite" and 1 c.c. of olive oil and the fourth 1 c.c. of olive oil but no "marmite," in addition to their daily ration of polished rice. The average results of the adrenal estimations in these series are given in Tables V and VI.

A further series of experiments were made between April 29 and the end of May. Three series of birds were fed with the addition, in Series 8 of 1 grm. of fat free casein, in Series 9 of 1 grm. of fat free casein and 1 grm. of "marmite," and in Series 10 of 1 c.c. of cod liver oil daily. The results of these experiments are given in Tables VI and VII. Finally, from the end of May till the end of June, several series of birds were fed, of which we are here concerned with Series 13 (Table V), in which a daily ration of 2 grm. of "marmite" was added to the diet of polished rice.

The Effect of the Addition of "Marmite" to a Diet of Polished Rice.

In order to ascertain the effect produced on the adrenals by the addition of "marmite" to a diet of polished rice comparisons may be instituted between the results of Series 4 and 5 and 6 and 7 recorded in Tables V and VI; between those of Series 8 and 9 in Table VII and finally between the figures given for Series 11, 12, and 13 in Tables III, IV, and V. The final comparison gives clear and definite information and will be discussed first.

In Table V, Series 13, the effect of the daily addition of 2 grm. of "marmite" to the diet of polished rice is shown. This dose is twice the daily amount required to protect the birds from polyneuritis.

The average weight of the adrenals per kilogramme of original body weight is 94·7 mgrm., and that of the adrenaline present in the suprarenals is 0·361 mgrm. as compared with 84·5 mgrm. and 0·338 mgrm. in normal birds (Table II, Series 11) and 158·4 mgrm. and 0·584 mgrm. (Table IV, Series 12) in birds with polyneuritis, observed at the same time and kept under the same conditions. This amount of "marmite" in addition to affording complete protection against the disease, enables the birds to gain in weight, and prevents the occurrence of adrenal hypertrophy and also of any definite increase above the normal in the store of adrenaline.

In Series 4 with a daily ration of 1 grm. of "marmite" (Table V) and in Series 5 with a daily dose of 0·75 grm. this result is also indicated, though there are no figures for normal birds which are strictly comparable with those obtained from these series.

The effect of yeast extract, in preventing the adrenal changes caused by a diet of polished rice alone is clearly shown; in the succeeding sections it will appear that it is much less effective in this direction when the basic diet contains protein or fat, free from accessory factors, as well as polished rice.

The Effect of the Addition of a Fat free from Accessory Factors to the Diet of Polished Rice.

As a fat free from accessory factors olive oil was selected and Series 6 and 7 (Table VI) show the effect of a daily ration of 1 c.c. of olive oil together with 1 grm. of "marmite," and 1 c.c. of olive oil without any "marmite," to the diet of polished rice. The pigeons fed on olive oil and rice without "marmite" (Series 7) exhibited symptoms of polyneuritis in between fifteen and thirty days.

One pigeon of this series, which had a higher-adrenal content than the others, had a trace of fluid in the pericardium at autopsy, and, in addition, the pectoral muscles were moister than normal.

Table V.—Effect of the Addition of "Marmite" to the Diet of Polished Rice.

	Number of series.	Number of birds in series.	Daily ration of marmite.	Number of days on diet.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(4)	6	1 grm.	30 (April 6 to May 7)	*312*	*349*	*20·3*	*65·1*	*0·087*	*0·279*
Maximal							26	84·7	0·105	0·356
Minimal							15	50·0	0·070	0·233
Average	(5)	6	0·75 grm.	30 (April 6 to May 7)	*306*	*322*	*26·0*	*85·0*	*0·109*	*0·356*
Maximal							31	101·1	0·129	0·469
Minimal							23	67·3	0·077	0·245
Average	(13)	6	2 grm.	30 (May 31 to June 30). In laboratory from May 5	*302*	*358*	*28·6*	*94·7*	*0·109*	*0·361*
Maximal							40	125·0	0·150	0·490
Minimal							23	71·9	0·084	0·269

Table VI.—The Effect of the Addition of Fat to the Diet of Polished Rice.

	Number of series.	Number of birds.	Diet.	Period for which diet given.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(6)	6	Polished rice + 1 grm. "marmite" + 1 c.c. olive oil	April 6 to May 5	*325·7*	*339·8*	*26·8*	*82·3*	*0·132*	*0·405*
Maximal							29	95·9	0·167	0·572
Minimal							25	75·5	0·092	0·309
Average	(7)	6	Polished rice + 1 c.c. olive oil	April 6 to May 5	*301*	*263*	*55·2*	*183·4*	*0·136*	*0·452*
Maximal							77	247·6	0·156	0·529
Minimal							43	145·8	0·092	0·296
Average	(10)	5	Polished rice + 1 c.c. cod liver oil	April 29 to May 20	*306*	*263*	*31·6*	*103·3*	*0·134*	*0·438*
Maximal							53	182·7	0·165	0·569
Minimal							22	70·9	0·108	0·354

It is evident that this dose of "marmite," when added to a diet of polished rice and oil, though it prevents the onset of symptoms and the enlargement of the suprarenals, is not enough to prevent some increase in the amount of adrenaline. The deficiency of fat in a dietary of polished rice has obviously no causal relation either to the hypertrophy of the adrenals or to increase in the content of adrenaline produced by feeding with such a diet.

The Effect of the Addition of a Fat Rich in Fat Soluble A, to the Diet of Polished Rice.

Although there is no evidence that fat soluble, A, is a necessary constituent of the normal diet of pigeons, the effect of the addition to the diet of polished rice of cod liver oil, a fat rich in this accessory factor, was investigated. Series 10 (Table VI) gives the results obtained.

Four out of the five birds in this series were killed on the twentieth day without having exhibited any symptoms of polyneuritis, though they showed some fall of body weight; there was no lowering of their cloacal temperature, and they were indistinguishable from normal birds. One was kept on the diet till the thirty-fifth day, and was then at the point of death, with extremely low cloacal temperature, great weakness and wasting, but with no definite symptoms of polyneuritis. This bird had been emptying its crop shortly after feeding, a tendency which was marked in all the birds of this group.

These pigeons had been caged for a month before the experiment was started, and the figures for weight of adrenals and of adrenaline per kilogramme of original body weight, *i.e.*, 103·3 mgrm. and 0·438 mgrm., may be compared with those of normal birds kept the same time under identical conditions, *i.e.*, 60·9 mgrm. and 0·255 mgrm. (Table II).

This experiment is complicated by inanition, which McCarrison (1919, *a*) has shown to cause both the suprarenal effects under investigation, but affords no evidence that fat associated with fat soluble A, behaves at all differently from fat free from accessory substances, so far as these suprarenal changes are concerned. It seems possible that the absence of definite symptoms of polyneuritis from these birds may be due to the presence of a trace of water soluble accessory factor in the daily ration of cod liver oil.

The Effect of the Addition to the Diet of Protein free from Vitamines and Fat.

The protein used for this purpose was commercial casein, which, after a preliminary extraction with cold ether, was repeatedly extracted under a

reflux condenser with boiling absolute alcohol. This treatment, in addition to removing all the fat, was efficient in removing the water soluble B, as will be shown by the experiment here described.

Two series of birds were fed, with the addition in Series 8 of 1 grm. of fat-free casein, and in Series 9 of 1 grm. of fat-free casein, and 1 grm. of "marmite" daily to the diet of polished rice. The birds of Series 8 rapidly acquired polyneuritis, while those of Series 9 remained normal. Table VII gives the results obtained.

The birds of these series were kept for about a month in the laboratory before the experiment was started, and the figures obtained are comparable with those obtained from the second series of normal birds in Table II.

The effect of the yeast extract in preventing adrenal hypertrophy in pigeons fed on polished rice is evidently not due to any protein or protein derivatives, which are present in small amount in such a ration, but to the water soluble B, which is present, though the influence of the salts in such a yeast extract is not excluded.

On the other hand, the addition of "marmite" to the casein-rice diet does not perceptibly prevent increase of the store of adrenaline, the figures for Series 8 and 9 being nearly identical, though the dose was adequate to prevent such increase in birds fed on a basic diet of rice alone. It becomes increasingly evident that the exhibition of symptoms of polyneuritis is not related to the increase in content of adrenaline, which occurs as a result of feeding pigeons on polished rice.

The Histology of the Adrenals of Polyneuritic Pigeons.

McCarrison (1919, *a*) has already made some important observations on the histological appearances presented by the adrenals of pigeons fed on polished rice. The chief points to which he has drawn attention are: the disproportionate enlargement of the cortex as compared with that of the medulla in these glands; the presence of congestion, which is neither sufficiently constant nor pronounced wholly to explain the enlargement; the occasional broken down appearance of the central part of the gland, together with general nuclear changes in both cortex and medulla, which suggest degeneration; and, finally, the degeneration which is sometimes present in sympathetic ganglia adherent to the gland.

I have examined the adrenals of a few normal and experimental pigeons in order to see if any further light could be thrown on the cause of the changes which occur in them as a result of deficiency diets. I have used two special methods of staining, the bichromate method of Kohn as described by Elliott and Tuckett (1906), using Scharlach, R, as a selective counterstain for the

Table VII.—The Effect of the Addition of Casein to the Diet of Polished Rice.

	Number of series.	Number of birds.	Diet.	Period for which diet given.	Initial weight in grammes.	Final weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	(8)	6	Polished rice + 1 grm. casein	April 29 to May 20	*328*	*285*	*35·5*	*108·2*	*0·122*	*0·372*
Maximal							47	134·2	0·153	0·483
Minimal							23	71·9	0·091	0·283
Average	(9)	6	Polished rice +1 grm. casein +1 grm. marmite	April 29 to May 20	*313·3*	*351·7*	*24·3*	*77·6*	*0·115*	*0·367*
Maximal							35	120·7	0·143	0·493
Minimal							15	55·5	0·077	0·285

Table VIII.—Estimation of Cholesterol by the Colorimetric Method.

Amount of cholesterol taken	2·52	2·64	2·76	2·88	3·12	3·24	3·36	3·48
Amount of cholesterol estimated	2·50	2·61	2·70	2·73	3·12	3·24	3·24	3·42

fats and lipoids of the cortex, and the osmic vapour method more recently used by Cramer (1918). Some of the frozen sections, prepared after treatment with bichromate solution, were stained with hæmatoxylin and eosin.

In addition to the changes described by McCarrison, most of which I have also noticed, there are two additional points which may be of importance. In the first place, the cortical enlargement appears to be accompanied, not only by degenerative nuclear changes, but also by a loss of definition of the lipoid granules present in it. This is well seen in frozen sections stained with Scharlach, R.

Secondly, the enlargement of the cortex appears to interrupt the continuity of the normal network of medullary substance, and gives it an appearance on section of a number of isolated nodes surrounded on all sides by swollen cortical tissue, and only connected with each other by fine strands of tissue. This appearance is well shown in the osmic vapour preparations which have been kindly photographed for me by Mr. Barnard (Plate 1, figs. 2 and 3).

Finally, it is of interest to record that the adrenals of pigeons fed on polished rice, with the addition of a daily ration of "marmite," differ histologically in no important respect from those of normal birds.

These histological appearances suggest two possible ways in which the output of adrenaline into the blood stream might be hampered or prevented in pigeons on such deficiency diet. The presence of degenerated sympathetic ganglia described by McCarrison indicates the possibility that the splanchnic fibres to the adrenals might also be degenerated, though I have not been able to show that this is the case. If this were so, the output of adrenaline from the glands would be greatly diminished (Elliott, 1912). On the other hand, the cortical swelling might conceivably act mechanically, by hampering the venous outflow from the gland, and so diminishing the output of adrenaline.

The Significance of Adrenal Enlargement of Pigeons fed on Polished Rice.

Various factors may come into play in causing the adrenal enlargement which occurs in pigeons fed on polished rice. Histological observations seem to exclude congestion of the glands as a constant factor in the production of this effect. Apart from congestion, some œdema of the gland tissues is consistent with the histological appearances presented by them, and part of the increase in weight is explained by an increase in the water-content of the adrenals.

The weight of the freshly dissected-out adrenals of a normal pigeon was 23·0 mgrm., and when these were dried to constant weight over sulphuric acid *in vacuo* they weighed 7·0 mgrm. The percentage of water in these

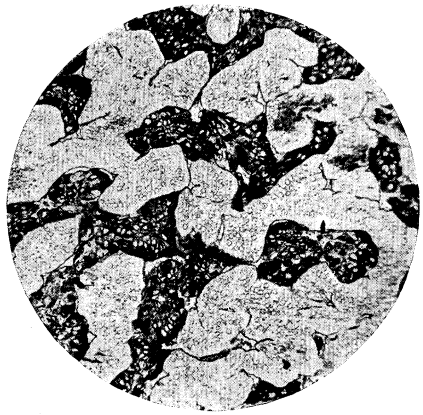

FIG. 2.—Normal adrenal of pigeon. Osmic vapour preparation (×140 diameters) showing the continuity of the medullary network.

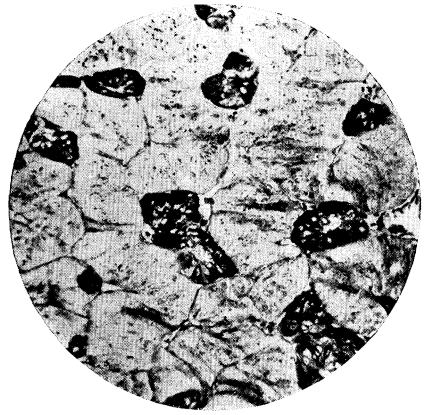

FIG. 3.—Adrenal from polyneuritic pigeon (×140 diameters) showing excessive cortical enlargement and interruption of medullary network.

glands was 69·6 per cent. The adrenals of a polyneuritic pigeon similarly treated gave wet and dry weights of 41·0 mgrm. and 10·0 mgrm., and the percentage of water in them was 75·6 per cent.

Apart from increase in water-content, the fact that enlargement of the cortex predominated over that of the medulla in the adrenals of pigeons fed on polished rice suggests a further possible reason for the increase in weight of the glands. McCarrison's observations on the occurrence of adrenal enlargement in pigeons suffering from inanition have already been referred to. In his feeding experiment on polished rice, the increase in weight of the adrenals is associated with loss of body weight, and in my own experiments loss of body weight and adrenal enlargement have been almost constantly associated.

Ellis and Gardiner (1912) have shown that in rabbits, which live normally on a diet poor in cholesterol, inanition causes a rise in the content of cholesterol in the blood, and Anitschow and Chalatow (1913) produced adrenal enlargement in these animals by feeding with excess of cholesterol. It was conceivable, therefore, that the enlargement of the suprarenals, which occurs in the production of experimental polyneuritis, was the result of inanition and was due to the storage in the adrenal cortex of lipoids which had been set free by the breaking down of the body tissues. The validity of this hypothesis was tested by observations on the cholesterol-content of the adrenals of normal and polyneuritic pigeons. For this purpose the colorimetric method of Grigaut (1911) was used and some concurrent estimations of the cholesterol-content of the blood of such pigeons were also made by the same method. Such colometric determinations were found to be most accurate when the standard contained approximately the same amount of cholesterol as the solution to be tested. The observations in Table VIII show the degree of accuracy of colorimetric estimations when this precaution was taken.

The cholesterol extracted from the adrenals was compared with a standard containing 1·2 mgrm. of the pure substance, while for blood a standard containing 3 mgrm. was used. The chief difficulty in making the estimations was caused by the presence in the blood and gland extracts of organic matter which altered the tint, making it greener than the standard. Screens of amber glass were found to be of use in making the colour comparison in such cases.

The average results obtained in normal and polyneuritic pigeons are given in Table IX.

In pigeons fed on polished rice with extreme symptoms of polyneuritis there is an increase in the percentage of cholesterol in the blood, but no

Table IX.—The Cholesterol-content of the Blood and Adrenals of Normal and Polyneuritic Pigeons.

	Diet.	Number of days on diet.	Initial body weight in grm.	Weight of adrenals in mgrm.	Cholesterol-content of adrenals in mgrm.	Percentage of cholesterol in adrenals.	Weight of cholesterol on 1 c.c. of blood in mgrm.
Average	Normal	—	*314·6*	*25·5*	*0·70*	*2·9*	*1 66*
Maximal				32·0	0·88	3·8	2·46
Minimal				21·0	0·44	2·0	1·06
Average	Polished rice	*18*	*320*	*47·7*	*0·79*	*1·9*	*3·06*
Maximal				66·0	1·34	3·3	3·69
Minimal				32·0	0·35	0·67	2·40

evidence of storage in the suprarenals. These results do not lend any support to the hypothesis of lipoid storage as far as cholesterol is concerned. Some observations were also made on rabbits and pigeons fed on a normal diet with the addition of cholesterol, in order to ascertain if the hypercholesteræmia produced in this way was associated with increase in the content of adrenaline in the glands.

Feeding Experiments with Cholesterol.

Eleven healthy rabbits, two months old, were selected for experiment. Of these, six were fed daily through a catheter with 0·1 grm. of pure cholesterol dissolved in 2·5 c. c. of olive oil. All the rabbits had the same basal dietary, consisting of bran, oats, and occasional greens. The experiment was continued for nearly seven weeks. All the rabbits remained healthy and gained weight, except one of the experimental animals, which became paraplegic after forty-two days' feeding, and which was discarded. At autopsy the animals fed with excess of cholesterol showed aortic lesions like those described by Anitschow and Chalatow, but only two had any adrenal hypertrophy.

Before the rabbits were killed, a sample of blood was taken from the ear for cholesterol estimation. The adrenals were carefully dissected out *post mortem*, weighed, and the residual adrenaline was estimated colorimetrically.

The average results obtained from the two series of rabbits are given in Table X.

There is a small increase in the content of adrenaline of the rabbits in which hypercholesteræmia had been produced and maintained, above the average content of normal rabbits investigated at the same time. A more rigid control would have been provided had these latter animals been fed daily with 2·5 c.c. of olive oil without cholesterol.

Table X.—The Effect of Excess of Cholesterol in the Diet of Rabbits.

	Diet.	Number of rabbits in series.	Number of days on diet.	Initial body weight in grammes.	Final body weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of final weight in milligrammes.	Weight of cholesterol per cubic-centimetre of blood in milligrammes.
Average	Normal	5	—	—	*1040*	*113·2*	*0·219*	*0·218*	*0·91*
Maximal						170	0·280	0·333	1·21
Minimal						86	0·175	0·159	0·63
Average	Normal	5	*41*	*780*	*1160*	*218*	*0·305*	*0·258*	*3·50*
Maximal	+0·1 grm.		46	960	1400	328	0·469	0·347	5·34
Minimal	cholesterol in 2·5 c.c. olive oil		33	605	900	128	0·201	0·163	0·90*

* No cholesterol given for forty-eight hours before death, at which time the estimation was made.

Table XI.—The Effect of Addition of Cholesterol to the Normal Diet of Pigeons.

	Period in laboratory.	Period of feeding with excess of cholesterol.	Initial weight in grammes.	Weight of adrenals in milligrammes.	Weight of adrenals per kilogramme of initial weight in milligrammes.	Weight of adrenaline in milligrammes.	Weight of adrenaline per kilogramme of initial weight in milligrammes.
Average	April 5	April 29	*325*	*16·0*	*49·2*	*0·105*	*0·323*
Maximal	to	to	360	18	57·1	0·142	0·406
Minimal	May 31	May 31	280	14	38·5	0·084	0·252

Six pigeons, which were fed on a normal diet with the daily addition of 1/25 grm. of cholesterol dissolved in 1 c.c. of olive oil, and which were kept for the same period and under the same conditions as the second series in Table II, were also investigated. The results are given in Table XI.

Here again there is an increase in the content of adrenaline, though the control would be more accurate if the normal birds had had olive oil in addition to their normal diet.

The production of hypercholesteræmia in normal rabbits and pigeons appears to be associated with a small increase in the content of adrenaline in the suprarenals.

The Significance of Increased Content of Adrenaline in the Suprarenals of Pigeons fed on Polished Rice.

The adrenaline in the suprarenal glands represents the balance between production and secretion into the blood stream. It is of course possible that the increased katabolism induced by feeding pigeons on deficient diets may provide more of the precursor or precursors from which adrenaline is built up by the gland, but this must remain a mere speculation. On the other hand, the evidence provided both by the present experiments and by the more extensive work of McCarrison suggests strongly that the increase of adrenaline in pigeons on deficiency diet is chiefly due to diminished output. The large increase which occurs in pigeons as a result of restriction of activity by caging, the increase which results from inanition and the association of such increase with the diminution of muscular and general metabolic activity in pigeons fed on deficient diets, whether polyneuritis were produced thereby or no, all point in the same direction.

The picture presented by pigeons on a deficient diet is in accord with this view. The progressively falling body temperature, the ruffling of the feathers, which do not lie down closely as would be expected if large quantities of adrenaline were being turned out into the blood stream, and the sluggishness exhibited by such birds, are all consistent with diminished output. The hyperglycæmia observed in pigeons fed on polished rice, which as Funk (1920) has suggested, might be accounted for by increased output of adrenaline, may easily be explained by the diminished use of sugar by the tissues together with the large excess of carbohydrate in the diet.

Whether the diminished output is itself due in the main to depression of metabolism, or whether the nervous and mechanical factors already discussed are chiefly concerned, is a matter on which no evidence is available.

In the present experiments the occurrence of increase in the store of adrenaline in normal birds, which in some cases exceeded the amounts found

in polyneuritic birds examined at a different time, makes it seem likely that, whatever the exact significance of the increase may be, it is not causally related to the polyneuritis which occurs in dietaries deficient in water soluble B. Further evidence in this direction is furnished by the experiments on feeding with casein and "marmite," and casein alone, in addition to polished rice, which yielded almost identical results for the store of adrenaline in two series of birds examined at the same time.

The Incidence of Œdema in Experimental Polyneuritis.

In my series of experimental pigeons there were very few examples of the œdema described by McCarrison. Of fifty birds with polyneuritis, only four presented signs of this œdema at autopsy. The œdema occurred either as a wetness of the pectoral muscles or as an effusion of fluid into the pericardium and peritoneum. There were no instances of œdema in the lungs or of the fatty band of tissue round the auriculo-ventricular junction on the surface of the heart. All but one of these œdematous birds gave values for the content of adrenaline which were higher than those given by birds in the same series. The association of œdema with very high content of adrenaline may possibly be explained by œdema of the glands themselves. It is more likely, however, that the factors which operate in producing œdema in the ill-nourished tissues of these birds are the same as those which give rise to diminished use of adrenaline.

McCarrison's early suggestion that the increased store might be evidence of increased output of adrenaline and that this increase might be an important factor in the production of œdema was put to the test of experiment.

Two pigeons—one on a normal diet and the other fed artificially with polished rice—were given daily an amount of adrenaline several times greater than the normal adrenaline-content of the pigeon's suprarenals, *i.e.*, 0·25 mgrm. of adrenaline chloride, which was injected into the pectoral muscles. The experiment lasted from May 31 to June 24, by which time the pigeon on the polished rice dietary was at the point of death with severe symptoms of polyneuritis. Its body weight had fallen from 300 grm. to 265 grm., and its cloacal temperature was 104·5°. It had had severe ataxia for the preceding forty-eight hours and was then unable to walk or fly. At autopsy there were necrotic patches in the atrophic pectoral muscles, but there was no evidence of sepsis and the heart blood was sterile. There was no trace of œdema. The internal organs were all greatly wasted except the adrenals, which weighed 75 mgrm. The left gland, which weighed 37·5 mgrm., contained 0·052 mgrm. of adrenaline and the right gland, which was of equal weight to the left, contained 0·216 mgrm. of cholesterol.

The bird on normal diet was killed on the same day. Its temperature was normal and it was indistinguishable from other normal birds. At autopsy there were necrotic patches in the pectoral muscles but the blood culture was sterile. All the organs appeared to be normal except the pancreas which was somewhat pale. The adrenals weighed 20·5 mgrm. The left gland, which weighed 9·5 mgrm. contained 0·042 mgrm. of adrenaline and the right, which weighed 11 mgrm., contained 0·192 mgrm. of cholesterol.

These birds then, gave adrenaline and cholesterol values for the adrenals, which lay within the limits of values obtained for other normal and polyneuritic pigeons.

In neither of these pigeons was there any œdema and the administration of adrenaline in this way did not appear either to hasten or retard the appearance of symptoms in the bird on the milled rice diet.

It seems unlikely that increased output of adrenaline, even supposing that this could be shown to occur in pigeons fed on polished rice and suffering from the consequent polyneuritis, could contribute to the development of œdema. On the other hand the ill-nourished state of the tissues in such pigeons would predispose them to œdema.

Conclusions.

1. McCarrison's observations on the occurrence of enlargement of the adrenals with increased store of adrenaline in pigeons fed on polished rice, are confirmed.

2. These changes still occur when either protein or fat is added to the diet, but are prevented by the addition of an adequate ration of yeast extract.

3. The addition of such a ration of yeast extract to a basic diet of polished rice with extra fat or protein, does not prevent the increase in the store of adrenaline, though in this case the glands are not enlarged.

4. It is suggested that the enlargement of the adrenals is due partly to congestion and œdema of the gland tissues and partly to the storage in the cortex of the gland, of lipoids set free by the breaking down of body tissues. The investigation of the cholesterol-content of the adrenals of normal and polyneuritic pigeons does not support this theory of lipoid storage, though a well-marked hypercholesteræmia occurs in the latter.

5. The artificial production of hypercholesteræmia in rabbits and pigeons by feeding with cholesterol appears to be associated with a small increase in the adrenaline-content of the suprarenals.

6. The increased content of adrenaline in the suprarenals of birds on deficient diets is attributed to diminished output of adrenaline as a result of lowered body metabolism.

7. The œdema which occurs in some cases of experimental polyneuritis is not due to increased output of adrenaline. Daily administration of adrenaline to birds fed on normal or polished rice does not cause œdema, nor does it accelerate or retard the onset of polyneuritis in pigeons on a polished rice diet.

I desire to express my indebtedness to the Medical Research Council for the facilities given me to carry out this work in their Department of Biochemistry, and especially to Dr. H. H. Dale, F.R.S., for much helpful criticism and advice.

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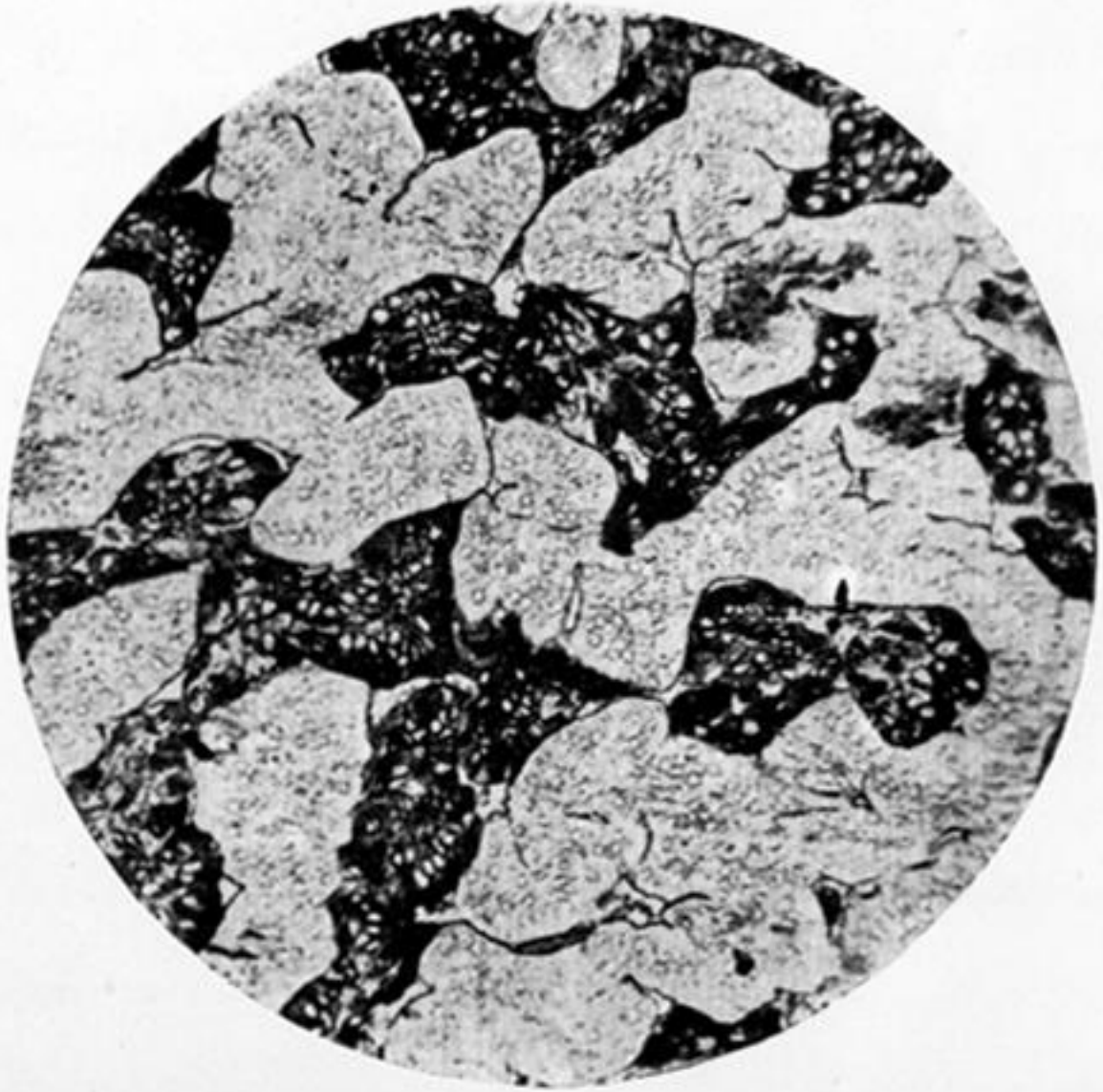

FIG. 2.—Normal adrenal of pigeon. Osmic vapour preparation (×140 diameters) showing the continuity of the medullary network.

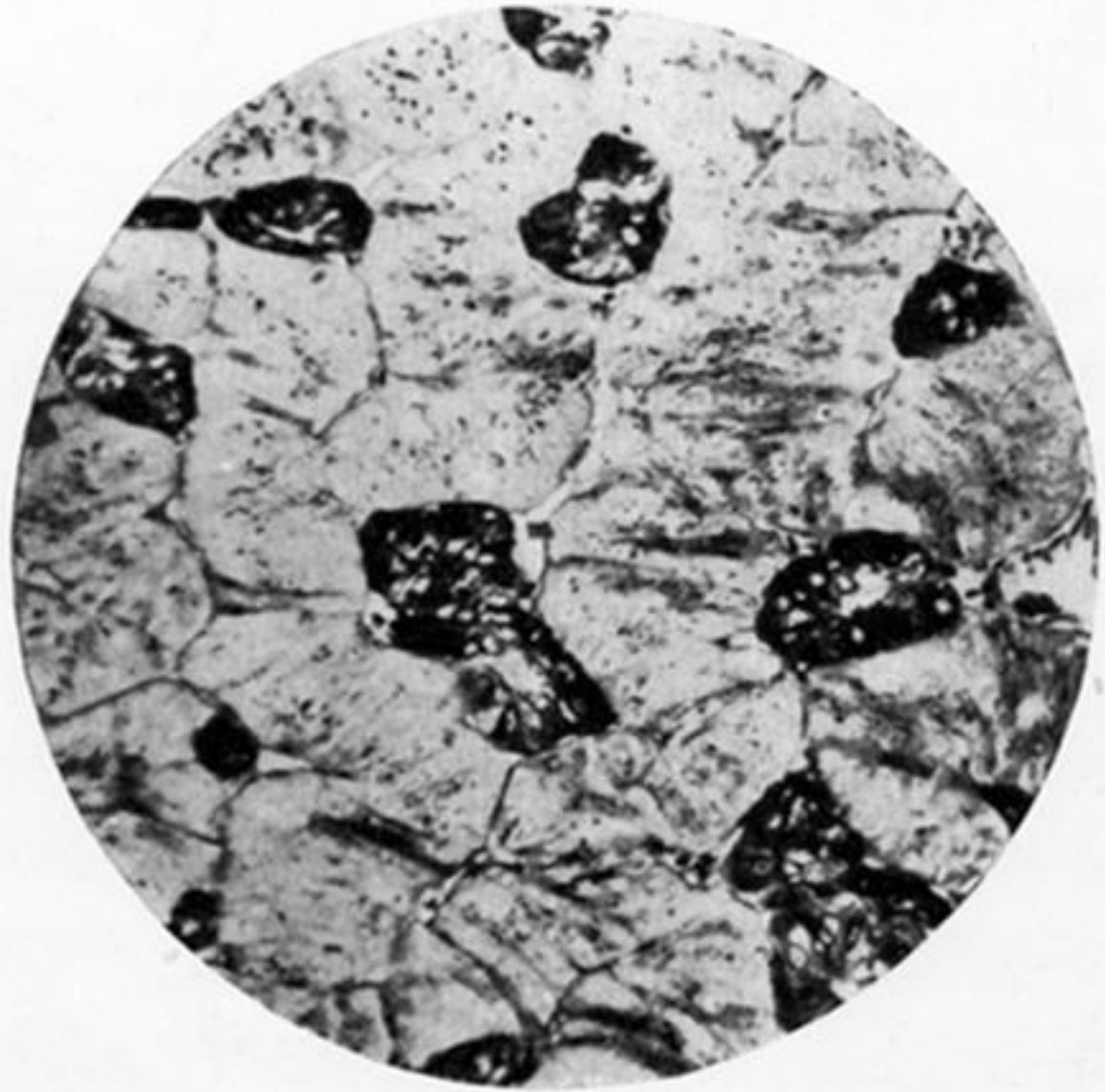

Fig. 3.—Adrenal from polyneuritic pigeon (× 140 diameters) showing excessive cortical enlargement and interruption of medullary network.