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Doctor of Medicine

in the University of Glasgow:

THE RELATIONSHIP OF THIAMIN TO THE VISUAL PATHWAY: A PHYSIOLOGICAL, PATHOLOGICAL, AND CLINICAL STUDY.

by

F. C. Rodger, M.B.,Ch.B.(Glas.),D.O.M.S.(Lond.)

Lecturer in Physiology, Medical School,

University of Durham.

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PART I.

A CRITICAL REVIEW OF THE CONTROVERSIAL EXPERIMENTAL EVIDENCE AS TO THE ROLE OF THIAMIN IN MAINTAINING THE INTEGRITY OF THE VISUAL PATH: AND THE PURPOSE OF THE THESIS.

The fundamental alteration in the visual pathway in thiamin deficiency might of course be biochemical and not cellular; yet, as there are no doubts that such a deficiency leads to a widespread degeneration of the vestibular neurones, it seemed reasonable enough at the start of this thesis to expect that the visual might be similarly affected.

The author's particular interest in cranial nerve II and its higher connections was fired by his experiences in the Far East during the Second World War, by his personal observations of nutritional Amblyopia in British Army personnel emprisoned by the Japanese. The plight of these wretched men once seen is not readily forgotten. The relative sick discharge rate of those who survived, and were repatriated, was as high as 6.8%; that is to say, 6-7 out of every 100 who returned home were blind, or nearly so*.

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* In 100 cases taken at random only 8% had a visual acuity better than 6/24.

The cause of this condition, to which the title "Camp Eye" was given, was generally attributed at that time to a deficiency in thiamin*. So conflicting was the evidence of this in the literature, however, that it appeared wise to test that view experimentally when the opportunity arose.

In 1949 this state of affairs presented itself, and mindful of the early promise, the thesis was started.

Apart from its probable clinical application, it was hoped that the work might furnish important information of a more fundamental scientific nature.

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It was indeed surprising in view of the possible relationship between thiamin and nutritional amblyopia to discover how little experimental work on the subject had been performed.

Observations on the effect on vision of a deficiency of the entire B complex were almost as rare.

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* While this was true of the locations where the author worked, it may not have been true elsewhere.

Most of the pioneers in this field were of course under the impression that thiamin filled the role earlier assigned to the hypothetical antiberiberi vitamin. They believed (wrongly) that they were inducing a thiamin deficiency by feeding polished rice.

Barletta (1932) was probably the first to describe optic nerve degeneration in an experimental series of this nature, although to Eijkman (1897) must go the honour of being the first to produce experimental beriberi. He made no mention of optic nerve degeneration, however. Barletta fed his birds (doves) on polished rice in the manner described by Eijkman. Subsequently he found that the fibres of the optic nerves, and chiasma, exhibited tortuosity, swelling, and varicosity, and that the myelin sheaths had many degenerative patches in them. He expressed doubts as to the validity of these changes in the axis cylinders for he found they also occurred in the optic nerves of the control birds. The myelin changes, nevertheless, he claimed were more pronounced in the deficient group.

Marchesini and Papagno (1935) repeated Barletta's experiment in pigeons. Apparently they were in ignorance of the papers by Prickett and Peters, written the previous year, for they do not mention them. The importance of the Italians' work lies in the fact that theirs is the only specific reference in the literature to involvement of the papillo-macular bundle*. As it is a somewhat awkward passage to construe, I quote it at length:

> "I cilindrassi presentano lesioni lievissime, estrinsecantesi solamente a carico di un fascio papillo maculare: queste aumentano in modo piu o meno regulare in senso ascendent fino al chiasma oltre il quale vanno poi scomparendo.
>
> Alterazioni di una entita notevole si riscontrano invece a carico della guaina mielinica lungo un fascio papillo maculare, ma non mancano altri focolai di degenerazione, per quanto rari e circoscritti, disseminati irregolarmente negli altri fasci del nervo ottico."

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* The pathway taken by the macular fibres in Aves is by no means proven.

Evidence of nerve cell degeneration was found to be of a minor nature - the axis cylinders being only slightly varicose, the retinal ganglion cells moderately shrunken and vacuolated with some irregularity of the cytoplasmic fibrils: but these latter observations were all made on non-controlled silver-impregnated sections, and such phenomena are common artefacts with this technique. Unlike Barletta, on the other hand, Marchesini and Papagno found the myelin changes to be constant and gross in all 12 deficient pigeons and not present in the control birds. The sheaths exhibited an ascending fatty degeneration, scattered around and within the papillo-macular bundle.

If we accept, however, the opinion of Swank (see later), myelin degeneration by itself can never be taken as the sole criterion. It must coincide with changes in the cell bodies and their processes. The retinal changes we have said already are not convincing; and the slight axon varicosity they described we have produced in healthy tissues by delay in fixation.

The authors stated the tissues were fixed without delay, and yet we must note that 4 out of 6 deficient pigeons allowed to die in their own time did so at night. It is difficult to believe that the brains of these birds were fixed within half an hour of death, after which time in our opinion autolytic processes will lead to distortion. In point of fact no exact time is specified, and so doubts arise as to whether Marchesini and Papagno were fully aware of the great necessity for speedy fixation.

In other respects, too, the experiment had limitations. The series was a small one. It was acute. It was not reversed. The visual paths were not examined above the chiasms and only three photo-micrographs, all Marchi preparations, are published. Furthermore, it has been shown by several workers that starvation leads to destruction of myelin, and birds on polished rice, it is well known, go off their feed badly. The Italians had to force-feed twice a day for that very reason, and yet as often as not, according to Shaw and Phillips (1945),

food placed in the pigeon's crop in such circumstances is regurgitated later. This probable complication is not mentioned.

The first references to involvement of the visual pathway in animals on a diet deficient only in the heat labile member of the B complex were those by Prickett (1934) in rats, and Peters (1934) in pigeons*.

The blindness which they describe, however, was a subjective finding in the terminal stages of an acute experiment. There might, then, be an explanation for it other than a cellular degeneration of the visual neurones. When animals are severely deficient, in our experience, they become no longer interested in their surroundings, and need a deal of arousing, fatigue and lethargy being dominant signs. For this reason, subjective investigation of an animal's response to visual stimuli in the terminal stages would appear to be of slight value. The rest of Peters' work, however, is of such a high order that perhaps more

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* All members of the B complex are in fact heat labile. Thiamin, however, is the least resistant being destroyed by autoclaving for 6 hours at 120°C. Pantothenic acid is the next least resistant, and excessive temperatures have to be carefully excluded, or fallacies will arise. The term "heat-labile member" traditionally obtains only for Thiamin.

stress has been laid upon this observation of his than was intended*.

The series of painstaking experiments on thiamin deficiency undertaken by Swank and his various collaborators constitute the main argument in favour of thiamin being an antineuritic vitamin.

These authors, Swank and Prados (1942a), describe degeneration within the central nervous system, of the optic fibres, in an avian thiamin deficiency induced by administering autoclaved yeast and a low protein diet. This was more marked in the chronic than in the acute deficiency. Ninety birds were used in their experiments, and no detail was too small to mention: in short, this is an excellent paper, and most of the criticism it has aroused would seem to be unjustified.

Although primarily interested in the vestibular system, Swank and Prados noted that the central terminations of the optic fibres in the deficient birds were either grossly thickened or fragmented; in the severe cases this was associated with sclerosis of the related cells (those in the

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* That was the impression gained on questioning Professor R.A. Peters in 1950.

optic lobes may be taken for practical purposes as being the homologues of the lateral geniculate body cells in man)*. These degenerative changes, they maintain, always precede changes in the myelin sheaths. This is in direct disagreement with Prickett (1934) who takes the opposite view.

Prickett also described the occurrence of shrunken and darkly staining cells in the neighbourhood of the vestibular nuclei, but noted their presence in some instances in his control animals. This unfortunately casts some doubt on his findings for we have produced a sclerosis of that nature in the cells of the dorsal nucleus of the lateral geniculate body of rats by alcohol fixation, and by inanition, and have otherwise never found it in our control animals.

But let us return to the work of Swank and Prados, for these authors like ourselves never found sclerosis of the visual cells in their control preparations. Furthermore, they state they took every precaution by force-feeding and by quick fixation to prevent such fallacies. If they are correct, moreover, in believing that myelin degeneration

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* This is not strictly correct. Primitive geniculate bodies are already present in teleosts which also possess optic lobes.

never occurs alone in thiamin deficiency, but is always preceded by a degeneration of nerve cells and their processes, it would seem very probable that, when demyelinisation does occur alone, it is evidence not of a specific vitamin deficiency but of inanition. Swank (1940) acquired proof of this in a group of rats on an inadequate caloric intake. The axons and somata remained intact, yet demyelinisation was marked. This is one of the main arguments against the experiments of Marchesini and Papagno.

The criticism levelled against Swank by Shaw and Phillips (1945) that food placed in the pigeon's crop is not always ultimately swallowed and digested would appear rather niggardly in the circumstances, for it is quite out of character with the good craftsmanship he exhibited in all other respects. More serious is the criticism that Swank's pigeons lacked in their diet many amino acids not required by mammals but essential to birds. Could lack of these be the causative factor in producing degeneration? We have no real evidence of this any more than had the critics. Swank and Prados (1942b) later in that year had the last word. They produced exactly the same type of degeneration

in mammals (kittens).

It is well known now, of course, that thiamin has a marked effect on a spontaneous paralytic disease of foxes. Evans, Carlson, and Green (1942) described this syndrome, first reported from the Chastek fur farm, and frequently given that name. The disease results from the presence in raw fish of a thiamin analogue, and may be cured by administering thiamin in excess. Degenerative lesions in the paraventricular regions were found on autopsy.

So, as it seems certain that thiamin deficiency can produce lesions in the central nervous system, there is less reason to doubt, surely, the optic tract degeneration described by Swank and Prados. Unfortunately, no account was taken in their experiments of the retinal ganglion cells, and so, according to their own high standards, they cannot be said to have provided evidence that the primary visual neurones are affected, a point not generally appreciated by other writers.

Such is the meagre positive evidence that exists.

A somewhat larger number of workers interested in this problem describe experiments in which they could find no significant degenerative changes within the visual pathway.

Engel and Phillips (1938) in a histopathological paper in which no photomicrographs are presented, claim that as long as β-carotene and riboflavine are maintained at a high level, no change occurs in any part of the central nervous system. In a group of rats made deficient in thiamin, they found no abnormality. In a group of chickens, 34% exhibited slight demyelinisation of the central nervous system, in which they include by inference the optic nerves. The importance of this finding they ignore (without explanation) in their summary, except to say that it did not occur when the two other vitamins, β-carotene and riboflavine, were administered in excess.

Perhaps the reason why Engel and Phillips did not manage to produce degeneration on a thiamin free diet was that the process was too acute. It seems, moreover, that the diet was much too well balanced. The recent work of Yudkin (1951) suggests that if the carbohydrate content had been raised at

the expense of the protein, degeneration might after all have been found. As biochemists following in the wake of Peters, they might, however, have evolved such a diet on their own initiative. The value of their work, moreover, would have been enhanced if they had taken into account the cell bodies of the upper two visual neurones.

McDermott and his four colleagues (1943) were others to conclude that the integrity of the visual fibres was not affected by thiamin deficiency. They produced the typical clinical signs of a chronic deficiency in rats by giving minimal doses of the vitamin, and by poisoning them with weekly doses of i.v. Tryparsamide (0.10 gm. per kg. body wt.). They were primarily interested, of course, in the aetiology of Tryparsamide Optic Atrophy.

Their animals, however, were not in individual cages, and had as a result access to the excreted pellets of their neighbours, whereby a certain amount of thiamin would be made available.

Like Engel and Phillips, they did not investigate the cells of the retina nor of the lateral geniculate body.

It is extremely interesting to note, however, and far from being irrelevant, that they did produce optic nerve degeneration with a diet deficient in the whole B complex, and in another lacking only β-carotene. The nerve fibres were varicose, or fragmented, the interstitial tissue loose and oedematous, and gliosis fairly marked. Here and there they observed well-defined vacuoles, which they considered were swollen oligodendroglial cells. Their technique, however, could not demonstrate the real nature of these vacuoles. It would seem, nevertheless, that the McDermott group had every reason to conclude that thiamin deficiency does not affect the visual path. They believe that at least two factors are concerned, β-carotene and some member of the B complex other than thiamin - and other than riboflavine, which they excluded in its turn. Thus do the opponents of Swank disagree among themselves.

Leinfelder and Robbie (1947), although they discovered slight myelin changes in rats - a few fine free droplets with the Marchi technique - also conclude that no real abnormality existed. This is true, they state, not only of an acute deficiency,

but of a chronic, and even when associated with a toxic element (continuous exposure to cyanide). The slight myelin degeneration found they believed to be due to inanition. It was reversible. The solitary chronic deficient group they induced exhibited the most marked signs of demyelinisation, although even they were slight. Each rat was given 0.2 mg. of thiamin hydrochloride daily. This with the small amount available by refection in the intestine is not, however, a truly inadequate dose. The authors themselves admit that when the rats were killed at the end of 21 weeks, they had not lost weight. Furthermore, the histological methods used were not nearly comprehensive enough. In short, these authors cannot be said to have submitted satisfactory evidence. They cannot at least be said to have excluded degeneration of the visual path in the chronic disability for it seems very much as if they had failed to produce it.

Finally, one must mention the positive opinions of Zimmerman (1941) whose brilliant studies on canine nutritional neuropathies are very well known to workers in this field. While there is no

specific experiment of his own devoted to a study of the visual path, he states nevertheless that he has never been convinced that optic atrophy occurred experimentally in dogs, pigeons, or rats as a result of thiamin deficiency. Such a statement leaves standing only the work of Swank and Prados published the following year.

Such, then, is the important literature concerned with the subject of this thesis. The scanty evidence, as you have seen, is extremely conflicting; discrepancies, and disagreements, exist in them all, some serious, some of lesser importance. The field in fact is wide open.

Before we will be able in our turn, however, to arbitrate, we must be able to exclude all possibility of artefactual change, and we must become familiar with the manner in which the visual neurones respond to lesions of a known order.

We must also learn how to induce both acute and chronic thiamin deficiencies; and if we cannot prevent our experimental animals from becoming anorexic, we must at least become familiar with the pathology of inanition, so that we can take

it into account in our final assessment.

As none of the ex-Ps.W. suffered from night blindness, we intend to neglect the probable complicity of vitamin A.

We must, however, determine whether or not riboflavine affects the pathogenesis of thiamin deficiency, as the indications are it might.

We might also investigate the possibility that an unknown member of the B complex, present in yeast, is the responsible factor.

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PART II.

A DISCUSSION OF THE METHODS USED TO INDUCE AND CONTROL THIAMIN DEFICIENCY IN RATS, AND OF SEVERAL PRELIMINARY TRIAL EXPERIMENTS.

I. THE PRODUCTION AND CONTROL OF THIAMIN DEFICIENCY IN RATS

1. The care and handling of the experimental animals.

(1) Feeding and control

The principle of paired-feeding was adopted, the control animal in nearly all cases being from the same litter. Occasionally, the cross-control of one deficient group with another had to be utilised; litter pairing was not then possible.

Force-feeding was never adopted. In the chronic deficiencies, we learned by our trial experiments how to prevent anorexia for the greater part. In those few cases in which loss of appetite persisted, we feel paired feeding adequately covers the probable effects of inanition. In our experience, anorexia is not such a striking symptom in chronic thiamin deficiency as it appears to be in a deficiency of the entire B complex (Drummond and Marrian, 1926). The appetite could be brought back by the administration of as little as 10 µg of thiamin hydrochloride. It rarely reached a severe degree (i.e. less than 2/3) until the weight had fallen to its original level. Even then, by careful nursing the animals could be made to eat as much

as 7.5 - 10.0 g. per 100 g. body weight, which is far from starvation level in a rat exhibiting a marked disinclination to exercise.

In the acutely deficient rats, so rapid was the onset of severe symptoms that force-feeding - a matter of great difficulty in rats - led to sudden death. It was as a result abandoned. In such cases, having carefully investigated the morphological lesions resulting from inanition, it was a simple matter to take them into account in the final assessment.

(2) Caging

The rats were housed in individual cages fitted with wide wiremesh floors. In this way, it was not possible for any animal to avail itself of the supply of thiamin present in its own or its neighbour's pellets by coprophagy. This is an important factor in the ordering of such experiments, for it would seem that little diffusion of thiamin occurs out of the bacterial cells in the caeca of rats. There is in consequence very little absorption of the vitamin. If, on the other hand, the pellets are swallowed, the animal digests the bacterial envelope, and absorbs the thiamin in its large intestine. According to

Abdel-Salaam and Leong (1938) samples of flora taken from rat caeca contained as much as 16 i.u. per g. of sample. Hence if coprophagy be prevented, it will be possible to produce the effects of thiamin deficiency despite the high degree of refection occurring in the gut, although there are, of course, exceptions.

The cages were kept in a room of fairly constant temperature (65-75°F), shaded, and draught free.

(3) Selection of animals

Prior to the onset of each experimental series, the rats (piebalds and albinos) were placed in their cages on standard rat cake for a week. They were then carefully screened for any signs of disease, or of temperamental unsuitability. In the entire group of experiments only 6 needed to be replaced at this stage, 1 with a benign rectal growth unnoticed in the first instance, and 4 who appeared to be unduly excitable. One was excluded because it was unusually quiescent. It died later in one of the stock cages. These rats were all replaced from a small reserve group that was maintained, and kept on standard rat cake during the first 2 weeks of the experiment - in addition to the control animals.

Equal numbers of bucks and does were used throughout.

(4) Weight and general condition

In nearly all our experiments, rats between 50-100 g. in weight were preferred. The individual weights will be given in detail, when we describe our results.

The animals were weighed on the first day of the institution of the experimental diet; at weekly intervals; and on the last day. On each occasion, the weighing took place before feeding. At the same time, the opportunity was taken to examine the eyes, skin, fur, gait, and posture. This was done in daylight on a raised examination table. Towards the termination of each experiment a general examination was undertaken daily.

(5) The Heart Rate

The criterion taken as being most characteristic of a chronic thiamin deficiency, however, was not the onset of clinical manifestations, but a reduction in the Heart Rate.

When the rate had fallen to the neighbourhood of 300 beats per minute, it was considered that the

animals were deficient, whether gross clinical manifestations were evident or not. To make absolutely certain, nevertheless, they were left until most of the members of the group were seen to be suffering from inco-ordination of the hind limbs. All the heart rates were then taken, and in very few instances indeed were they found now to be above 300 beats a minute.

The bradycardia of experimental thiamin deficiency is generally accepted as being pathognomonic. It cannot be caused by inanition unless it be very prolonged. This was certainly never the case in our experiments. It was, moreover, never found in any of the control animals. Without altering the diet, the bradycardia can be eliminated by administering intraperitoneal thiamin hydrochloride (1 mgm.). Below a rate of 200 beats a minute, we were less successful in obtaining such recovery.

The cause of this bradycardia is not known. Harris, and his associates, however, although producing in some rats a slight increase in rate by vagal section, failed to restore the heart rate to the normal values. One must presume, therefore, that it is of sinus origin.

The method used to record the heart rates was based on that first described by Drury, Harris, and

HEART RATE RECORDING AMPLIFIER

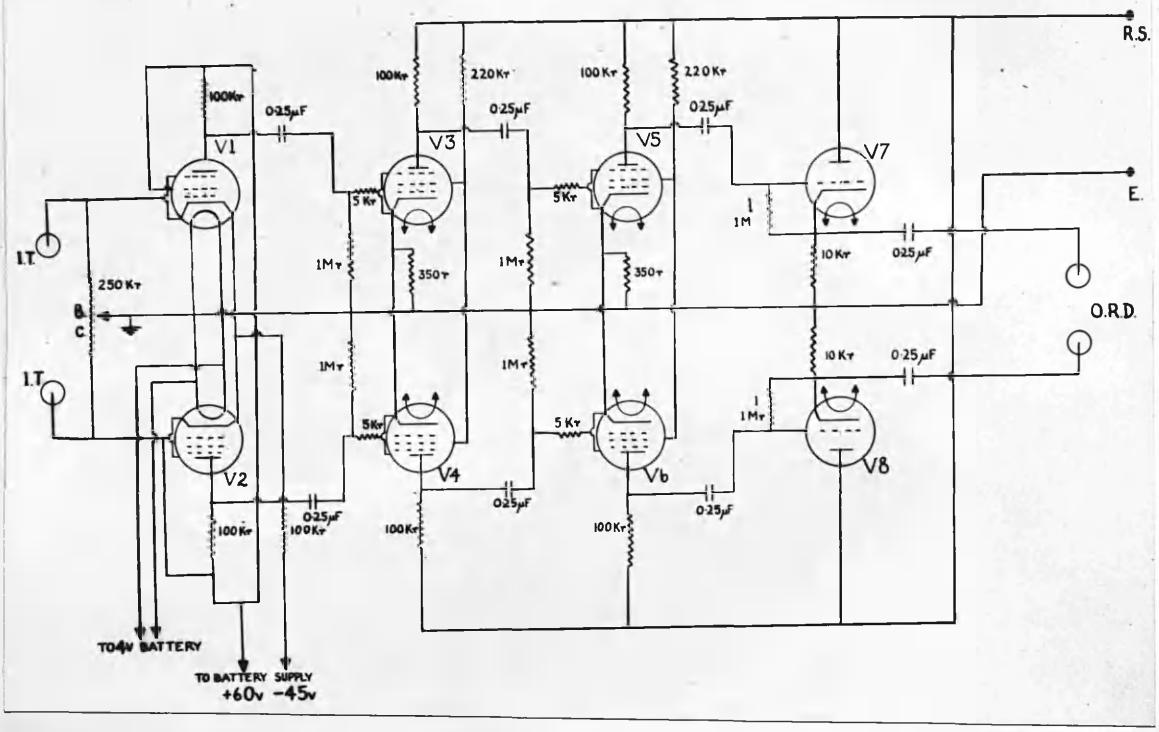

Fig. 1.

I.T. Input Terminals B.C. Balance Control

V. V1 and V2, EF37 used to avoid microphony
V3, V4, V5, and V6, SP61
V7 and V8, 6SN7
All valve heaters except V1 and V2.
Supplies from 6.3 volts A.C.

R.S. Regulated Supply, H.T. from an electrically stabilised power pack of low output impedance.

E. Earth.

O.R.D. Output recording device, which consists of a Seimens' high speed relay. A pointer (1 inch long) is soldered on the armature. This pointer is heavily damped by means of a rubber band. Its end is bent to an angle of about 35°, and records on a kymograph revolving at 25 mms./sec.

Maudsley (1930). Our apparatus was built in the department by Messrs. Catton and Farrier. The details are given in Fig.1.

In order to overcome the high resistance of the fur and skin, No.20 Record Syringe needles were used as electrodes. They were placed a few millimetres under the skin, one in the right foreleg, and the other at the lower end of the thorax, midway between the sternum and the left axillary line, in the manner described by Harris. Care must be taken not to press on the neck of the rat during the recording, as we found this gave rise to a marked slowing of the heart rate. When we became familiar with the method, the entire procedure proved extremely simple, and a recording could be obtained within 5 minutes of the start. Some of the rats, however, proved fractious, and would not remain quietly restrained in the head clamp (Plate I). Fearing this complication, we decided to investigate the effect of administering a hypnotic.

It was noticed early on that the deficient rats required lower dosages of Urethane than the controls, so we took this into the reckoning and chose as the standard dose 60 mgms. per 100 g. body weight. This meant that some of the controls were extremely

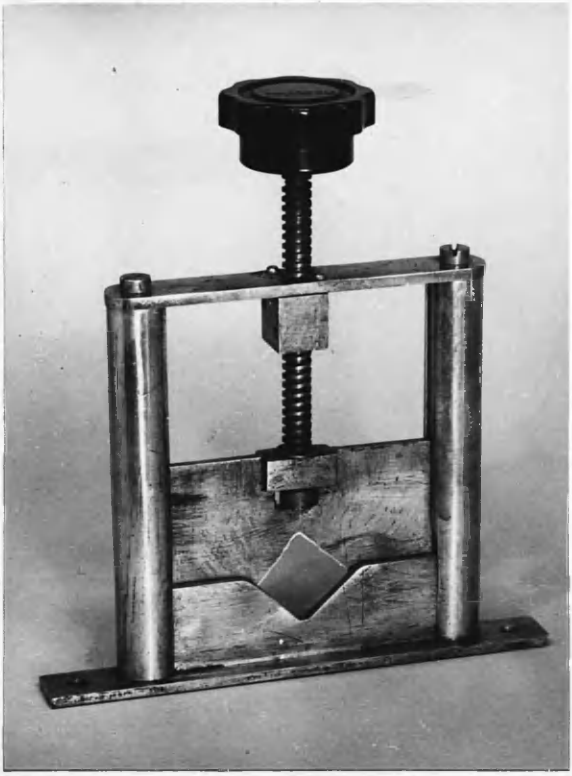

PLATE I

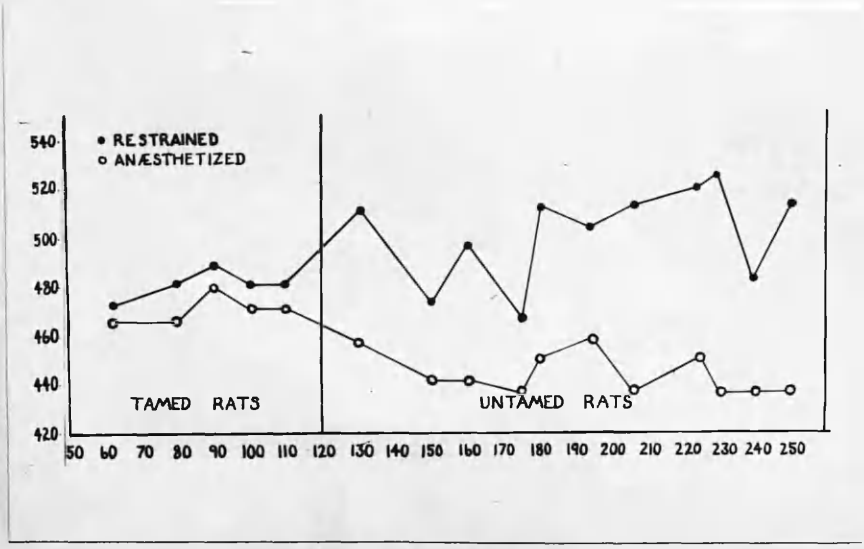
Heart Rate in beats per min.
• RESTRAINED
○ ANÆSTHETIZED
540
520
500
480
460
440
420
TAMED RATS
UNTAMED RATS
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250
Weight in gms.

Fig. 2.

lightly anaesthetized, but at the same time it gave us a truer estimate in the case of the deficient animals, as the action of Urethane on the heart rate in light hypnosis is thought to be negligible.

Sixteen normal healthy rats of varying weights were chosen at random from stock, and recordings taken (a) with the animals restrained by hand, and (b) 3 minutes after administration of the standard hypnotic dose of Urethane. The results (Fig.2) proved extremely interesting.

The question as to which of the sets of figures (restrained or anaesthetized) more nearly represents the true value is a difficult one to answer. Without the anaesthetic, every rat presumably undergoes a certain amount of emotional excitement, the heart rate as a result being elevated. This was particularly true of the larger rats we used, which had been left in the stock room, breeding, without ever being handled very much. The smaller rats by comparison were much more tame. The average heart rate of rats below 120 g. appears to be in the vicinity of 500 beats a minute: in rats above this weight, there is a steady fall down to the vicinity of 440. The emotional unrestraint of the larger rats explains the apparent paradox of our figures as seen in

Heart rates of Tamed and Untamed anaesthetized Rats

Nature of Exp.	Leblond & Hoff		Author	
	Beats/min.	S.D.	Beats/min.	S.D.
Untamed Restrained	494.85	38.60	506.36	23.30
Untamed Anaesthetized	462.85	9.00	430.90	11.95
Difference	32.00	29.60	75.46	11.45
Tamed * Restrained	453.00	24.35	480.00	7.00
Tamed Anaesthetized	431.00	21.00	468.00	8.00
Difference	22.00	3.35	12	1.00

TABLE I.

* The average of Leblond and Hoff's figures for male and female rats has been taken, as we did not thus discriminate the sexes. In the lower experiment, there is no correlation of weights.

Fig.2 (upper graph). With hypnosis, the true state of affairs emerges (lower graph).

Our results show that hypnotic doses of Urethane depress the heart rate. Whether this is a direct effect, or merely the result of eliminating the emotional factor, we do not know. Probably there is an element of truth in both. As the rats we intended to handle in the main experiments (a decision influenced by the results of trial experiments) were expected to be 100 g. or below, it was estimated that the depressant effect of Urethane at such weights would be by not more than 20 beats a minute.

In Table I, the findings of Leblond and Hoff (1944) are shown with our own. These workers compare the heart rates of restrained with nembutalised rats. For the sake of comparison we have divided ours into corresponding groups, as shown in detail in Table I. Considering that there is not an exact correlation between the two experimental series, the results show a remarkable similarity. It is not understood why the figures of Leblond and Hoff show the heart rates of young rats to be lower than old, however.

To return to the work of Harris and his co-workers, we find that temperature is another factor they believe affects the heart rate. Between 40°C

and 30°C, a 1°C fall causes a 15 beat decrease, they state. We have been unable to confirm this finding by rectal temperatures. Readings have varied in our control animals only by about 2°F. Although we took our recordings at a constant external temperature, at no time did we find a significant correlation between rectal temperature and heart rate in either deficient or healthy animals on the Fahrenheit scale.

From these premises, we might be excused in concluding that the Heart Rate is not a very good criterion for assessing the degree of thiamin deficiency. We must not forget, however, that when the recording is done under standard conditions conclusive evidence may be obtained by comparing them with control rats, whose heart rates are recorded at the same time under identical conditions.

(6) Termination of the experiment

In the Acute deficiencies, only 1 or 2 heart rates were recorded, the animals being killed when they were seen to be dying. Only 6 rats out of our many experimental groups died at night, and had to be discarded.

In the Chronic deficiencies, the diets were fed until one (or two) in a group were seen to be mildly anorexic. A week later these same rats were removed,

the heart rates recorded, and compared with the initial values. If it had fallen to the neighbourhood of 300 beats/minute, surveillance of that group was increased.

They were sacrificed subsequently at the very first signs of severe anorexia in any one of the group, the heart rates being taken in each animal prior to death, if possible without a hypnotic.

(7) Sacrificing and fixation of tissues

All the rats were decapitated while under Urethane anaesthesia.

The brains, optic nerves, and eyeballs were removed and fixed within 10 minutes in 10% Neutral Formalin. Such speedy fixation is essential if autolytic distortions are to be prevented, as we shall show later. Occasionally the sciatic nerves were similarly treated.

We would have liked to have investigated all the sciatic nerves, but such a digression from the main theme (itself involving so much practical work) would have prolonged matters unduly.

The pathological alterations in the peripheral nerves in thiamin deficient animals, it appears, are not as clear-cut as are the clinical manifestations we have earlier described. (It is this finding which

suggests that beriberi is not due solely to an absence of thiamin). Examination of the peripheral nerves of rats fed on diets containing autoclaved yeast has revealed no significant morphological differences from control animals according to numerous authors (Prickett, 1934: Davison and Stone, 1937: Engel and Phillips, 1938: and Prickett, Salmon, and Schrader, 1939). The slight changes occurring are ascribed to inanition. Berry, Neumann, and Hinsey (1945) placed cats on a thiamin-free diet for as long as 116 days but found no trace of degeneration in the peripheral nerves. The same conclusion is reached by Follis and his co-workers (1943) and by Wintrobe and his (1944) in swine*.

This fact is not, we feel, generally appreciated, but the evidence is too strong to ignore. To have used peripheral nerve preparations as a means of controlling the experiment, therefore, would appear somewhat pointless.

2. The constitution of the diets

(1) Daily caloric requirements

A rat of 75 g. will thrive on approximately 10 g. of food daily. A rat of 150 g. requires 15 g. Rats above 200 g. vary in their demands. All rats in

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* This is a controversial theme which will be developed later.

the experiment - if we exclude the preliminary trial experiments to be described next - were less than 150 g. in weight, and so 15 g. of food was fed to each irrespective of weight. Thus, in the vital experiments, where a complete histopathology of every animal in the group was undertaken, no rat, control or otherwise, was fed at too low a level. As a corollary we may add that if less than 5 g. of the daily ration was left in rats 75 g. or under in weight, it was considered quite within normal.

(2) The composition of the diets

(i) Casein	15
(ii) Salt mixture	4
(iii) Dextrinised Starch, OR Glucose	70
(iv) Autoclaved yeast, OR the water soluble vitamins (W.S.V.) ...	10
(v) The fat soluble vitamins (F.S.V.)	1
	100

Hereafter this diet will be known as "STOCK".

(3) Detailed description of dietary components

(i) Casein (lactic casein, unextracted)

Moisture	10.0%
Fat	2.1%
Nitrogen	13.5%
Calcium	0.04%

It should be noted that the composition of the diet is based on dry weights, an allowance being made for the 10.0% of water present in casein.

The quantity of protein is low, of course, as

the proportion of carbohydrate is required to be high. This precipitates thiamin deficiency (Yudkin, 1951). The protein is quite sufficient, however, for growth. It is vitamin-free.

(ii) Salt mixture

Sodium Chloride	22	g.
Calcium Phosphate.. ...	130	g.
Potassium Citrate.. ...	125	g.
Magnesium Sulphate. ...	30	g.
Iron Citrate	6	g.
Trace mixture, as below:	0.7	g.
	312.7	g.

Potassium Iodide ...	12	g.
Sodium Fluoride ...	10	g.
Manganese Sulphate..	2	g.
Cuprous iodide ...	1	g.
Potash alum ...	1	g.
Zinc Sulphate ...	1	g.

The Manganese and Magnesium proportions are higher than most workers prescribe to ensure good growth, and the normal excitability of the nervous system. There is nothing unusual in the quantities of the other salts present*.

(iii) Starch or Glucose

The dextrinisation of the starch was undertaken in the manner described by Coward (1938). Maize starch was used. It is cheap, and proved quite satisfactory.

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* This mixture corresponds to DL6 a salt complex prepared by Glaxo Laboratories, Greenford, Middlesex, and was purchased from them. In the initial planning of the diets, the helpful advice of Dr. W.F.J.Cuthbertson of that same laboratory is gratefully acknowledged.

By adding water, a consistency as of dough is obtained; it may be moulded into small cakes and baked in an ordinary gas cooker for an hour and a half. At the end of this time it is brittle, and can be ground up into a coarse powder. Allowance was made for the presence of about 5% water.

Dextrinised starch is required in a thiamin deficient diet because if raw starch is fed the rats are more able to refect. This is thought to be due to the fact that in the caecum, the bacteria responsible for the synthesis of the thiamin do so while they ferment sugars. Raw starch which is not well digested higher up will as a result reach this level, Glucose or Sucrose not.

When Glucose was incorporated in the diet, only the pure medicinal variety was used.

(iv) The water soluble vitamins

Choline Chloride	10.0	g.
Inositol	1.1	g.
Nicotinic acid	0.5	g.
Calcium-d-pantothenate	0.5	g.
Pyridoxine hydrochloride	40.0	mg.
d-Biotin	1.0	mg.
Folic acid	1.0	mg.
Para-aminobenzoic acid	0.375	g.
Distilled water to	250	mls.

150 mg. of Riboflavine was then dissolved in the least amount of boiling 5% Acetic acid, shaken in 50 mls. of nonmethylated 95% Alcohol and added.

Finally, distilled water brought the total volume up to 500 mls.

The daily dose of W.S.V. per rat was 1 c.c.

The absence of ascorbic acid from this complex is not an oversight. Rats need not be given vitamin C, as they synthesise it readily in the intestine. On a diet deficient in this factor considerable quantities are found in the livers, even after a long period (Coward, 1938).

When the relationship of riboflavine to thiamin was investigated in the later experiments, the former vitamin was made up separately, the composition of W.S.V. being adjusted accordingly. Riboflavine itself was administered at the same level (150 mg. in 500 mls. Daily dose per rat 1 cc.) as in the original W.S.V.

The full maintenance dose of thiamin required by rats above 50 g. in weight is in the region of 10 µg. a day. Woolley and White (1943) claim mice require 2 µg: Arnold and Elvehjem (1938), chicks, 10 µg: and Swank and Bessey (1941), pigeons, 20 µg.

Nevertheless, we administered it at the extremely high level of 500 µg. daily.

As an alternative to W.S.V. dried brewer's yeast was autoclaved in shallow layers at 120°C for 6 hours. Most of the arguments against the use of

autoclaved yeast are based on the point of view that not all of the thiamin is destroyed. The opposite state of affairs, it would appear, is the more likely to occur. By Coward's reckoning, 10 parts of autoclaved yeast in the diet would as a result be on the low side, as she believes that the process destroys about 50% of some other factor in addition to all the thiamin. What this factor is she does not state, but after a preliminary trial, we considered 10 parts of autoclaved yeast quite adequate. Three rats we placed on autoclaved yeast and full thiamin revealed no signs of deficiency at the end of three months. We examined them extremely carefully, especially looking for signs of pantothenic acid deficiency.

Thiamin was administered as follows:-

a. Full maintenance dose (Thiamin full)

Thiamin hydrochloride 250 mg.
Distilled water to 500 mls.

Daily dose per rat 1 cc. (0.5 mg.)

b. Minimal maintenance dose (Thiamin minimal)

Thiamin hydrochloride 5 mgms.
Distilled water to 500 mls.

Dose, when directed, $\frac{1}{2}$ cc. (5 µg)

(v) The fat soluble vitamins

β-carotene (1.2×10^6 i.u.)		5 mg.
Calciferol (0.2×10^6 i.u.)		5 mg.
α-tocopherol acetate		2 mg.
Arachis Oil	to	100 g.

Daily dose per rat 0.1 cc. (or about 3 drops)

Vitamin K (2 methyl-1, 4-naphtha-quinone) was not included, for apart from the physiological defects associated with haemorrhage, no other changes have been detected in the tissues of vitamin K deficient animals.

Ferraro and Roizin (1943), in an interesting paper on vitamin K deficiency in rats, describe the presence of multiple haemorrhages in the brain. Best and Taylor (1950), on the other hand, will not accept this, and maintain that it is not possible to induce a haemorrhagic tendency in rats owing to the high degree of synthesis going on in the intestine.

As the absence of K does not affect the central nervous system, it was omitted, a practice in keeping with other workers.

II. PRELIMINARY EXPERIMENTS TO ASCERTAIN THE MOST EFFECTIVE THIAMIN-DEFICIENT DIETS

1. Acute thiamin deficiency

According to Harris and Kodicek (1946) in refected rats a symbiotic synthesis of B vitamins occurs in the gastro-intestinal tract which prevents them developing the corresponding deficiency diseases. This is not true of B_1, as we have already seen, although there may be exceptions. The delayed onset in some cases, the modifications in others, and the absence of the characteristic manifestations in a few, on diets inadequate in or even free from thiamin would appear to depend upon a varying degree of bacterial synthesis in different rats.

As a general rule, synthesis of thiamin in rats is of a sufficiently high order to prolong the onset of an acute deficiency to some 4 or 5 weeks depending on the weight of the animals. In an attempt to produce a more acute lesion, therefore, we resorted to the use of the chemical analogue of thiamin, the antivitamin, pyrithiamin*. This substance, 2-methyl-1-amino-5-pyrimidyl methyl - (2-methyl-3-hydroxyethyl)

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* This was very generously supplied by the Research Department, Merck and Company, Rahway, New Jersey. Prepared in their laboratories, and renamed Neo-pyrithiamine, we found it most effective. At the time, it was not available in this country.

pyridinium bromide, has been shown by Woolley and White (1943) to produce in mice a fatal disease with many of the characteristic symptoms of thiamin deficiency. It may be prevented or cured by giving large amounts of thiamin. Pyrithiamin, as it is called for the sake of brevity, acts by reason of its structural resemblance to the vitamin, competing with thiamin for its position in the metabolism of carbohydrate, and antagonizing as a result its free action.

We were not certain, of course, in an acute deficiency such as pyrithiamin induces, whether or not the lesions we might expect to find would appear. Theoretically, they would be more likely to occur as the result of some longstanding abnormality related to a chronic deficiency. However, it was decided the result would be of much scientific interest.

EXPERIMENT I.

Series 1 - Minimal maintenance dose of thiamin plus pyrithiamin

Diet

STOCK (Glucose + W.S.V.)	15 g.
Pyrithiamin *	100 µg.
Thiamin minimal	5 µg.

PLATE II

PLATE III

PLATE IV

Exp.I.S1 Rat	Weights		Heart Rates *		Disposal
	Initial	Final*	Initial	Final	
1	90	80	440	200	All
2	85	87	460	200	killed
3	75	95	480	180	on
4	100	95	480	200	16th
5	100	85	440	180	day
6	85	90	480	180	
* at end of 16 days			Duration of expt. - 16 days		

General observations

Three days before death, all the rats became moderately anorexic. Due to our experiences with Series 2 (see below) we did not attempt to force-feed. On the day they were killed all the animals were found to exhibit the same clinical picture. On picking them up by the tail a convulsion appeared, and, on recovery, spasticity of the hind legs was marked (Plate II). Occasionally they fell over sideways (Plate III). Both sides seemed equally affected. Two of the animals were ultimately (minutes) unable to move, assuming what later proved to be a characteristic pose, lying flat on their abdomens with their limbs extended outwards (Plate IV).

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* Under hypnotic.

Spontaneous convulsions appeared in 2 others. This group proved unusual in that all the signs appeared in all the rats at one and the same time. In the other groups, we experienced variations in the time of onset even in rats from the same litter.

Series 2 - No thiamin administered plus pyrithiamin

Diet

STOCK (Glucose W.S.V.) 15 g.
Pyrithiamin 100 µg.

Exp.I.S2 Rat	Weights		Heart Rates		Disposal
	Initial	Final	Initial	Final	
1	130	120	420	200	allowed to recover
2	165	175	420	220	allowed to recover
3	110	107*	440	-	died 12th day
4	100	97*	440	-	died 10th day
5	95	77	460	-	killed, dying, 12th day
6	85	65	480	-	killed, dying, 12th day
* at end of 7 days			Duration of expt. - 12 days		

General observations

The characteristic signs of an acute deficiency (see Exp.I Series 1 above) appeared in all the animals by the 12th day.

Anorexia ensued suddenly on the 10th day, Rat 4 being first affected. While attempting to force-feed it, it died. On the 12th day we had the same experience with Rat 3, and so we did not attempt to use this method again. Rats 5 and 6 who were prostrate on the 12th day were killed; to Rats 1 and 2, who were spastic, we administered 1.0 mgm. of thiamin intra-peritoneally. Both were much better next morning, and were placed once again on the STOCK diet with a full complement of synthetic vitamins. They made a good recovery, and were killed 10 weeks later, when their weights were 175 g. and 235 g. respectively.

Series 3 - No thiamin, but no pyrithiamine

Diet

STOCK (Glucose W.S.V.) 15 g.

Exp.I.S3 Rat	Weights		Heart Rates		Disposal
	Initial	Final	Initial	Final	
1	55	47	480	300	Killed 33rd day
2	53	52*	480	-	Died 30th day
3	55	40	460	250	Killed 33rd day
4	60	59*	440	-	Died 31st day
5	52	48	480	200	Killed 33rd day
6	53	44	480	310	do.
* At end of 28th day			Duration of expt. - 33 days		

General observations

Anorexia was severe by the end of the 4th week. It was impossible to make the animals eat by subminimal doses of thiamin hydrochloride (≠1 µg), and it seems highly probable that in the end they succumbed to starvation. The clinical manifestations were in all respects exactly the same as Series 1 and 2 otherwise. (The heavier the rat, the longer it takes to produce the acute deficiency).

Discussion on results of Experiment I.

The most effective diet for the production of an acute thiamin deficiency is one in which the animal receives no thiamin from an exogenous source, while it is being fed pyrithiamin. The latter, administered orally, proved extremely effective at a level of 100 µg. daily per rat. It retains its potency for 6 months, if kept in ice.

The signs of the deficiency should occur in from 10 - 16 days. Anorexia may be expected after the 10th day, but is never severe until the day before death.

The loss of weight is not related to the loss of appetite, and is never great. Some animals, in fact, who became paralysed had actually gained a few grammes. It can be of no value, therefore in controlling the experiment.

The Heart Rate in the closing stages only becomes markedly reduced. This occurred so consistently that there seems as little point in recording the Heart Rates as there is in recording the weights. In short, the onset may be judged purely on clinical grounds, the most important sign being the sudden loss of appetite. Although the possibility exists that the animals may die overnight at this stage, one must take that risk, for anorexia by itself cannot be taken as the end-point of the experiment. At the first signs of inco-ordination, however, the animals should be sacrificed.

2. Chronic thiamin deficiency

The amount of thiamin required to give rise to the signs of chronic thiamin deficiency (when a high carbohydrate diet is being administered) has been variously estimated. We felt the only possible way of ascertaining this was by preliminary trial.

We also required information on several other scores:

While remembering that the main purpose of this thesis is to discover the relationship of thiamin to the visual pathway from the physiological aspect, it would be of extreme interest to compare the effects of

administering the known members of the B complex in synthetic form with yeast in an attempt to discover whether or not there exists in the latter yet another unknown member of the ever-enlarging B family.

The problem of symbiotic synthesis in the rat intestine is another problem we found of interest. By comparing the effect of diets incorporating starch with those in which glucose has been substituted, we hoped to provide further evidence as to whether or not thiamin is formed (during the fermentation processes occurring in the alimentary tract) to a degree sufficient to prevent the onset of the characteristic nutritional disease.

Finally, by running such a series of experiments we hoped to gain practical experience before embarking on the trials of making a histpathological survey.

EXPERIMENT II.

Series 1 - Control group, starch and fresh dried brewer's yeast

Diet

STOCK* (Dextrinised starch and fresh dried brewer's yeast) 15 g.

Exp.II. Sl Rat	Weights		Heart Rates		Rectal Temperatures	Disposal
	Initial	Final	Initial	Final		
1	192	285	420	420	100^{5}	All
2	167	200	480	480	98^{8}	killed at
3	175	180	480	480	99^{5}	end of
4	205	205	420	440	98^{5}	6th
5	256	225	480	480	100^{2}	week
Duration of experiment - 6 weeks						

General observations

These animals throve. The results suggest that 15 g. daily was not large enough for rats whose weights averaged 44.2 g. more at the end of the sixth week than the deficient animals in this experiment. Such a view is borne out by the voracious way in which they attacked their food. They were always hungry, and always active.

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* Attention is drawn again to the composition of the STOCK diet on page 30.

Series 2 - Control group, starch and the synthetic vitamins

Diet

STOCK (Dextrinised starch and W.S.V.) 15 g.
Thiamin (full) 1 cc.

Exp.II. S2 Rat	Weights		Heart Rates		Rectal Temperatures	Disposal
	Initial	Final	Initial	Final		
1	285	256	420	420	98^2	All
2	255	275	440	420	99^2	killed at
3	251	250	420	420	100^2	end of
4	239	240	420	420	98^2	6th
5	235	210	480	480	100^o	week
Duration of experiment - 6 weeks						

General observations

The animals of Series 2 did not do so well as those fed with yeast inasmuch as they exhibited an average weight loss of 5.8 g. at the end of 6 weeks, as compared with an average gain in the case of Series 1 rats of 20.4 g. Nevertheless they remained well and active, and were always hungry. At the start of the experiment, they were on the average 54.0 g. heavier than those in the first control group.

The heart rates and rectal temperatures were unaffected.

On taking all these factors into consideration, there can be little doubt that the loss of weight was due to the fact that they were not getting enough to eat. The diet otherwise was quite adequate.

The administration of thiamin hydrochloride to the deficient groups

In the series of deficiency experiments which follow thiamin was administered to all the four groups from week to week in the manner described below. The weekly dosage was suggested in each instance by the activity, appearance, appetite, and growth of the animals. It is not surprising, therefore, in view of our unfamiliarity with the course of these experiments, that we cut down the vitamin too zealously. This matter will be discussed with the other results later.

THIAMIN PLAN A.	
Administration of thiamin hydrochloride	
Week	Daily trial dosages
1	5 µg.
2	2 µg.
3	2 µg. every second day
4	none
5	none
6	2 µg. every second day

Series 3 - Deficient group, glucose and the synthetic vitamins

Diet

STOCK (Glucose and W.S.V.) 15 g.
Thiamin (minimal) Plan A.

Exp.II S3 Rat	Weights		Heart Rates		Rectal Temperatures	Disposal
	Initial	Final	Initial	Final		
1	168	155	420	-	-	Died on 40th day.
2	179	135	420	180	97^{0}	Killed.
3	181	157	440	150	97^{2}	Killed.
4	160	150	420	220	97^{2}	Killed.
5	262	240	480	-	96^{4}	Died during Heart record.
Duration of experiment - 6 weeks						

General observations

During the first week, all rats gained a few grammes in weight: thereafter before any loss of appetite occurred there was a steady loss of weight. At the end of the 3rd week, a disinclination to exercise and a marked desire to sleep was observed. The fur became bedraggled, and erected in a characteristic fashion (porcupine effect - Plate V). Towards the middle of the 4th week, anorexic symptoms were first recorded. As it was slight, it was allowed to continue for another week when all the

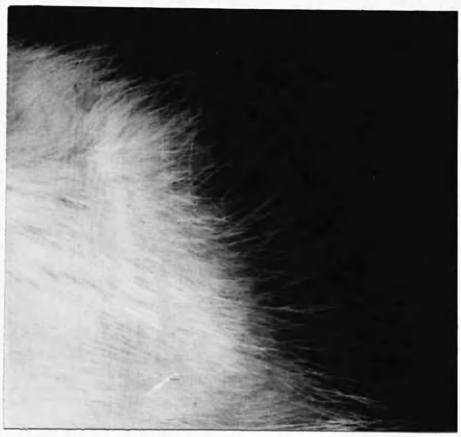

PLATE V

PLATE VI

above signs became marked. Small doses of thiamin during the final week made for no improvement. Rat 1 was found dead on the 40th day, and there was marked inco-ordination in the hind limbs of the others. Heart recordings were taken two days later at the end of the 6th week, all animals by now being spastic. Rat 5 died during the recording, and the others exhibited the convulsive seizures obtained in the acutely deficient animals. Prior to sacrificing Rats 2, 3, and 4, we noticed the presence of ptosis (Plate VI). This appeared to be a true paralysis of the upper lid, for, even if frightened, the rats were incapable of increasing the aperture of the palpebral fissure - the normal response.

The residues of food left were weighed. In the 4th and 5th weeks, it averaged 5 g. $\pm$ 2. In the last week the residue was as high as 10 g. $\pm$ 3.

The results will be discussed in the light of the other 3 deficient Series, run in conjunction with this one.

Series 4 - Deficient group, glucose and autoclaved yeast

Diet

STOCK (Glucose and autoclaved yeast) 15 g.
Thiamin (minimal) Plan A.

Exp.II. S4 Rat	Weights		Heart Rates		Rectal Temperatures	Disposal
	Initial	Final	Initial	Final		
1	189	155	420	300	96^{8}	All
2	191	205	460	440	97^{8}	killed at
3	187	180	440	400	97^{4}	end of
4	192	155	420	320	96^{8}	6th
5	209	205	420	420	98^{0}	week
Duration of experiment - 6 weeks						

General observations

This group fared much better than the previous one. Anorexia was only slight up to the beginning of last week, and even then each rat ate at least 10 g. The fur was bedraggled: the porcupine effect striking: they were inactive: slept a great deal: and when killed all five exhibited only slight inco-ordination of the gait.

Series 5 - Deficient group, starch and the synthetic vitamins

Diet

STOCK (Dextrinised starch W.S.V.) 15 g.
Thiamin (minimal) Plan A.

Exp.II S5 Rat	Weights		Heart Rates		Rectal Temperatures	Disposal
	Initial	Final	Initial	Final		
1	189	160	420	360	98^4	Killed
2	182	164	420	360	98^4	Killed
3	187	162	420	-	-	Died during recording
4	169	160	420	-	-	do.
5	199	185	420	380	99	Killed
Duration of experiment - 6 weeks						

General observations

The clinical course was exactly as in Series 3 up to the beginning of the last week, although anorexic symptoms were never so severe.

Although Rat 4 exhibited signs of spasticity on conclusion of the experiment, the other four were only slightly inco-ordinated.

Series 6 - Deficient group, starch and autoclaved yeast

Diet

STOCK (Dextrinised starch and autoclaved yeast) 15 g.
Thiamin (minimal) Plan A.

Exp.II S6 Rat	Weights		Heart Rates		Rectal Temperature	Disposal
	Initial	Final	Initial	Final		
1	188	205	420	360	984	Killed
2	190	185*	440	-	-	Died 5th wk.
3	181	200	420	420	988	Killed
4	204	198	420	360	984	Killed
5	182	169	420	420	978	Killed
* 6 days before death			Duration of experiment - 6 weeks			

General observations

Rat 2 in this group became paralysed 4 days before the end of the experiment. It died next day. The others although shaky did not exhibit spasticity. In short the state of the deficiency paralleled Series 4 and 5.

Discussion on results of Experiment II.

It is obvious that the speed in reduction of exogenous thiamin has been too great in these experiments. That will have to be altered. It is also apparent that the use of younger rats would be an advantage.

Assessment of Effectivity of thiamin-deficient diets by comparing clinical manifestations

Exp.II Series No.	Diet	Mean Heart Rate (beats/min)	Mean T° (°F)	Mean Wt. loss or gain (gms.)	Incoordination	Spasticity	Death
3	Glucose Syn. vits.	183.3	97^{2}	-22.6 none gained	5 severe	5	2
4	Glucose Autocl. yeast	378.0	97^{4}	-13.6 1 gained	5 slight	none	none
5	Starch Syn. vits.	366.6	98^{7}	-19.0 none gained	5 moderate	1	2
6	Starch Autocl. yeast	390.0	98^{3}	+2.4 3 lost	none	none	none

TABLE II.

The diet in Series 3 (Glucose + W.S.V.) was the one most effective, nevertheless, in producing signs of the deficiency. It also appeared to be the diet the composition of which we could best control.

The heart rates in Series 3 all fell to about 180 beats a minute. The average loss of weight after 6 weeks growth was 22.6 g., none of the rats exhibiting a gain. The rectal temperatures although varying showed an average fall to 97^{2o}F. Two out of the group of 5 died. Inco-ordination and spasticity occurred in every rat.

None of the other 3 Series could compare in the severity of their symptoms at the conclusion of the experiment, as can be seen in Table II.

It is interesting to note that the least successful diets were those in which autoclaved yeast replaced the synthetic vitamin B complex (less thiamin). It is difficult to say why.

One cannot claim to have obtained by this finding indirect evidence that another member of the B group as yet unknown exists in yeast. Two other explanations are much more probable: either the autoclaving process did not destroy all the thiamin, or, if it did, in addition it destroyed another constituent member. It is

possible that in the absence of a hydrogen carrier (such as this other member might very well be) the carbohydrate will not be used so quickly. As a result, there will be a lesser need within the central nervous system of thiamin, and life will be prolonged. In retrospect this latter explanation is the one favoured. Later we obtained evidence supporting such a view (Part IV). At this stage, of course, it is only conjecture.

Comparison of the Heart Rates affords further interesting data. The heart rate when Glucose and the synthetic vitamins are used (Series 3) is much lower (180/min.) than when Starch and the synthetic vitamins are used (Series 5) - (360/min.). We have already shown how the presence of dextrinised starch in the caeca of rats, where no glucose is likely to be found, promotes the symbiotic synthesis of thiamin. The discrepancy in heart rates supports such a view. In other respects, however, these two series do not differ so greatly. Two died in each series, and all were incoordinated, although the Series 3 rats were the more so.

It seems probable that the grosser nervous manifestations are not due directly to the absence of thiamin, but rather to the loss of appetite which occurred during the last days of the experiment.

Yet, we cannot be certain on this point for in the case of Rats 1 and 2, Series 2, Experiment I (acute deficiency), a good recovery was made 24 hours after administering a full dose of thiamin. The nervous manifestations, gross in these two animals, vanished without leaving a trace of residual paralysis. Assuredly this did not arise as a result of a return of appetite. Such a recovery favours the view that the cause of the disorder is a biochemical one, of a kind we know is produced in the absence of thiamin (Peters, 1936).

It would appear, then, that bradycardia is by far the most specific criterion of thiamin deficiency. Anorexia, although important and indicative of a biochemical disorder, is not specific. Any rat suffering from any deficiency will lose its appetite in the end. Within the context of our experiments, however, anorexia is suggestive. Two other points can help us. First, the loss of weight occurred in the chronic deficiencies prior to loss of appetite. (We have already discussed this in the acute deficiencies). Our records show that this loss occurred up to two weeks before anorexia was noticed. A careful weekly weighing is, then, likely to be an added means of controlling the chronic deficiency.

Secondly, and lastly, how can we get round the vexed question of "starvation lesions" in these deficiencies?

It seemed we might, by making the chronic experiments less drastic, get over this difficulty. In the acute experiments however, the onset of all the nervous signs which advise us, as we have seen, that the experiment has been successful, is extremely sudden. Might it not be that in that last 24 hours irreversible anatomical damage results from inanition, which would completely confuse the evidence we seek?

It was this problem, and also the desire further to convince ourselves that the nervous manifestations are caused by starvation, that forced us to design one other preliminary experiment, whereby these questions might be better answered.

EXPERIMENT III

Series 1 - Caloric inadequacy, without Exogenous vitamins

Diet

Potato Starch	70%
Wheat Straw Pulp	20%
Agar-Sucrose	10%

On this diet* 6 rats - of average weight 200 g. - died at the end of 11 days. They were perfectly healthy

* The diet was suggested by Dr. R.A.Armstrong, Department of Agriculture, King's College, University of Durham.

until the morning of the 10th day when they were seen to be very inactive, and to have assumed a crouching position. They could be roused only with difficulty. Their movements were inco-ordinated, and their postural sense appeared diminished. Next morning they had violent convulsions while on the examination table and within half an hour were dead.

The tissues of the visual pathway were immediately obtained for subsequent histological examination (Exp.IV, Series 7).

Series 2 - Caloric inadequacy associated with full vitamin intake

Diet

Potato Starch	70%
Wheat Straw Pulp	20%
Agar-Sucrose	10%
W.S.V. (less thiamin)	6 ccs.

These rats were fed the usual 15 g. daily, unlike those of Series 2 who had been fed ad libitum.

The 6 rats - of average weight 160 g. - were members of the same litter. They were left on the above diet for 24 hours. Then they were divided into two groups, one of which (A) remained on the same diet, the other of which (B) received 2.0 mgms. of thiamin (i.p.) in addition.

On the 18th day, the rats in Group A all died, as did one of the rats in Group B. Two days later the remaining 2 rats in Group B, now moribund, were killed. The manner of death in those permitted to die was exactly as described in Series 1 above.

These brains were saved also (Exp.IV, Series 8), as were the sciatic nerves.

Discussion on results of Experiment III.

The similarity of the violent nervous disorders in the two groups of rats of Series 2 and in the rats of Series 1 confirms us in the belief that their cause is inanition, and has nothing to do with a specific vitamin deficiency.

We did note, however, that the convulsions in the rats suffering from inanition were of a much higher order than in the acutely thiamin deficient rats. This may not, of course, have any significance.

Conclusions (Experiments I, II & III)

1. The most effective means of inducing an Acute thiamin deficiency is to feed the following diet:-

Casein, vit. free	15
Salt mixture	4
Glucose	70
The Water soluble vitamins (less Th.)	10
The Fat soluble vitamins	1
	100

15 g. daily per rat with 100 µg. of pyrithiamin.

2. The most effective means of inducing a Chronic thiamin deficiency is to feed the following diet:-

Casein, vit. free	15
Salt mixture	4
Glucose	70
The Water soluble vitamins (less Th.)	10
The Fat soluble vitamins	1
	100

15 g. daily per rat with at least 5 µg. of thiamin hydrochloride, as suggested by the appetite, appearance, activity, and weight of the animal.

3. Idiosyncrasy (where an animal dies or thrives contrary to the rule in its group) occurs, but is rare. It probably depends upon the degree of symbiotic synthesis occurring in that particular rat's caecum. Such a rat may be discounted.

4. The nervous manifestations which occur prior to death are apparently due to inanition.

In Acute deficiencies complete loss of appetite may produce such functional disturbances within 24 hours of the last meal. In Chronic deficiencies, anorexia is more gradual and less severe, and may be controlled. When it coincides with bradycardia, thiamin deficiency may be said to have been induced. It is possible, then, in such cases to sacrifice the animal at this stage, thereby avoiding the complication of inanition.

5. If the role of thiamin in maintaining the integrity of the visual path is to be revealed by histopathological methods, it will be possible to obtain material in the case of Chronic deficiency. In Acute deficiency, however, it will be very difficult to exclude lesions due to inanition. For that reason we must examine the brains of such 'starved' animals, and familiarise ourselves with any structural alterations that might be present.

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PART III.

THE ANATOMY OF THE RAT VISUAL PATHWAY: A DESCRIPTION OF THE HISTOPATHOLOGICAL TECHNIQUES, AND THE FALLACIES INHERENT IN THEM: AND EXPERIMENTAL STUDIES OF THE REACTION OF THE VISUAL NEURONES TO DISEASE.

I. The anatomy of the visual pathway in the rat, including some personal observations.

As the rat appears to have a large monocular field of about 90°, it is not surprising that the macular area of the retina is not very well developed. There is only a slight increase in the number of ganglion cells halfway between the disc and the temporal rim just above the horizontal meridian.

The ganglion cells (Plate I,a) are multipolar nerve cells with a large, clear, round, sometimes oval, nucleus, somewhat eccentric, and set in a cytoplasm rich in a coarse chromophile substance. The nucleolus is well marked. These cells are large (30 μ), and are intermingled with glial cells (15 μ) in the ratio of 1 to 6 or 7 in 7 μ sections. The dominating process (3 μ) is not the axon, but one of the dendrites.

In the rat retina (Plate I,b) the ganglion cells are 1.5 rows deep; the bipolars (12 μ in diameter) are about 8 deep; and the rod and cone granules (8 μ in diameter) about 16 deep. At the macula these figures are almost doubled. In short, the nuclear layers contain twice as many cells as their homologues in man.

PLATE I

a. Retinal Ganglion cell. Giemsa x 1600.

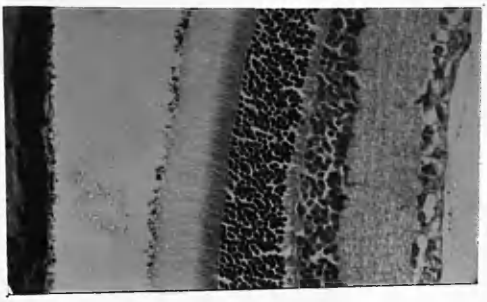
b. Rat Retina. Giemsa x 175.

c. Rat Optic Tract after enucleation of one eye. The uncrossed fibres occupy as much space as do the crossed. Author's silver x 640.

d. Healthy myelin sheaths viewed by polarised light. Optic Nerve x 350.

e. Optic Tract encircling the cerebral peduncle, and flattening out as it runs from the ventral to the dorsal surface (from right to left in the photomicrograph). Loyez x 200.

a(x1600)



b(x175)

d(x350)

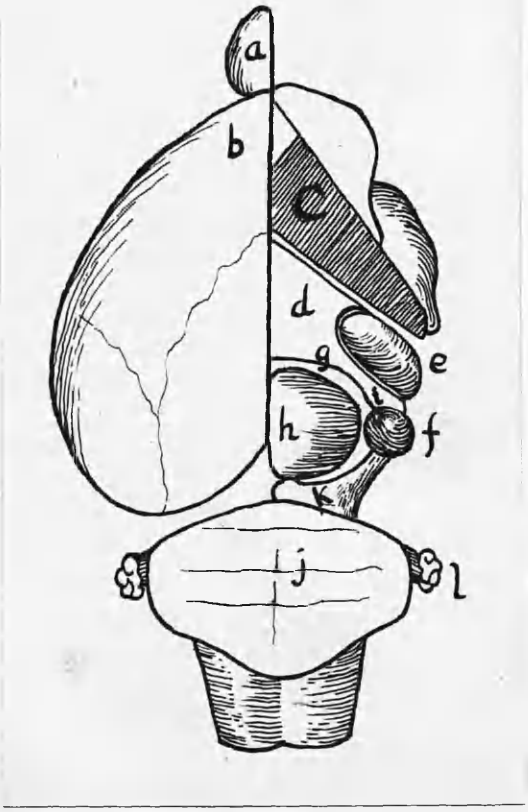
c(x640)

e(x 200)

PLATE I

Despite the limited nature of the binocular field, the proportion of crossed nasal fibres to uncrossed temporal in the optic nerve appears to be rather greater than one would have expected. In the proximal third of the optic tract they have separated out (Plate I,c) but up to that point there is little separate congruity. Lashley (1934) has supplied evidence that there is an individual reproduction of the four retinal quadrants in the lateral geniculate body, but this we did not attempt to confirm as it was scarcely necessary to our purpose. He further claims that the central retinal fibres are projected on to the central region of the dorsal nucleus. Nauta and van Straaten (1947), could find no definite localization of the degenerating terminals in any one part of the nucleus. We ourselves burned the macular region in one eye, and 10 days later investigated both nuclei with a similar lack of success. More evidence, it would appear, is required before a clear cut macular representation can be stated to exist.

We are not concerned in the connections made by the visual fibres with the superior colliculi,



Dorsal Aspect

a. Olfactory bulb

b. Cerebrum

c. Capsula interna

d. thalamus

e. lat. gen. body

f. med. gen. body

g. Pretectum

h. Sup. Colliculus

i. pulvinar

j. Cerebellum

k. inf. colliculus

l. paraflocculus

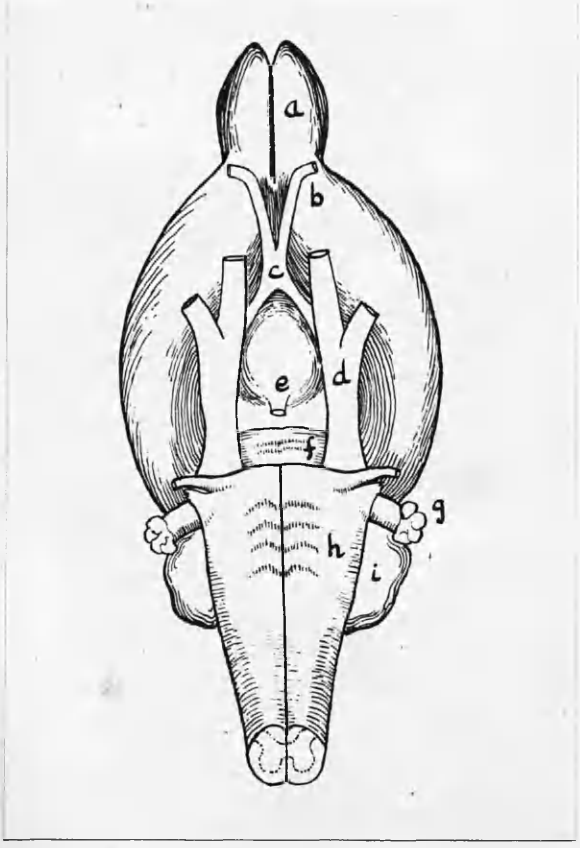
Fig. 1.

PLATE II

the pulvinar, the pretectum, Cajal's nucleus, nor the deep lying nucleus opticus tegmenti, for it is certain that the visual impulses underlying the form sense are for the greater part relayed to the cortical area striata, and then only by the dorsal nucleus of the lateral geniculate body. The other relays we have mentioned are concerned with such primitive visual reflexes as the pupillary, blinking, etc. These diencephalic structures may be seen when the cerebral hemispheres are removed from the dorsal aspect of the rat brain (Fig.1 and Plate II).

The optic nerves emerging from the eyeball are some 10 mms. in length and 1 mm. thick. They enter the cranium through the optic canals, and pass back on the ventral surface of the brain, converging towards each other until they fuse to form the chiasma at the level of the cephalad border of the hypophysis (Fig.2 and Plate III). Only a few of the optic nerve fibres are non-myelinated; in the rest, the myelin sheaths, as viewed by the polarising microscope, are surprisingly thick (Plate I,d). This is true also of the tracts.

From the chiasma, each tract passes dorsilaterally deep to cranial nerves 3, 4, 5, and 6 to encircle the



Ventral Aspect

a. Olfactory bulb

b. Optic nerve

c. Chiasma

d. Trigeminus

e. Hypophysis (cut)

f. Pons

g. Parafloccculus

h. Medulla

i. Cerebellum

Fig. 2.

PLATE III

cerebral peduncle (crus) of that side (Fig.3 and Plate IV). They pass, next, rostral to the medial geniculate body and the colliculi, and running forwards, external to the pulvinar - having now performed a half circle - reach the lateral geniculate nuclei. Throughout most of their course, the tracts lie under cover of the large smooth cerebrum, which is such a characteristic feature of the rat brain.

As the tracts pass round the lateral border of the brain stem they are seen to expand and flatten, thereby becoming closely approximated to the outer surface of the lateral geniculate body (Plate I,e). The visual fibres are thus enabled to leave the tract along its entire length and breadth, synapsing with the cells in the peripheral and central regions of the dorsal nucleus. Many of the fibres leave the tract earlier, and pass through the substance of the ventral nucleus, and, as Gurdjian (1927) first pointed out, the dorsal nucleus itself is traversed by optic fibres (many in meridians at right angles to each other) which pass through to a different destination. We need only concern ourselves, however, with those fibres terminating in this region (Plate V,a).

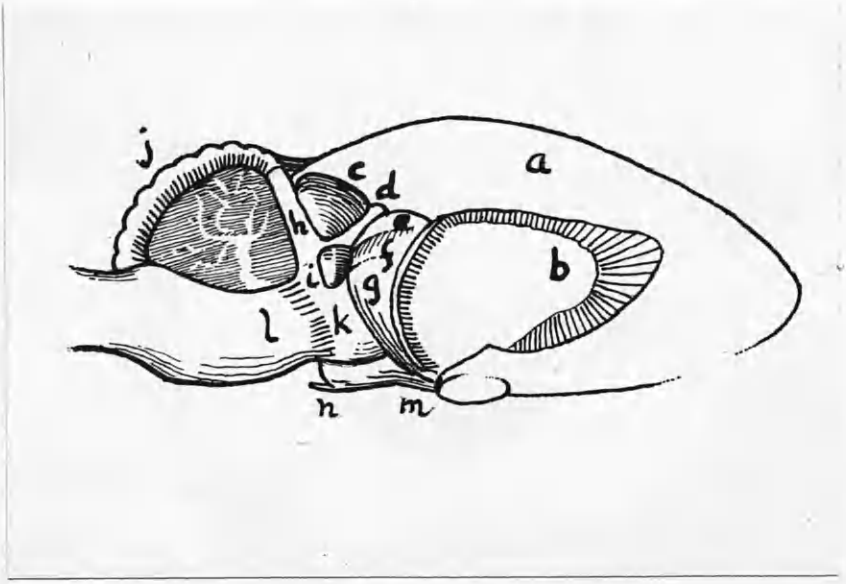


Fig. 3.

Lateral Aspect

a. cerebral hemisphere
b. Corpus Striatum
c. Sup. colliculus
d. Pretectum
e. Pulvinar
f. L.G.B. (dorsal nucleus)
g. L.G.B. (ventral ")
h. inf. colliculus
i. med. gen. body
j. Cerebellum
k. C.M.S.
l. Pons
m. Optic tract
n. Hypophysis (cut)

PLATE IV

The intranuclear fibres, peripheral and central, crossed and uncrossed, and those traversing the nuclear substance, all intermingle freely, and there is as a result no subdivision into layers as occurs in the primates.

A further difference between the rat pathway and the primate lies in the mode of termination of the fibres. Le Gros Clark (1941) has shown that in primates each of the 3-5 terminal branches of a visual fibre makes contact with a single geniculate cell, which receives no afferents from any other source. Glees (1941) describes how in the cat the terminals of a single optic tract fibre cover an area embracing some ten geniculate cells, each cell having approximately 40 synaptic contacts, whose origins are obscure. In the rabbit Glees (1942) finds 10 synaptic contacts with each geniculate cell, and an immeasurable quantity of axodendritic contacts. From our experience, we feel little weight should be placed upon such high figures. The terminations after all are restricted by the thickness of the section, and the whole picture is extremely complicated. In the rat, Nauta and van Straaten (1947) express doubts as to whether the 'bouton' method used by

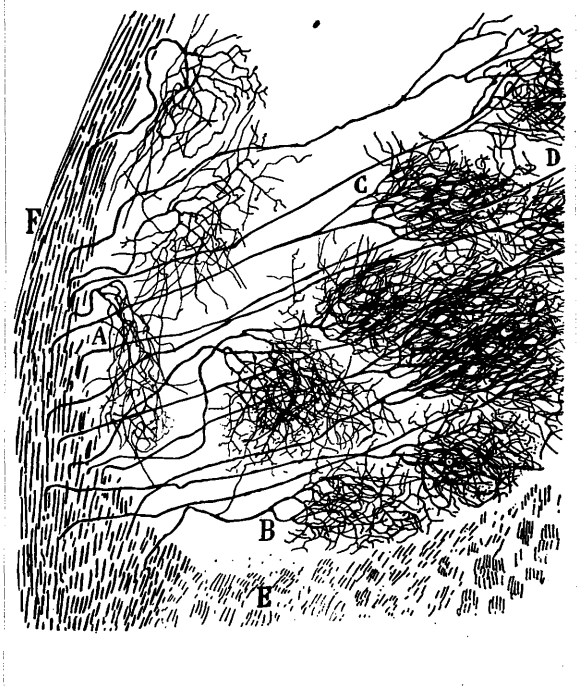


Fig. 4.

Lateral Geniculate Body of Cat (after Cajal)

A - Superficial arborisations of optic fibres.

B,C,D - Deep arborisations.

E - Lower extremity of lat. gen. body.

F - Optic Tract.

Le Gros Clark and Glees is of value for establishing such point-to-point relationships, except as a gross measure. They did not state why, but we expect it is for the reasons we ourselves have already given. We have found it impossible in the rat to count all the axosomatic and axodendritic synapses by the 'bouton' method. They appear to be legion even in very thin sections (Fig.4).

The nerve fibres found within the geniculate body are not in our opinion beaded, and their terminations are probably of the same diameter as the fibre. That is to say that we do not believe in the existence of end-buttons, either solid or ringshaped. All the workers already mentioned, and many others, have remarked at one time or another on the difficulty experienced in demonstrating them in the visual centres of normal healthy animals, but they still believe they exist. Whether or not the isolated argentophil ring (Plate V,b) that is occasionally seen in well stained silver preparations represents a normal button, whose continuity with its nerve fibre has been severed by the microtome knife, is a matter of personal opinion. We have elsewhere expressed, and given some proof of, our scepticism (Rodger, 1950). We shall enlarge on this point later.

The rostral extreme of the dorsal (visual) nucleus of the lateral geniculate body, according to Gurdjian, extends further cephalad than the ventral nucleus. Viewed transversely it is somewhat elongated, or oval shaped. The corticotectal fibres separate its medial aspect from the thalamus, which extends ventrolaterally. The multipolar cells of the visual nucleus - the final neurones of the optic path - are large (20 μ), and elongated, with a clearly demarkated axon hillock (Plate V,c). The cells in the central region are slightly the larger, and are less elongated than those in the periphery (Plate V,d). The visual cells are generally larger and less densely packed than the non-visual cells in the ventral nucleus. The nuclei are round, with well defined nucleoli, and are not always centrally placed. As eccentration is commonly considered to be significant of a degenerative process this is important to know. While an eccentric nucleus as a normal feature is uncommon in the central nervous system, it is accepted as a normal feature in the cells of Clarke's column, Goll and Burdach, etc. As long as we can distinguish extrusion from eccentration, the latter phenomenon in our opinion should be ignored.

PLATE V

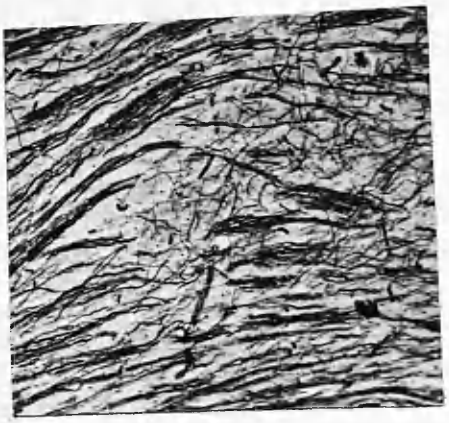
a. Dorsal nucleus of the lateral geniculate body of an albino rat. Author's silver (not counterstained) x 160.

b. Isolated argentophil ring apparently attached to a 'stump' of axis cylinder. Author's silver x 1500.

c. Final visual neurone with axon hillock clearly demarkated. The swelling of the processes is abnormal. Author's silver x 670.

d. Normal visual cells in lateral geniculate body. Note clearly outlined nucleoli, large nuclei (often eccentric) and slight flattening of cells on the right (peripheral). Giemsa x 320.

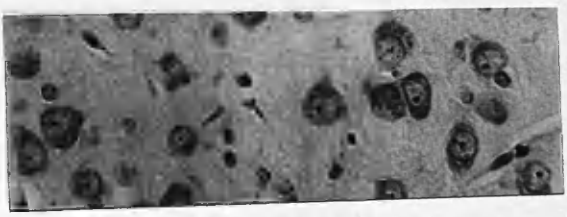
. & f. The relative size of optic and sciatic nerve fibres compared under polarised light at the same magnification x 250.



a(x160)

b(x1900)

c(x670)



d(x320)

e(x250)

f(x250)

PLATE V

It appears too frequently in healthy cells to be pathological. Nissl granules in the geniculate cells are plentiful, being scattered generously throughout the cytoplasm, save of course in the axon hillock.

The optic radiations leave the lateral geniculate body in a rostral direction. To demonstrate them is difficult. In the rat, the geniculo-cortical bundle is extremely small, and is sandwiched between the thalamo-cortical fibres and the auditory radiations. If there is to be any comparison, however, between our physiological findings, and nutritional amblyopia (affecting the nerve head) in man, then it should not be necessary to examine either the optic radiations or their cortical projections. The ground will be quite adequately covered, if we investigate up to the lateral geniculate body level. Here, we observe not only the cell bodies of the final visual neurones, but a not inconsiderable portion of their axis cylinders.

Krieg (1946) gives a most accurate account of the cortical areas of the rat, in which, however, we have no special interest. The visual area proper corresponds to Brodmann's area 17 in the ape. Throughout the visual pathway, the nerve cells and fibres are

packed around with neuroglia. We made a differential count in the dorsal nucleus, and found in the neighbourhood of 200 ganglion cells, 431 glial cells, a 1:2 relationship. Of the latter, 163 were astrocytes, 127 oligodendrocytes, and 141 cerebral histiocytes (microglia). Within the tract, the oligodendrocytes evinced a tendency to form short proliferative chains. It has been suggested the function of this cell is the maintenance of myelin nutrition. We never found satellitosis in normal pathways.

Dissection of visual path.

We found the visual pathway readily dissectable, even when the brain was comparatively soft. The following method was adopted.

After 48 hours in Formalin, and 24 hours in 50% Alcohol, the cerebral hemispheres are removed by hand. Then, splitting the chiasma with a knife, and laying the brain on its lateral border, the optic nerve, half-chiasma, tract, and lateral geniculate body are excised in one piece. A portion of the thalamus and cerebral peduncle are included.

Reference to Fig.1 will show the angle at which the incision is made (from f to d). The section runs back medially from the lateral border of the hemisphere at an angle of 45°, midway between the

transverse and sagittal meridians. As the lateral geniculate body is cut longitudinally, an opportunity is afforded of observing the nerve, tract, and dorsal nucleus in the same plane, and of following the visual fibres in to their synaptic connections with the geniculate cells. For our purposes, it is ideal. The section may be described as having anteroposterior and dorsoventral diameters. As it is possible to examine by serial sections the entire length of the nucleus, observations may, therefore, be made in the medio-lateral diameter as well.

TECHNIQUES

Part of Visual Path concerned	(1) Nissl's	(2) Giemsa's	(3) Triple	(4) Polarised Light	(5) Marchi's	(6) Weigert-Pal's	(7) Loyez'	(8) Silver Nitrate
Retina	X	X	X					
Optic nerve (axons)				X				X
Optic nerve (myelin)				X	X	X	X	
Optic tract (axons)				occasionally ~~rarely~~				X
Optic tract (myelin)				dits. ~~rarely~~	X	X	X	
Lateral geniculate body (cells)	X	X						
Lat.gen.body (processes)								X
Lat.gen.body (myelin)							X	

TABLE I.

II. The histopathological techniques employed.

It was obvious to us from the outset of this thesis that as far as the experimental side was concerned, the main criticisms would be levelled (as in the case of other workers) at two things, the manner in which the diets were fed and controlled, and the adequacy of the histological procedure. The former of these we have already dealt with at some length. We now propose to deal with the latter.

Table I gives a detailed plan of the various methods used to investigate the integrity of the visual path in the deficient animals and their controls.

The fact that we chose to fix the eyes, and brains, and when required the sciatic nerves, all in 10% Neutral Formalin simplified matters considerably. Except in the case of the specialised stains for myelin, it meant that we could obtain serialised paraffin sections available for each of the other techniques (Table I, 1,2,3,7, and 8).

The eyeballs and brains were left in formalin for 48 hours. The lenses were then extracted from the eyeballs - through a coronal corneal incision, and the visual paths, to be dissected in the manner previously

described, were placed (if the technique so permitted) in 50% Alcohol. Thereafter, the tissues were brought up in short spells through the alcohols, cleared in chloroform, and embedded in the usual manner for 2 hours*.

The sections were 7-10 μ thick except in the case of those cut for the polarising microscope on the freezing microtome, which were 10-12 μ thick.

The details of the different methods employed will now be given.

(1) Nissl's

Flood with Methylene Blue 1% for 5 minutes (3-4 drops of aniline oil is added to the solution before use).

Differentiate in 70% Alcohol, using the staining microscope.

This stain did not prove so effective as Giemsa's, as the ganglion cells differentiated at a different rate from the bipolar and granule cells.

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* We are much indebted to Mr. A. Harvey, the Department of Pathology, Royal Victoria Infirmary, Newcastle on Tyne, for permitting the blocks to be prepared by the histokine. Much time was saved by using it. Mr. Harvey also made himself responsible for all the Loyez' staining. Otherwise the material in the main experiments was prepared by ourselves.

(2) Giemsa's

Place in Giemsa's Solution for ½ hr.

Differentiate in 50% Alcohol (to which Eosin may be added if desired), and then in 90% Alcohol.

Giemsa's Solution

Gurr's R.66 (azure blue)	1.5 ccs.
Methyl alcohol, pure	1.5 ccs.
Sodium Bicarbonate 0.5%	2 drops
Distilled Water to	50 ccs.

The mixture is buffered with phosphate buffers, the optimum pH being found by trial and error. It was always on the acid side, ranging from pH 6.8 to pH 4.4.

It is an excellent method.

(3) Triple

This is a controlled modification of Mallory.

Stain in Celestin Blue solution 10-20 mins.

Rinse in water.

Filter on Mayer's haemalum 5-10 mins.

Rinse in 95% Alcohol.

Stain in Orange G 0.2% (in 80% Alcohol saturated with picric acid) 5-15 mins.

Rinse in water.

Filter on Ponceau-fuchsin 3 mins.

Decolourise in "Strong differentiator" under the staining microscope.

Stain with Soluble blue 2% (in acetic acid 1%) 2 mins.

Rinse in water.

Decolourise in "weak differentiator".

Celestin Blue Solution

Iron alum	2.5 g.
Distilled water	50 ccs.
Leave overnight	
Add Celestin Blue	0.25 g.
Boil for 3 minutes, and filter.	
Add Glycerol	7 ccs.

Mayer's Haemalum

Haematoxylin	1 g.
Sodium iodate	0.2 g.
Powdered Potassium alum	50 g.
Distilled water to	1000 ccs.
Leave overnight	
Add Chloral hydrate	50 g.
and Citric acid	1 g.
Boil for 5 minutes.	

Strong differentiator

Stock decolourising solution	70 ccs.
Absolute Alcohol	30 ccs.

Weak differentiator

Stock decolourising solution	30 ccs.
Distilled water	70 ccs.

Stock decolourising solution

Phosphotungstic acid	5%
Phosphomolybdic acid	5%
95% Alcohol	25%

This method afforded the best opportunity of studying the nerve fibre layer of the retina.

(4) Polarised Light

The famous prism made from Iceland Spar, and devised by William Nicol in 1828 has two disadvantages. Optically perfect crystals of large size are practically

unobtainable so that this device is limited to very small apertures, and, secondly, it is costly.

We had a Watson microscope adapted, therefore, to act as a polarising microscope by inserting in it synthetic polaroid film*. Polaroid film consists of thin plastic with submicroscopic crystals of a dichroic substance, Herapathite, embedded in it in such a way that the optic axes of all the crystals lie parallel. As a result, it acts optically exactly like a thin single crystal plate of Iceland Spar.

One of the films was mounted between two discs of glass, and held together by a thin metal ring on which the plane of vibration of the polarised light transmitted was marked. This polaroid (the polariser) was placed under the condensor in the filter holder.

The other film (the analyser) was mounted in a modified eyepiece, and screwed into the lower extremity of the draw-tube.

The polarised light transmitted by the polariser will be transmitted by the analyser only when the two vibration axes are made parallel. When perpendicular (that is, crossed) no light will pass, as the analyser

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* Made for us by Messrs. W.B. Nicolson (Scientific Instruments) Ltd., Bath Street, Glasgow C.2.

absorbs it all. On using a very bright source, however, for photomicrography, we observed that some of the blue and some of the red end of the spectrum was transmitted. The field as a result appeared a deep claret colour. This could be eliminated under high power only - by closing the iris ring.

The use of Nicol prisms for the study of neurohistology was suggested first by Baldi (1929). Setterfield and Sutton (1934) put the method to the test, describing in an excellent paper the appearances accorded by experimental myelin degeneration.

Prickett and Stevens (1939) repeated this work in a paper that is beautifully illustrated. In the same volume is a paper by Prickett, Schrader and Salmon describing the appearances in acute and chronic B_1 deficiency in the rat. All these papers, however, were based on studies of the peripheral nerves.

Because the optic nerve fibres are extremely small it is difficult to obtain clear-cut photomicrographs (Plate V,e and f).

Engel and Phillips (1938) failed to obtain results. Although it is not nearly so easy, however, as in the case of large peripheral fibres, we have shown it is possible to obtain sections thin enough to interpret the results with accuracy. We believe with more time that we could further improve this technique.

Such is the meagre literature, concerning a method which appears to us to be ideal for experimental work, although even yet one not in general use. The procedure is simple. 10-12 μ longitudinal frozen sections are cut straight from formalin into distilled water. They are then mounted in Glycerine Jelly, and allowed to clear for 15 minutes, by which time they are ready for photographing.

The appearances will be described later. We used the method exclusively for optic nerve, rarely for tract.

(5) Marchi's

Handle the tissues with care and use only well-cleaned vessels.

After 24 hours in Formalin, the tissue is suspended in gauge in Potassium Dichromate 2.5% for 7 days.

Place next in Marchi Solution for a further 7 days.

Wash in running water at least 48 hours.

Rapidly dehydrate.

Marchi Solution

Osmic Acid 1%	1 part
Potassium Dichromate 2.5%	2 parts

This method was used for nerve and tract.

(6) Weigert-Pal's

Transfer from Formalin direct to Weigert's rapid fixative for 4-7 days.

Wash in running water for 12 hours.

Dehydrate and embed as usual.

Cut 10 μ sections and bring to water.

Mordant a second time in ½ sat. Copper Acetate for half-an-hour.

Wash well in distilled water.

Place in Pal's haematoxylin for 4-6 hours.

Wash in water (to which a little saturated lithium carbonate 1.33% has been added).

Differentiate in Kultschitzky's solution for 2-6 hours.

Weigert's rapid fixative

Potassium dichromate	5 g.
Fluorochrome	2 g.
Distilled water	100 ccs.

Prepare by boiling the dichromate in water, then adding Fluorochrome. Filter when cool.

Pal's Haematoxylin

0.75% aqueous soln. Haematoxylin	100 ccs.
Add sat. lithium carbonate	2 ccs.
Absolute Alcohol	5 ccs.

Kultschitzky's solution

Sat. lithium carbonate	100 ccs.
1% aqueous Potassium Ferricyanide	10 ccs.

This well-tried technique is inferior to Loyez' in our opinion.

(7) Loyez'

Place in 4% Iron alum for 24 hours.

Wash well with distilled water.

Stain in Loyez' Haematoxylin at 37°C for 2-4 hours*.

Wash in water.

Differentiate as required under the staining microscope in acid alcohol.

Wash well in water.

"Blue" in a solution of 95 ccs. tap water plus 5 ccs. of 5% sodium carbonate.

Loyez' Haematoxylin

Stock Solution:

Haematoxylin	1 g.
Absolute Alcohol	10 ccs.

This keeps 6 weeks.

Before use, make up as follows:

Stock Solution	10 ccs.
Saturated lithium carbonate	4ccs.
Distilled water	90 ccs.

Loyez' stains the individual sheaths so distinctly that early changes (thickening, globulation, etc.) may be clearly seen.

---

* The usual procedure is to stain in the embedding oven. The lower temperature gave better results.

(8) Silver Nitrate (author's)

In the present state of our knowledge of silver staining, the empirical approach suggested here appears the most sensible. Fundamentally, our technique is not new. It is merely one of the countless modifications, which has never reached publication. Without doubt the protargol method of Bodian (1936) is superior, but it is longer, and, at the time we started experimenting with silver preparations, protargol was difficult to obtain. By the empirical approach, we can eliminate the inconsistencies which are the main source of trouble in the many silver nitrate techniques. We have found it quite satisfactory, and capable of producing really good pictures, if one is patient. It is not necessary to make up fresh solutions each day.

The sections are firmly fixed to the slide with Mayer's Albumen, and brought to water.

Filter on (through Whatman No.40) 20% Silver Nitrate - leave for 10 minutes.

Decant excess.

Place in glass agitator in 1% Formalin for 10 minutes.

Rinse in Formalin 'preparator', and filter on the Ammoniated-Silver mixture while the slide is still wet. Leave for 5 minutes, or until the first signs of brown tinting.

Remove the excess by dipping in distilled water, and place in 1% Formalin a second at a time. The depth of the staining can be controlled by the staining microscope. To a certain extent, over-staining is prevented by washing rapidly in distilled water. If the nerve fibres are found to be under-stained, go back to the 'Formalin preparator' stage, and repeat.

Tone with Gold in the usual manner.
Counterstain with Delafield's Haemalum by the method of regression, if desired.

Ammoniated Silver Mixture

The proportion we found most generally useful was 1 part conc. Ammonia to 3 parts 20% Silver Nitrate. As alternatives 1:2, and 1:4 may be tried.

Formalin Preparator

Any one of 3 concentrations may be used, 1%, 3%, or 5%. The solution most successful in our hands was the 3%.

With 3 Formalin Solutions, and 3 Ammoniated-Silver Mixtures, a permutation of the order of 9 procedures is involved. As indicated above, the procedure of choice was to begin by using the 3% Formalin preparator, and the 1:3 Ammoniated-Silver Mixture. From this central point, a technique suitable to the tissue may be quickly found. The blocks should be uniformly prepared on every occasion.

Chemical changes in Rabbit Brain
2 hrs. after death

Rabbit Brain	Mgs. in 100 g. of fresh tissue			
	Glycogen	Lactic acid	Cerebro-sides	Phosphorus
Fresh	244	38	434	35
After 2 hrs. at 40°C.	137	176	383	55

TABLE II.

III. A description of the fallacies inherent in the preparation of histological specimens.

(1) Artefacts due to Autolysis.

We have shown elsewhere in the case of the corneal nerves how readily the axis cylinders react to changes in their environment (Rodger, 1951). One is often left wondering as a result how accurate the picture obtained in human post mortem material really is.

A few seconds after death the acid metabolites within the Central Nervous System increase at an enormous rate : at the same time the free fermentable sugar disappears, and most of the glycogen stored in the cells is lost. According to Weil (1945) these changes are maximal after 30 minutes. Some idea of the degree of this chemical dissolution is given in Table II, which is taken from the paper by Jungmann and Kimmelstiel (1929). It seems very probable that such gross chemical changes must sooner or later distort the cytoarchitecture of the nervous system, and alter the affinity of the tissues towards the various chemicals used in staining.

In living animals it is known we can obtain a more intense stain if vital dyes be injected along with chloroform, presumably because the latter partially dissolves the fatty membranes of the cells concerned.

We have every reason to suspect that such changes may also be induced in dead partly fixed cells. Where there is a probability that there has existed before death a biochemical disorder one might expect a similar alteration in the affinity of the tissues. This no doubt explains many of the discrepancies in staining such material.

With these considerations in mind, we investigated the structural changes due to autolysis at the end of 30, 60, and 120 minutes, as compared with those already present, if any, at the end of 10 minutes.

a. After 30 minutes

The outer limbs of the rods and cones were less uniform than normal, and the bodies showed some vacuolation. The granules, however, were unaffected. Although the bipolar cells were swollen, no other abnormality was noted at this level. The ganglion cells, on the other hand, were greatly distorted, exhibiting swelling, vacuolation, and aggregation of nissl bodies.

The fibres in nerve and tract stained well with silver. They exhibited only a minor degree of thickening and tortuosity. The nerve endings were

PLATE VI

a. Early autolytic changes: slight swelling of nerve endings. Author's silver x 570.

b. Early autolytic changes: granular isotropism in myelin sheaths. Polarised light x 250.

c. Autolytic changes in retina at the end of 1 hour: shrinkage involving the receptors. Triple x 520.

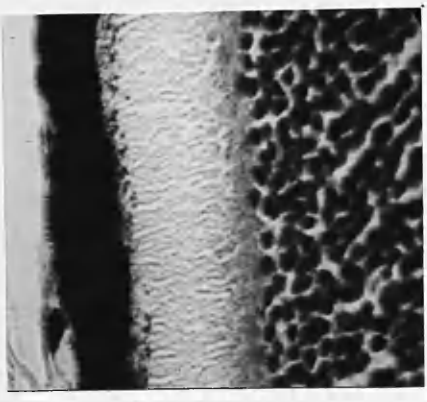
d. The same: sclerosis of ganglion and glial cells, and shrinkage of the molecular layers. Triple x 820.

e. Gross autolytic distortion: extrusion and hyperchromatism of ganglion cells. Nissl x 720.

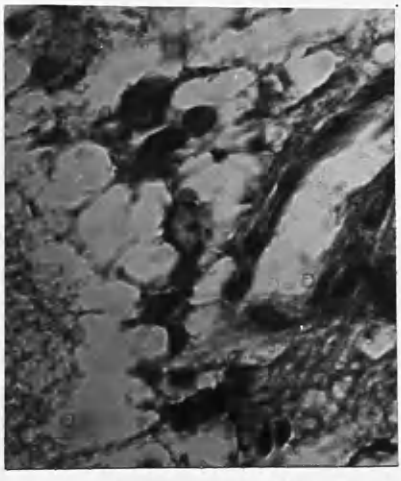
f. Gross autolytic changes: globulation of myelin in optic nerve. Polarised light x 250.

a(×570)

b(×250)



c(×520)



d(×820)

e(×720)

f(×250)

PLATE VI

slightly swollen (Plate VI,a). With the polarising microscope, the myelin sheaths (Plate VI,b) were seen to be somewhat granular (small scattered areas of isotropism). With Marchi and Loyez no changes could be clearly distinguished. In fact the sheaths showed an increased affinity for Loyez.

The geniculate cells revealed changes similar to those found in the retinal ganglion cells, although scarcely of so gross an order. That the cell nuclei were swollen was suggested by a diminution in the cytoplasm.

b. After 60 minutes

The outer limbs of the rods and cones had now shrunk. The body membranes were disintegrating. The pericellular spaces around the granules were very wide (Plate VI, c). The bipolar cells were markedly swollen; in places the cell membranes had ruptured, and the Chromophile substance extruded. The ganglion cells of the retina were also badly distorted. Most of the cells were sclerosed, and hyperchromatic. A few were swollen. There were similar changes in the cell processes. The shrinkage gave rise to many spaces in the molecular and nerve fibre layers (Plate VI,d). Vacuolation of all cells was gross, and nuclear extrusion could be seen (Plate VI,e).

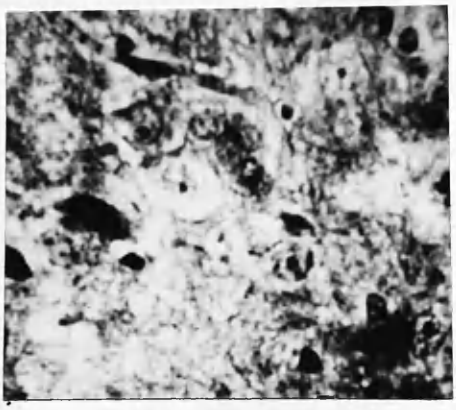
PLATE VII

a. Fatty debris within geniculate body due to autolysis. Loyez x 640.

b. Very severe varicosity of optic tract fibres after 2 hours autolysis. Author's silver x 1300.

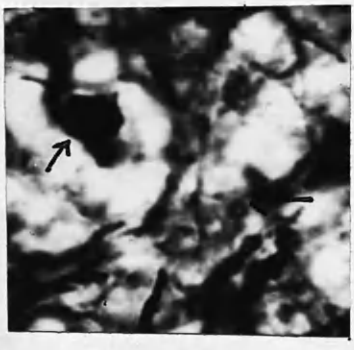
c. The same process affecting the terminal branches. Gross swelling of the endings, many dense argentophil rings, and much debris can be seen. Author's silver x 1300.

d. After 2 hours, autolytic hyperchromatism and sclerosis of the geniculate cells. Giemsa x 640.

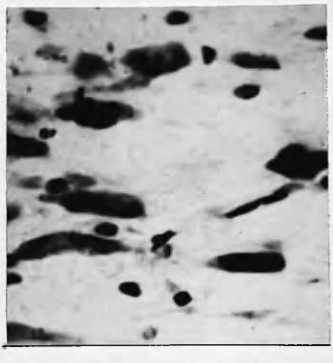


a(×640)

b(×1300)



c(×1300)



d(×640)

PLATE VII

The fibres in optic nerve and tract were moderately tortuous, and their endings now exhibited a marked enlargement. There was looping within the geniculate body, and argentophil rings were more common. The myelin changes were also more advanced. By polarised light, the sheaths were seen to have broken up into fatty globules, although some unaffected segments persisted. The nodes were swollen. (Plate VI,f). The majority of the sheaths within the geniculate body exhibited similar changes when viewed by Loyez, and there was a lot of fatty debris lying free (Plate VII,a). The cells in the geniculate body were remarkably unaffected, although a small degree of hyperchromatism was seen in addition to the cloudy swelling noted in the early stages.

c. After 120 minutes

The structural distortions were now gross. The rods and cones were badly fragmented. The granules, which possess only a small amount of cytoplasm, were sclerosed. They appear to have a very high resistance. Disruption and disintegration of the bipolar and ganglion cells was common.

Varicosity, tortuosity, and thickening of the nerve fibres was marked (Plate VII,b). Large beads

could be seen along their course, and the nerve endings now were extremely large. Many more darkly staining and thickened isolated rings could be picked out (Plate VII,c). There was a lot of argentophil debris. The myelin changes paralleled those in the nerve fibres.

In the dorsal nucleus, some of the cells were heavily vacuolated; in the majority, the nuclei were dark and elongated. This was also true of the glial nuclei (Plate VII,d).

As a result of these findings, we saw that the tissues were always fixed within 10 minutes of death. This was a rule we never relaxed.

(2) Artefacts due to fixation, and the preparation of blocks.

The fixative we chose was 10% (Neutral) Formalin. The Formalin was stored over calcium carbonate in brown bottles to prevent the formation of formic acid. In this way the destructive action upon the tissues was minimised.

Weil suggests that the mucoid bodies sometimes found in brain tissue, and which stain metachromatically with toluidine or azure blue may arise as a result of formalin over-fixation. We obtained pictures of this in tissues left only 14 days in formalin (Plate VIII,a).

PLATE VIII

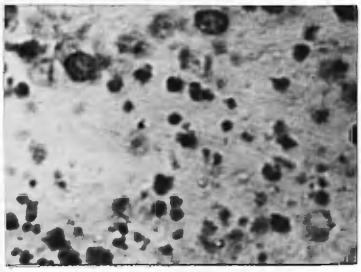
a. Over-fixation in Formalin. Mucoid bodies in dorsal geniculate nucleus. Giemsa x 300.

b. The same. Foamy vacuolation and hyperchromatism of glial nuclei. Giemsa x 575.

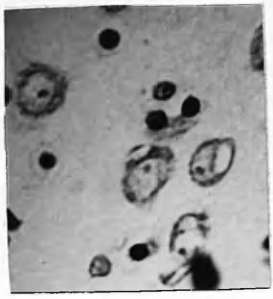
c. The same. Marchi bodies. Marchi x 650.

d. Alcohol fixation. Diffuse staining of background and vacuolation of geniculate cells. Giemsa x 640.

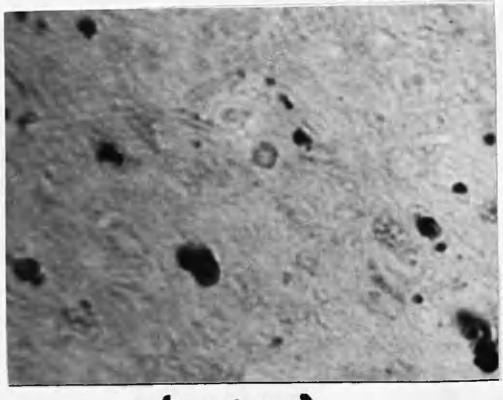
e. Over-embedding. Fragmentation of the retina after only 1½ hours in wax. Giemsa x 640.



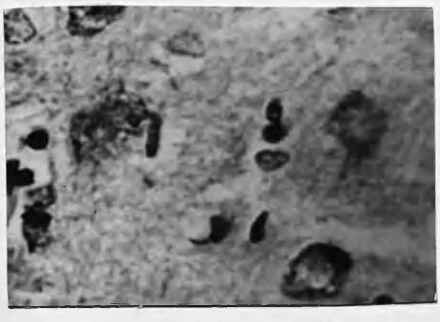
a(X 300)



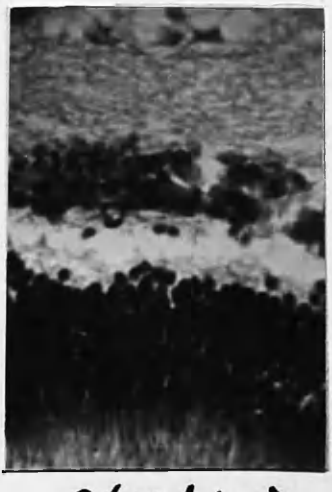
b(X 575)



c(X 650)



d(X 640)



e(X 640)

PLATE VIII

In over-fixated brains, the marginal cells exhibited a foam-like vacuolation and a greater affinity for Giemsa (Plate VIII,b). This was also true of the retinal cells. We eliminated this deceptive fallacy by cutting down the fixation time to a maximum of 2-3 days.

Over-fixation in Formalin also led to fallacies with the Marchi technique, some fine black precipitates being found (Plate VIII,c). It is thought this results from a change in the colloidal nature, and hence the solubility, of myelin lipoids.

Increased shrinkage of the retina was also a feature of over-fixation. Here, also, vacuolation was common.

The ill-effects of Alcohol fixation are even greater. In alcohol-fixed sections stained with Nissl or Giemsa a diffuse staining of the background occurs, which is very difficult to eliminate (Plate VIII,d). This suggests alcohol also leads to a process of lipid dissolution. The chief cause for complaint, however, lies in the high incidence of cyto-sclerosis which occurs. Some workers on the subject of our thesis used this means of fixation. Nissl's original manner of cutting the alcohol-fixed tissue was to embed it in gum arabic,

and cut directly. We do not know if workers like Swank and Prickett adhered to this rule.

Alcohol fixation also leads to much cellular vacuolation, to shrinkage of interstitial tissues, to tortuosity of nerve fibres, and fragmentation of all the retinal elements.

As a result of these observations, we made it the rule (in an endeavour to obtain equivalent pictures) to fix our tissues in neutral formalin for a period not exceeding 72 hours.

Changes due to dehydration, clearing and impregnation with wax were eliminated by restricting the times to a minimum. If they were too lengthy, the affinity for the stains increased. The sections became extremely fragile, and distortions similar in nature to those just described occurred, if the tissues were left 4 hours or more in Absolute Alcohol, 20 hours or more in Chloroform, or longer than 1½ hours in wax.

In the case of the retina, 1½ hours was too long (Plate VIII,e). The sections broke up, and exhibited gross vacuolation, and disruption of the cell membranes unless the impregnation-time was restricted to one hour or less. Several of our earlier experiments were made worthless for this reason, and had to be repeated.

(3) Artefacts inherent in the techniques.

1 and 2. Nissl and Giemsa. The principal source of fallacy in the Nissl and Giemsa techniques lies in differentiation. A degenerating cell undergoes three stages. First, there may be swelling with a normal affinity to the dye. Second, there may be shrinkage with an increased affinity. Third, there may be disintegration with a decreased affinity. No ganglion cell, therefore, could be designated as hyperchromatic or hypochromatic unless the serials of choice each exhibited a degree of it. As this fallacy was due to imperfect differentiation, the use of the staining microscope very soon taught us how to eliminate it as an artefact.

3. Triple. We found no difficulty in interpreting the results of Triple staining. As already indicated, we used this technique almost entirely to view the nerve fibre layer of the retina (Plate IX,a).

We were only interested in the morphology, and so the degree of differentiation did not much matter.

4. Polarised Light. The use of polarised light brought many new problems. The prime difficulty arose as a result of the small size of the visual fibres. The specimens were all photographed at a magnification of

PLATE IX

a. Normal retina. The nerve fibre-layer stains a rich plum colour. Triple x 75.

b. Frozen sections cut at 20 μ. Confusion arises at this thickness as a result of the overlapping of adjacent birefringencies. Polarised light x 250.

c. & d. Uncrossing the prisms (c) reveals that the gap in the myelin sheath as seen with crossed prisms (d) is not an artefact, but is due to degeneration. Polarised light x 400.

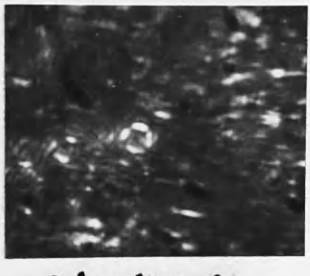
e. Maltese cross formation. Polarised light x 400.

a(x75)

b(x250)

c(x400)

d(x400)



e(x400)

PLATE IX

X250 at the point of greatest birefringency between crossed polaroids.

A purely technical difficulty arises in the cutting of frozen sections as thin as 10 μ, and yet if cut thicker, the adjacent birefringencies overlap, and make it almost impossible to obtain a clear picture (Plate IX,b). Every area of the section had as a result to be examined in detail under high power. The proportion of myelin to axon, the width of the incisures of Schmidt-Lantermann, the thickness of the myelin at the nodes, all had to be minutely observed. Early changes, that is variations in relative size, were extremely difficult to interpret.

When degeneration was moderate, but segmentation and increased isotropism both present, it was important to eliminate artefacts. By uncrossing the polaroids we could, however, determine whether these factors were in fact due to fragmentation and degeneration or not. An isotropic area, for example, may represent degenerating myelin, or may be a gap (Plate IX,c and d).

By the same method we could determine whether thin sheathing indicated a perifascicular degeneration, or merely a smaller fibre.

The pictures were invariably confused by the presence of many doubly refracting substances which according to Duguid and Mills (1928) are free fatty acids formed from the tissue lipoids by hydrolysis after death. Fixation in formalin although delaying the onset does not completely eliminate their development. Some of these isolated birefringent structures had the appearance of maltese crosses (Plate IX,e). Pearse (1951) considers these may be cholesterides or lipins.

5. Marchi. Artefacts with Marchi when the possibility of minimal degenerative changes exist are difficult to eliminate.

If the tissue is not handled gently, or if, as we showed earlier, formalin-fixation has been over-prolonged, many fine black precipitates composed of particles of uneven size and shape may appear. Cleanliness is also essential. We made it our rule always to control Marchi preparations with the polarizing microscope. It saved us from making wrong conclusions on two or three occasions.

6 and 7. Weigert-Pal and Loyez. Weigert-Pal and Loyez are not prone to any specific artefact. One must be careful, however, when differentiating. We never saw

PLATE X

a. Imperfect silver staining: incrustations. x 720.

b. Understaining simulating degeneration. Ring shaped 'boutons terminaux' can be seen in two distinct regions. x 520.

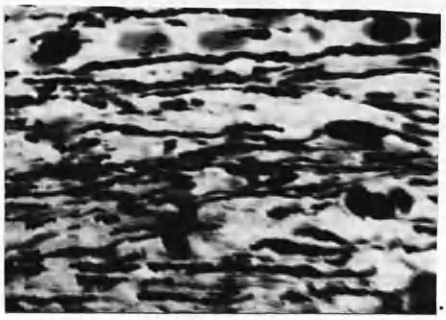
c. Looping of adjacent fibres simulates (under a lower magnification) the appearance of ring shaped 'boutons de passage'. x 1050.

d. & e. Looping of the free cut-ending of a nerve fibre simulates a ring-shaped terminal 'bouton'. x 600.

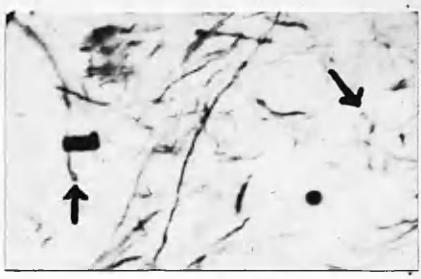
f. Absence of axial staining in normal nerve fibres, as seen longitudinally. x 1050.

g. Absence of axial staining in normal nerve fibres; as viewed transversely. x 1500.

(All Author's silver preparations)



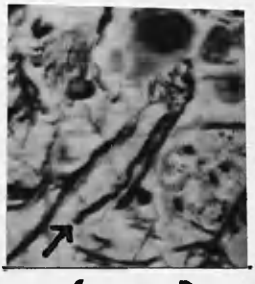
a(X720)



b(X520)

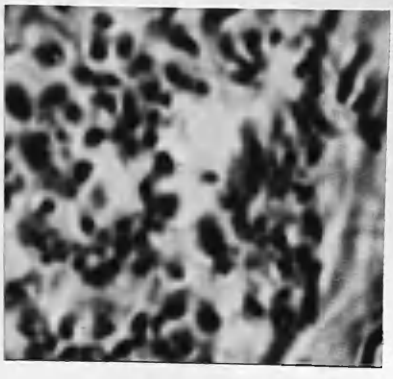
c(X1050)

d(X600)



e(X600)

f(X1050)



g(X1500)

PLATE X

patchiness ever appear in tissues stained by these methods, although it crossed our minds as a possibility. When it occurred, as it did in our main experiment, we examined the next few in the series before accepting the result.

8. Silver. Artefacts due to imperfect silver staining are many. We consider it dangerous, for one thing, to stress unduly the presence of argentophil debris. Incrustations are commonly cited as evidence of neuronal disease (Plate X,a). We are certain now they are artefacts. The non-acceptance of silver is another source of fallacy. By our empirical method we have obtained perfectly stained specimens in the end although 3-4 of the earlier sections exhibited incrustations or hypochromatism. When a nerve fibre accepts the silver badly it bears a remarkable resemblance to a degenerating fibre (Plate X,b). Lastly, there is the controversial question of the ring shapes. We have already shown they may arise as a result of autolytic changes. The "boutons de passage" variety are also included in this category. Where a minor degree of such autolytic 'beading' is associated with imperfect staining, a ring shape might easily occur. Rings are present, however, even in the best stained sections both in the course of and at the end of the

nerve fibres. In some instances without doubt, the appearance is suggested by the looping of adjacent nerve fibres. Where two fibres run closely together a slight separation (i.e. looping) gives the appearance of ring shaped boutons de passage (Plate X,c). Similarly the cut ending may loop around itself. In one such 'ring' we managed to straighten the loop forcibly by pressing on the slide. The ring-shape was present at the end of an abnormally thickened fibre, and might well have been mistaken for an enlarged terminal ring (Plate X,d and e). In our finest sections, moreover, there always appear some fibres which do not accept the silver axially, (Plate X,f). This in itself, of course, may represent an early degenerative change. It is, however, we believe, just another possible explanation of the isolated rings. When such a fibre is cut transversely, a ring shape is exactly reproduced (Plate X,g). We cannot say why the affinity of the axial neuroplasm for silver varies from the peripheral.

IV. A short review of the structural alterations found by other authors in disease of the visual neurones.

Before describing how the visual neurones reacted in response to certain morbid processes we produced experimentally, it will be as well to mention briefly the structural alterations that other authors have found.

(1) The general reaction of neurones to disease

When a nerve cell body is damaged it becomes in the first instance pale and swollen, the nucleus being thrust to the side. The Nissl substance then loses its shape, and breaks down into fine dust-like particles. If the degenerative process continues the eccentric nucleus will be extruded, the cell body thereafter rapidly disintegrating and disappearing.

Following the early cellular changes, the axis cylinder - usually at its furthermost point - becomes tortuous, and swells, first of all in a regular manner, and then in an irregular. The axonal ending enlarges to form a large swollen end-bulb. Although not so common an occurrence in toxic processes, the distorted nerve fibre soon disintegrates and

becomes absorbed.

The interstitial tissue (the astrocytes, it is thought) will now proliferate, and occupy the position previously filled by the disappearing axis cylinders.

The disturbance in the nerve fibre, as we might expect, is reflected in the myelin sheath, which breaks down in similar fashion into fat globules. These are ingested, and removed by the microglial cells, which, thus engorged, can be seen aggregated around the capillaries.

The changes just described may of course occur in the reverse direction. A lesion in the visual cortex, for example, will lead ultimately to degeneration of the parent cells in the lateral geniculate body (Le Gros Clark, 1941). It is the Wallerian type of degeneration, however, according to Boyd (1948) which is to be expected as a result of an avitaminosis.

A third type of degeneration may also be found, especially in the visual path. A degeneration affecting the neurones of the second order (Ganglion cells) is reflected in the neurones of the third order (lateral geniculate body cells). The conditions and

significance of such a transneuronal degeneration are not understood. From this point of view it would seem that the most important region for our purposes will be the lateral geniculate body, as it is likely to reflect lesions affecting the visual pathway in any part of its course, provided the duration of the adverse conditions be long enough.

In toxic degenerations, however, there is another factor to be considered. The response of the nerve cell will differ, depending on whether the process be an acute one, or a chronic. If acute, the cells will swell; the nucleus will become pale and enlarged; and the Nissl substance diminish, especially around the nucleus. The nerve fibres, as stated previously, will become swollen and varicose, although there is nothing specific in that. If chronic, the lesion, as it affects the cells, differs quite remarkably. The latter shrink, and in place of a loss of chromophile substance, there will be an exaggeration of its staining reaction. The nerve fibres, too, are much more likely to go beyond the stage of cloudy swelling, and disintegrate. Prior to death of the cell, of course, the picture will become less typical, vacuolation, chromatolysis and destruction of the cell envelope occurring as they

would in response to any necrotising agent.

Both acute and chronic cell changes will of course be found together in the latter case.

Such, then, is the pathology we might expect on general principles.

(2) The reaction of the visual neurones to disease

Wolff (1950) states that the ganglion cells are the least resistant of all the cells in the retina to a toxic process. There is a lot to support this view, so it is not remarkable. What is more remarkable in our opinion is to find that the cells of the outer and inner nuclear layers are so rarely mentioned as being affected by lesions of any kind.

The ganglion cells undergo chromatolysis in the characteristic fashion - as in such conditions as methyl alcohol poisoning - and may disintegrate leaving a space behind, which can be readily confused with the cystic spaces so commonly found as a degenerative process at the periphery of the retina.

Oedema of the outer molecular layer occurs very readily as a post mortem artefact, and may be so gross that detachment of the anterior half of the retina occurs. Those authors, therefore, who stress retinal oedema as a feature of any particular pathology,

especially in Henle's layer, may have been misled. Gliosis affecting the nerve fibre layers has been remarked only in inflammatory processes.

The rods and cones, it is known, respond to certain intoxications by a swelling of their outer limbs, the pigment frequently being seen to have migrated downwards to the external limiting membrane. In the end the receptors may break up into many small refractile granules.

In the pathology of the retina, nevertheless, it is the response of the ganglion cells and their processes of which most is known. It will be important, then, for us to look for structural changes in these cells as changes elsewhere in the retina appear to be much less probable.

We have a more precise knowledge of the responses of the visual fibres and the cells of the lateral geniculate body. Among the best known workers in this field we must rank Le Gros Clark (1941), Glees (1941), and Nauta and van Straaten (1947). According to these authors, the larger fibres are affected first. After enucleation of the eye, they become tortuous and varicose, and start to break up. Many terminal, or isolated, argentophil rings can then be seen. Glees and Le Gros Clark believe that these

rings thicken, until the lumina become obliterated, and solid end-bulbs arise, matching the size of the geniculate cells so greatly do they swell.

Degeneration of the visual fibres and their myelin sheaths takes place otherwise in the characteristic fashion, and the changes arising in the geniculate cells as a result of transneuronal or retrograde degeneration present no new features.

(3) The specific reaction of neurones in thiamin avitaminosis with particular reference to the visual.

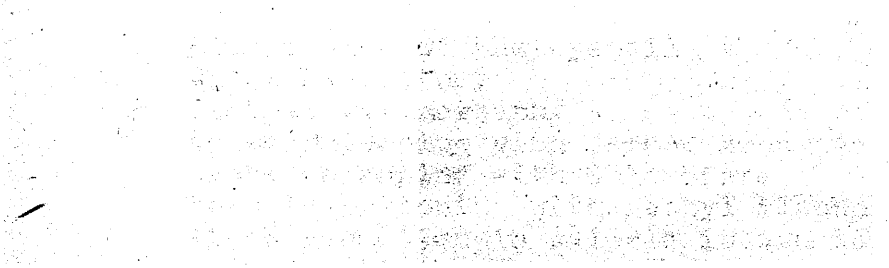
Prickett (1934) in a study of thiamin deficiency in rats describes damage to the cells of the vestibular nuclei. This was of two types, sclerosis and pyknosis, associated in places with pericellular incrustations, and, secondly, swelling, associated with central chromatolysis and nuclear eccentration. However, as the darkly staining shrunken cells also appeared in the control specimens, their appearance suggests they are artefacts. We have shown that the prolonged use of 95% Alcohol as a fixative can cause it, and this is the fixative that Prickett used. Prickett describes, in addition, the occurrence of marked demyelinisation in the central terminations of the vestibular fibres. He did not stain the nerve fibres, as he considered axonal degeneration always succeeded

the myelin changes. There is considerable doubt whether this is in fact the case.

Later, in association with Salmon and Schrader (1939) the same author describes changes in the peripheral nerves of rats, as seen by the polarising microscope. The incisures and nodes were enlarged; dark granulations occurred in the myelin sheaths in the acute deficiency, and the axis cylinders had rough margins. In chronic thiamin deficiency, a few large droplets only were found. Presumably this was myelin that was no longer doubly refractile.

Swank (1940) describes in both acute and chronic deficient pigeons, the characteristic appearance and course of a retrograde degeneration in the peripheral nerves. He claims that these changes corresponded in intensity with the clinical behaviour. In addition, he claims that degeneration of the axis cylinders preceded demyelinisation. In association with Prados (1942) he found that the optic system was not affected (with one exception) by an acute deficiency, but that in a chronic, there was marked thickening and varicosity of the nerve terminals, which proceeded in a retrograde direction until in the end sclerosis of the parent cells arose.

Leinfelder and Robbie (1947) describe minimal changes only in the optic nerve in acute experiments on rats: a few fine droplets around the myelin sheaths with Marchi, slight tortuosity of the fibres, and swelling of the retinal ganglion cells. All these changes are almost certainly fallacious. They could not be said, however, to have produced a chronic thiamin deficiency, any more than did MacDermott and his associates (1943). Nevertheless, the latter by feeding rats a diet deficient in the entire B complex obtained signs of degeneration in the optic nerve: vacuolation of the myelin sheaths, and oedema of the interstitial tissue.



V. The reaction of the visual neurones to disease.

EXPERIMENT IV.

The reasons why we undertook the various procedures (which we have collected under the title of Experiment IV) are three. We wanted to possess complete confidence in our histological techniques; we wanted (having already excluded the fallacies) a clear-cut picture of the signs and stages of degeneration: and we were interested to know whether or not there was any truth in the claims of such authors as Gagel and Bodechtel (1930), and Scherer (1931, 1932), who stated that each neurone exhibits a specific cytoarchitectural reaction to disease, no matter what the cause of the degeneration may be.

The procedures we employed were of the following nature:-

(1) Enucleation of the eyeball
(2) Optic neurectomy
(3) Central tarsorrhaphy
(4) Acute poisoning with Carbon Monoxide
(5) Acute poisoning with Chloroform
(6) Chronic poisoning with Methyl Alcohol
(7) Acute reduction in caloric intake to a level incompatible with health
(8) Subacute reduction in caloric intake.

Series 1 - Traumatic degeneration (enucleation)

Enucleation of one eye only was undertaken as it gave results that were similar to those following removal of both. Several rats were used, and the visual pathways subsequently examined at intervals ranging from 1 to 21 days. We did not expect to obtain in our main deficiency experiments signs of degeneration as gross as the ones obtainable by this method. The experiment was a useful means, however, of confirming the boundaries of the dorsal nucleus, and of enabling us to obtain evidence of the changes occurring as a result of transneuronal degeneration.

Pathological observations

At the end of 24 hours, there were signs of commencing degeneration in the myelin sheaths. With the polarizing mic roscope, irregular oedema and enlargement of the incisures could be seen (Plate XI,a). The nodes were slightly swollen. The nerve fibres were tortuous.

On the next day, the changes were more striking. The terminal branches within the lateral geniculate body were affected most (Plate XI,b). They were beaded, tortuous, varicose, and, in the case of the larger fibres, fragmented. The nerve endings were

PLATE XI

a. Trauma. Enlargement of the incisures and nodes in early stages. Polarised light x 250.

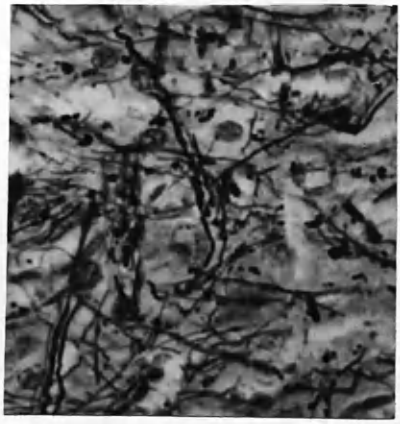
b. Trauma. Degeneration affecting the terminal branches of the visual fibres. Author's silver x 540.

c. Trauma. Distortion in the nerve. Author's silver x 670.

d. Trauma. Ballooning and globulation of myelin sheaths in optic tract. Loyez x 1600.

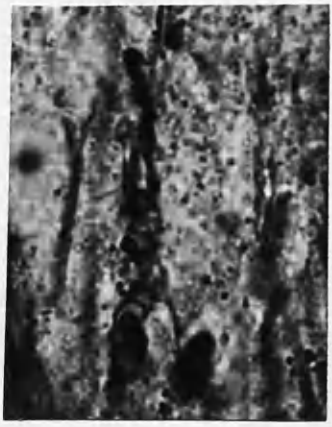
e. Trauma. Almost complete destruction of myelin in optic nerve 6 days after enucleation. Polarised light x 250.

a(X250)



b(X540)

c(X670)



d(X1600)

e(X250)

PLATE XI

swollen, and argentophil rings were present in the peripheral regions of the dorsal nucleus. In the tract and nerve, the fibres were now thickened as well as tortuous (Plate XI,c).

The distortions described above were well seen in the Loyez preparations (Plate XI,d). In addition the sheaths themselves were globulated. With osmic acid, many black-staining marchi bodies were noted. The nodes were now swollen.

By the sixth day, the process had advanced considerably. The larger, and many of the smaller fibres, in nerve and tract had fragmented. Gitter cells were appearing. The argentophil rings were larger and coarser. There was generally an increased affinity for silver, and much debris. The nerve endings shared in the gross swelling of the nerves, striking end bulb formations having formed.

The myelin sheaths no longer existed as such. Small swollen segments alone could be seen here and there. There was much fatty debris, most of it birefringent when viewed by the polarising microscope (Plate XI,e).

By the twenty-first day, destruction was almost complete. In nerve and tract, fragmentation

was the rule. There were many gitter cells engorged with fat and neurofibril remnants (Plate XII,a).

The argentophil rings, if they existed, were represented by small dark homogeneous bodies. The nerve endings were as large as the glial nuclei (Plate XII,b).

The myelin when viewed by polarised light, was totally destroyed (Plate XII,c), and there was still a great deal of fatty debris.

The first evidence of transneuronal degeneration was seen on the sixth day. Central chromatolysis was fairly common, and vacuolation at the periphery of the cells was of pathological dimensions (Plate XII,d). A few of the cells were hyperchromatic, but there was little shrinkage. By the tenth day many more hyperchromatic and sclerosed cells were present (Plate XII,e), and by the twenty-first day this was even more noticeable. The nuclear and cellular outlines were grossly distorted.

The response of the glial cells was equally well marked. There was a noticeable increase in the number of gitter cells when the first and last day specimens were compared (Plate XII,f). The astrocytes were occasionally observed to have enlarged, their cytoplasm staining homogeneously, and the nuclei becoming markedly eccentric. It was extremely interesting to

PLATE XII

a. Trauma. The nerve fibres in the tract by 21st day are completely destroyed. Many Gitter cells are present. Author's silver x 200.

b. Trauma. Enlargement of the nerve endings, severe fragmentation, and many solid rings are now present. Author's silver x 1000.

c. Trauma. Complete destruction of the myelin sheaths by 20th day. Polarized light x 250.

d. Early changes in transneuronal degeneration. Vacuolation and central chromatolysis in lateral geniculate body. Giemsa x 510.

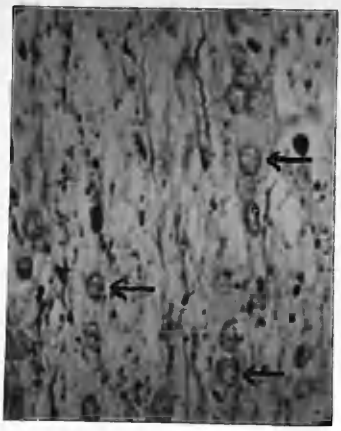
e. Late changes in transneuronal degeneration, sclerosis and hyperchromatism. Giemsa x 160.

f. Trauma. Gitter cells in region of disintegrating terminal branches within the geniculate body. Giemsa x 700.

g. Trauma. Reaction of oligodendrocytes. Chain formation in tract. Giemsa x 195.

h. Trauma. Aggregation of cerebral histiocytes around cerebral capillary. Giemsa x 920.

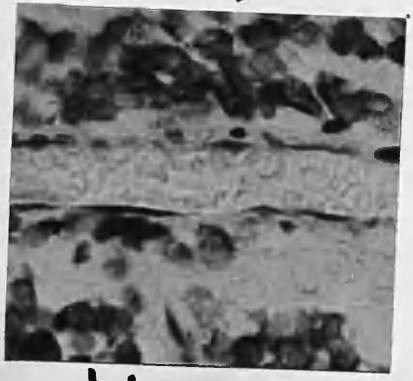
(Part III)



a(X200)

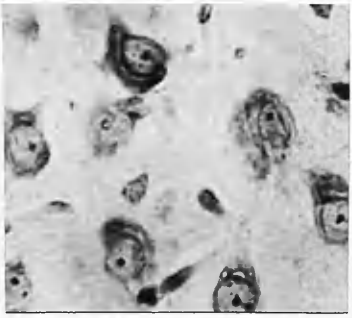
c(X250)

e(X160)

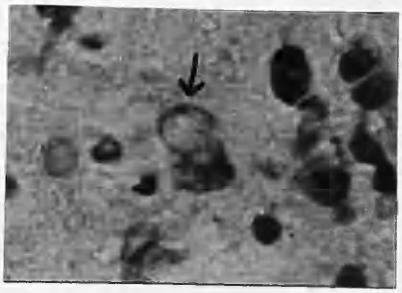


h(X920)

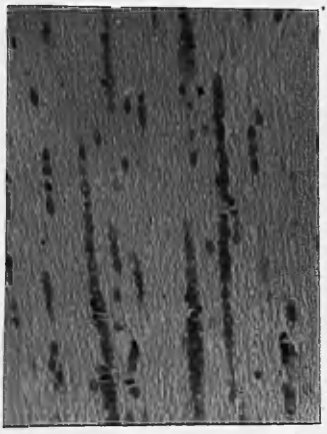
b(X1000)



d(X510)



f(X700)



g(X195)

PLATE XII

find in this same specimen that the oligodendrocytes in nerve and tract had proliferated, lying beside the degenerating processes in long parallel chains (Plate XII,g). This formation would appear to occur as a defensive measure, although the function of oligodendrocytes is debateable. In the 21-day specimen a marked aggregation of cerebral histiocytes around the blood vessels could be seen (Plate XII,h).

Series 2 - Traumatic degeneration (optic neurectomy)

This procedure was performed to enable us to observe the reaction of the retina to a retrograde degeneration. So small is the rat eye that it is not easy to obtain good results unless great care is taken to maintain an adequate blood supply to the eyeball. Otherwise phthisis bulbi rapidly ensues. The changes were sometimes complicated by infection. In the end, we did obtain one specimen that satisfied in all these counts. The rat was killed 12 days after the operation.

Pathological observations

The changes discovered affected all the retinal layers.

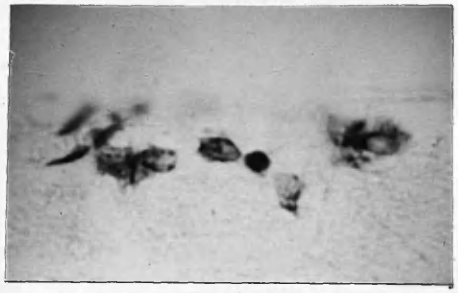
The ganglion cells were in various stages of disintegration. Vacuolation and chromatolysis were

PLATE XIII

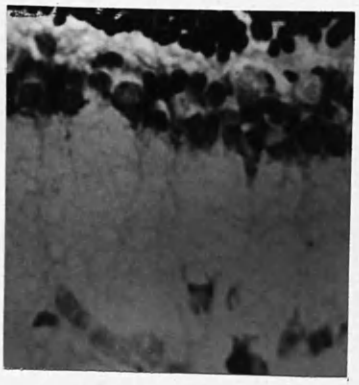
a. Trauma. Extrusion of nuclei in retinal ganglion cells 12 days after cutting the optic nerve. Nissl x 620.

b. Trauma. The same. Cloudy swelling in some and sclerosis in other bipolar cells. Note the swelling affects the bipolar processes. Giemsa x 670.

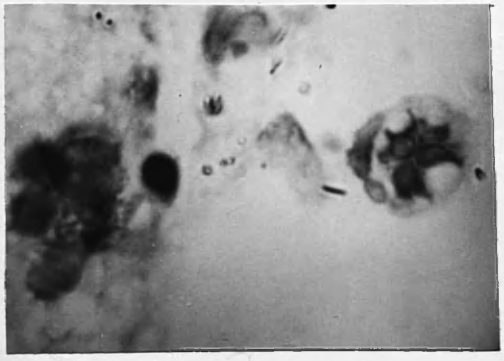
c. Trauma. Disintegrating ganglion cells in the retina, and a large compound granular corpuscle engorged with chromatin. Giemsa x 1500.



a(x620)



b(x670)



c(x1500)

PLATE XIII

common. Some cells were sclerosed. Others fragmented the cell membrane being lost, and the nucleus extruded (Plate XIII,a). An increase centrally in the number of cystic spaces suggested these spaces had previously been occupied by ganglion cells.

The cell bodies and processes of the bipolar cells and neighbouring glial nuclei were markedly swollen. Chromatolysis in some, sclerosis in others, were separate features (Plate XIII,b).

The rod and cone granules did not appear affected, but the bodies had fragmented, and the outer limbs were contracted.

The pigment epithelial cells had withdrawn their processes from the outer limbs, in the manner of the chromatophores in the Iris (clumping).

In the ganglion cell layer, there were several engorged gitter cells, in which fat and chromatin substance could be seen (Plate XIII,c).

Series 3 - Disuse atrophy (central tarsorrhaphy)

It is not known whether an amblyopia of disuse can lead to atrophy of the visual cells. If it does, that might be a source of fallacy in the rat, especially in the geniculate body where a certain amount of form

sensibility exists. It is the form sense, and not the light sense which is lost in amblyopic vision.

The argument that the non-recovery of central vision in man in cases treated late (despite prolonged occlusion of the sound eye) is due to disuse atrophy is, however, fundamentally unsound. The recovery of vision by occlusion in the majority of amblyopic eyes, and the ease with which the occluded eye may in turn become amblyopic are arguments all in favour of its functional nature. Despite this conclusion we decided to exclude any possibility of it. A central tarsorrhaphy was, therefore, performed on 4 rats, in 2 of which it was successful. The Pomfret Kilner method of lid suturing was used. At the end of six months, the two rats were sacrificed. No abnormality was found.

Series 4 - Acute poisoning (Carbon Monoxide)

As a means of producing an acute anoxic state, carbon monoxide is ideal. According to Duggan (1941), the retrobulbar neuritis of unknown aetiology is due to an acute tissue anoxia. Poisoning by coal gas used to be a common method of sacrificing experimental animals, for it was believed that acute intoxications of this kind did not produce any histological changes. This we

found was not quite true.

Pathological observations

Cloudy swelling of the retinal ganglion cells, the glial cells, and some of the bipolars was present (Plate XIV,a).

With Giemsa, there was a noteable increase in the affinity of the surrounding tissue. It was very difficult as a result to differentiate the cells from the background.

The geniculate cells exhibited an indefinite degree of chromatolysis. Otherwise there was no abnormality.

The nerve fibres were slightly varicose. Increased separation suggested oedema of the interstitial tissue. The affinity of the fibres for silver had altered: it was only with the very greatest difficulty that we were successful in staining them. As in the case of Giemsa, however, there was an increased affinity of the background. This phenomenon occurred in all rats, all blocks, and all sections, and was not a fallacy.

The myelin sheaths (Marchi and the polarising microscope) were normal (Plate XIV,b).

There was no alteration affecting the neuroglia.

PLATE XIV

a. CO poisoning. Cloudy swelling involving retinal bipolars. Giemsa x 1600.

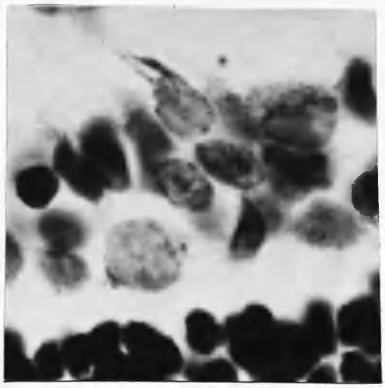
b. CO poisoning. 'Roughening' of myelin sheaths. This is considered within normal. Optic nerve. Polarised light x 250.

c. Chloroform poisoning. Central chromatolysis and some sclerosis of geniculate cells. Giemsa x 510.

d. Chloroform poisoning. Central chromatolysis and satellitosis as above. Giemsa x 450.

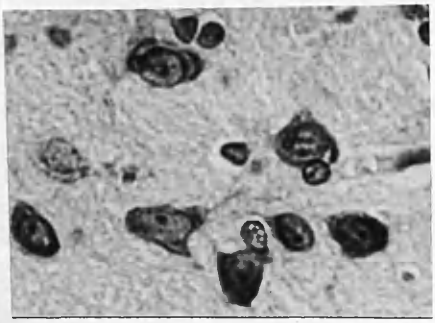
e. Methyl Alcohol poisoning. Dorsal nucleus of lateral geniculate body. Increased ring formation and affinity of background to silver. Much debris. Author's silver x 1500.

f. Methyl Alcohol poisoning. A moderate degree of sclerosis and hyperchromatism of the geniculate cells. Giemsa x 200.

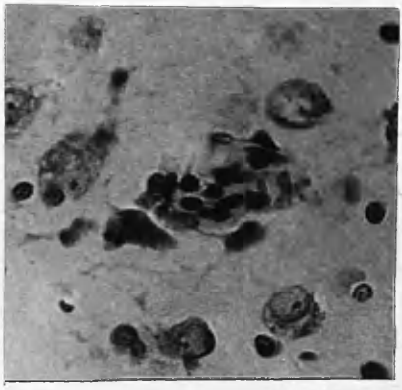


a(x 1600)

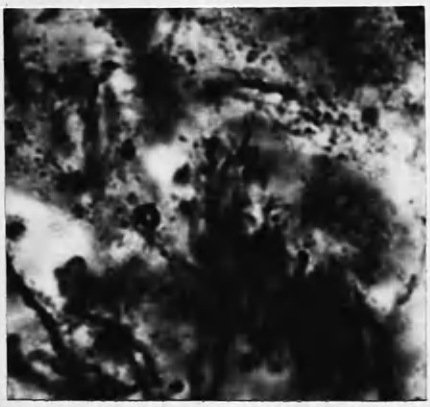
b-(x 250)



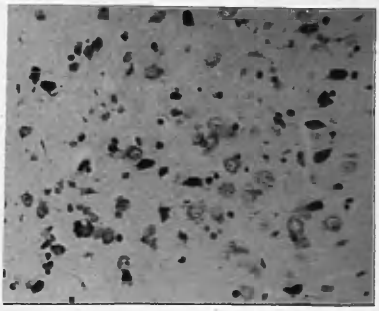
c(x 510)



d(x 450)



e(x 1500)



f(x 200)

PLATE XIV

Series 5 - Acute poisoning (Chloroform)

As some authors (Prickett and Stevens, 1939) made their observations on the tissues of animals killed by chloroform, we considered it would be of use, if for this reason only, to compare the effects with animals sacrificed by decapitation under light urethane anaesthesia. The latter revealed no abnormality. Chloroform poisoning, however, produced signs of cellular regression.

Pathological observations

There was gross cloudy swelling of the retinal ganglion and glial cells, and chromatolysis was common. In places the cell membranes appeared to have ruptured. The rods and cones were also involved. Their outer limbs had shrunk, and their bodies in some cases disintegrated.

The geniculate cells revealed a high degree of central chromatolysis and were swollen in addition. Many were sclerosed (Plate XIV,c). The nerve fibres and myelin sheaths were within normal, although we suspected slight myelin changes.

The oligodendrocytes were moderately swollen, and as the tissues had been fixed in the usual way within 10 minutes, it could not be considered a post

mortem artefact. In addition, satellitosis was seen here and there (Plate XIV,d). There was no alteration in the affinity for silver or azure blue, as found in the case of gas poisoning.

Series 6 - Chronic poisoning (Methyl Alcohol)

It is well known that this alcohol, also known as wood alcohol or Columbian spirits, produces in man a sudden amaurosis. Small amounts may produce in some permanent blindness, while in others large amounts lead to no visual defect. In experimental animals an inconsistency of species rather than of individual appears to exist. Some animals (e.g. rabbits) have a great resistance to it. For instance, we administered wood alcohol intravenously (2 ccs. per kg.) daily for 2 months, and found no change in rabbit retinas. This finding agrees with that of Friedenwald and Felty (1935), McCord (1931), and Scott, Helz, and McCord (1933). Birch-Hirschfeld (1901, 1902) on the other hand claims to have obtained vacuolation and disintegration of the ganglion cells after 5 days. These changes may have been due to artefact. The rat, however, according to McCord and his associates is very susceptible to methyl alcohol poisoning, and for

this reason we were encouraged to administer it despite our lack of success in the case of rabbits. Four rats were given 1 cc. daily in their drinking bottles. As they drank this with apparent relish, after a week the daily dose was doubled. The drinking bottles continued to be emptied.

We did not favour as methods of administration skin absorption or inhalation, because the former leads to marked irritation and the latter to inflammation of the air passages (McCord, 1931). The rats were killed at the end of 8 weeks, and the eyeballs fixed immediately as post mortem changes in the retina occur very rapidly. At death, the rats were well.

A discussion as to the cause of the toxicity would serve little purpose here. Let it suffice briefly to quote Fink (1943) who, in the course of a most comprehensive paper, suggests that the pathological changes are not directly due to Methyl Alcohol; it is too long, he says, before it takes effect. He suggests that a toxic substance interfering with metabolism arises as a result of the slow breakdown of Methyl Alcohol. Such a process interferes seriously with the more highly differentiated cells. The intermediate product causing the damage he believes is formic acid.

The changes we found were not gross but we consider them to be evidence of the early reaction of the visual pathway to a toxic process. These changes might well prove to parallel those expected in thiamin deficiency.

Pathological observations

The retinal ganglion cells were markedly vacuolated. The nuclear and cellular outlines were distorted, and the nissl substance had aggregated at the periphery. Many of the bipolar cells exhibited cloudy swelling. There were no other abnormalities in this region.

There was a moderate degree of tortuosity and thickening in the nerve fibres of the nerve and tract. Within the geniculate body, this was more marked, and there was a great number of heavily staining rings. The background exhibited an increased affinity for silver, so it was difficult to obtain a well contrasted picture (Plate XIV,e). The myelin was unaffected, and there were no signs of oedema.

A moderate number of the geniculate cells were sclerosed, and hyperchromatic (Plate XIV,f). Within the nerve and tract the glial cells had proliferated.

Series 7 - Acute reduction in caloric intake

This is an account of the changes found in the eyes and brains of the rats sacrificed in Experiment III, Series 1.

Pathological observations

Vacuolation of the ganglion cells of the retina, and the geniculate body, was the only cytological change found (Plate XV,a). As vacuolation of the retinal cells is a very common artefact, more common than swelling, we are inclined to consider that this is the explanation of the finding.

The myelin sheaths viewed by polarised light were hazy in outline and their substance somewhat granular. Loyez and Marchi revealed no abnormality. We found no signs of any Wallerian degeneration involving the nerve fibres.

Series 8 - Subacute reduction in caloric intake

All the rats of Experiment III, Series 2, A and B, exhibited the changes described below.

PLATE XV

a. Acute inanition. Vacuolation of the final visual neurones. Giemsa x 720.

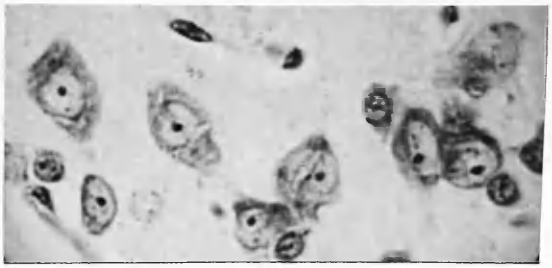
b. Subacute inanition. Regressive changes in the retinal ganglion cells. Giemsa x 570.

c. Subacute inanition. Severe neuronal disease in the geniculate cells. Nissl x 180.

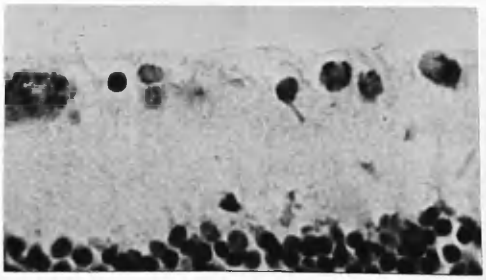
d. Subacute inanition. Cloudy swelling of all cellular elements in the geniculate body. Giemsa x 470.

e. Subacute inanition. Rod cells in the geniculate body. Giemsa x 670.

f. Subacute inanition. Granular isotropism of myelin sheaths. Optic nerve. Polarised light x 250.

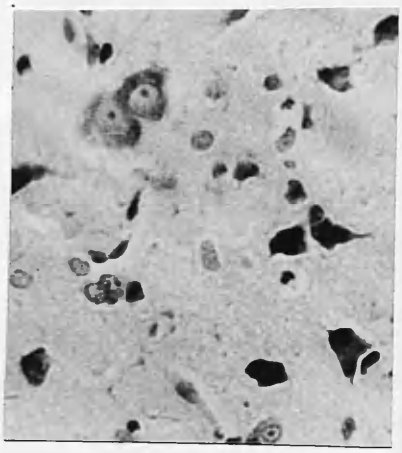


a(x720)

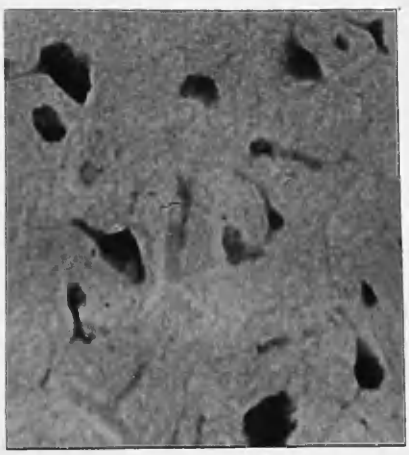


b(x570)

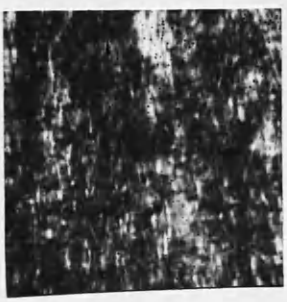
c(x180)



d(x470)



e(x670)



f(x250)

PLATE XV

Pathological observations

In the retina, the outline of many of the cells was indistinct, the cytoplasm staining homogeneously, and the nucleus containing dark-stained granules. Some of the cells, ganglion and bipolar, were much swollen (Plate XV,b).

The geniculate cells similarly exhibited signs of severe neuronal disease. The nissl bodies were in many cells clotted, and darkly stained. The cell outlines were frequently badly distorted, (Plate XV,c). Some of the astrocytes were swollen (Plate XV,d), and there were many hypertrophied cerebral histiocytes (rod cells) with dark staining nuclei (Plate XV,e). Long oligodendrocytic chains lay in the fibre pathways.

The nerve fibres themselves were tortuous, but not grossly distorted. The endings were unaffected.

The myelin sheaths revealed more advanced changes than in the previous series. These changes were, however, in view of the advanced cellular damage present, much less than we expected. There was a slight degree of demyelinisation in the sciatic nerves in both Groups A and B, revealing itself in polarised light as a granular isotropism (Plate XV,f).

The last two experiments were of great value. They covered possible fallacies in our main acute experiments. It was not necessary to investigate the effects of prolonging inanition beyond 20 days for we knew that such an exigency would never arise. In our chronic experiments we were confident we could control inanition by the judicious use of minimal doses of thiamin. In addition paired feeding was to be instituted, and would adequately control the experimental findings.

Demyelinisation, never gross, appeared to affect the peripheral nervous system much more than the central.

CONCLUSIONS

It cannot be said that the neurones of the visual pathway reacted to these divers lesions always in the same manner. The integral parts did, and within that limited context the views of Gagel, Bodechtel, and Scherer are correct. Nerve cells swell, undergo chromatolysis, and then, as they shrink, become distorted and hyperchromatic: myelin breaks up into fatty globules, balloons, segments, and is ingested: axons become tortuous, thicken, undergo distortion, and then fragment - all that is true no matter the nature or duration of the morbid process. We must, however, consider the three neuronal elements togéther, and here we find the rule is broken. Even more so is this true when we consider the neurones of the visual pathway collectively.

If, for example, we had concluded as a result of considering the effect of trauma on the cell processes that the myelin sheath and nerve fibres always degenerate side by side, we would be wrong. In chronic methyl alcohol poisoning the nerve fibres are affected, but the myelin is not. In starvation the myelin sheaths appear to be affected before the nerve fibres. In short, one cannot prophesy with any degree of accuracy

how the visual neurones may respond, if they respond at all.

Experiment IV, however, does enable us to describe the reaction of neurones to disease in general terms.

It would seem that an acute toxic process leading to the death of the animal will affect the cell, and to a lesser degree the myelin but not the fibre. A chronic process of mild nature, on the other hand, is more likely to produce preferential changes in the latter. Only if the lesion be prolonged and severe will all the neuronal elements be affected together.

The changes taking place in the retina were the ones we felt least certain about. It is very difficult to obtain good preparations of this tissue by embedding in paraffin wax. We did our best to control our results by thoroughly investigating the artefacts. It seems that the ganglion cells rapidly advance to a stage of complete dissolution. Seldom could we be certain in the retina that we had obtained signs of chronic neuronal disease. The geniculate cells on the other hand have a much greater resistance, so that the classical stages in degeneration were well

seen. The bipolar cells corresponded to the geniculate in their reactions. The rod and cone granules - in contradistinction to the retinal ganglion cells - have an extremely high degree of resistance, although we must not forget that changes in their cytoplasm are reflected in the rod and cone bodies rather than in the small protoplasmic envelope enclosing the granules. Distortion of the outer limbs is a common artefact, and a dangerous criterion to adopt.

Several histopathological features generally accepted as significant, we prefer to neglect, among them vacuolation, eccentration of nuclei, and oedema of the interstitial neural tissue. These signs are too readily open to fallacy.

The changes in the glial cells we found of great personal interest. The oligodendrocytes appear to be particularly sensitive to changes in the intraneural environment. Chain formation, satellitosis, and hydropic swelling are the signs we look for, although at times the cerebral histiocytes combine in these activities. We are inclined to suggest that the stimulus for such changes must be a biochemical one. The stimulus to microglial phagocytosis (true neuronophagia) on the other hand would appear to depend on

the death of cells or their processes. In a bio-chemical lesion, therefore, at a time when the changes are reversible, microglial proliferation by this argument is most unlikely. The reaction of the microglial histiocytes to disease appears to vary in other respects. As a preliminary to the anatomical destruction of cerebral neurones they become elongated, and their processes hypertrophy (rod cells). Changes of this order coincide with the proliferative phase undergone by the oligodendrocytes. They precede the appearance of Gitter cells.

None of our procedures was of sufficient length to enable us to observe a fibrillary gliosis. Changes in the astrocytes were extremely difficult to determine. Occasionally these cells were swollen. Seldom did we observe clasmatodendrosis*. The gemastete cells of Nissl we believe we observed on occasion, especially in the retina, but they are easily confused with distorted ganglion cells. Their appearance, therefore, becomes a matter for debate, a state of affairs we avoided wherever possible.

The significance of argentophil rings we have discussed several times already in the course of this thesis. We have inferred, and it is our belief,

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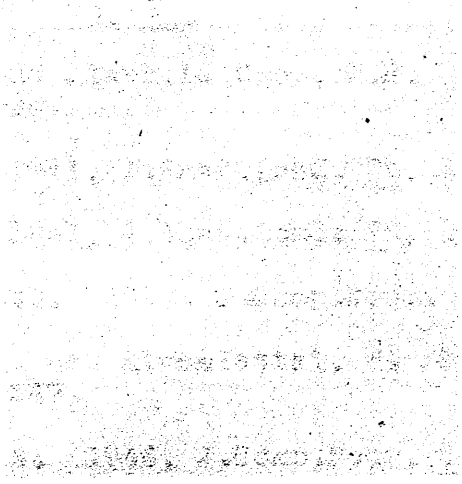
* Overstaining with azure blue revealed the processes.

that they do not exist as anatomical entities. We might here summarize our conclusions in this matter. We believe that the axial stream of neuroplasm in healthy nerve has a low affinity for silver. Such a fibre (or the loop of such a fibre) cut transversely will as a result present the appearance of an isolated argentophil ring. In the early stages of degeneration when nerve fibres swell, and tortuosity is common, the opportunity of finding rings of this nature is correspondingly increased. Later, when, as is well known, the affinity of the degenerating fibres for silver is greatly increased, the axial neuroplasm may accept the stain, hence the observation that the rings have apparently swollen, or are now completely homogeneous! Although for a different reason, we are still able to say, nevertheless, that an increase in the number of argentophil rings (as a result of an increased thickening and tortuosity) is important indirect evidence that a degenerative process exists.

In conclusion, we would like to stress one other point, and that is that the increased affinity between abnormal nerve fibres and the stains is also reflected in the surrounding glial tissues. The existence of this phenomenon in chemical poisoning suggests that this may also occur in a biochemical.

Its possible presence, therefore, in our main experimental material would be suggestive.

In the last analysis, of course, it must be apparent that the only way by which we can ascertain whether or not structural changes have in fact occurred in our experimental material is by comparing the tissue in question with the corresponding tissue of the control animal, treated in the same way with the same solutions at the same time.



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PART IV.

THE MAIN EXPERIMENTS : A CRITICAL DISCUSSION OF THE RESULTS : AND THE CONCLUSIONS DRAWN.

We now propose to give in detail the experiments we performed with a view to discovering whether or not there exists between thiamin and the visual path a physiological relationship.

We planned to reduce the thiamin intake in three ways, totally and suddenly, moderately and fairly rapidly, slightly and gradually. We planned also to reverse certain of the experiments with a view to ascertaining whether recovery of damaged neurones was possible. We also planned to withhold riboflavine, as well as thiamin, for reasons given elsewhere.

We were fairly confident as a result of the experience we had gained in our preliminary experiments that we could control inanition in the experiments of longer duration. In the acute experiments, although we could not prevent anorexia in the terminal stages, we now had a clear picture of the pathology produced by such a state.

In the acute experiments we used no control animals, intending to utilise the pathological data we already possessed.

In the series of experiments of moderate duration (subacute deficiency) we used, however, a system of cross control, 4 deficient groups being paired with 2 control groups: and in the experiments of prolonged duration (chronic deficiency), paired feeding was made the rule.

The basic diet of choice we have already given*. It incorporates glucose instead of starch, and the synthetic B complex rather than autoclaved yeast. As we were to use younger rats, however, we decided to repeat the preliminary variety of diets** in one of the experiments (Experiment VIII).

The essential details of the diets, a description of the clinical behaviour of the experimental animals, and our pathological observations will now be given without comment. In view of the detailed information presented in Part II, our descriptions will be brief and to the point.

A discussion of our results, and the conclusions made, will be treated separately at the end of this part, and photomicrographs illustrating the changes described are collected in Appendix A***. The material was examined by all 8 histological methods before opinions as to the pathology were expressed.

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* See page 58.

** As in Experiment II.

*** Plates XIII - XXI.

EXPERIMENT V - Acute thiamin deficiency, non-recovered

Diet:- Glucose, the synthetic vitamins less thiamin plus pyrithiamin

Exp.V Rat	Weights		Diff.	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
1	90	80	-10	500	250	-250	killed
2	80	87	+ 7	500	240	-260	"
3	75	95	+20	510	310	-200	"
4	75	75	0	500	-	-	"
5	75	75	0	500	-	-	"
6	85	90	+ 5	500	300	-200	"
7	80	77	- 3	500	-	-	"
8	117	100	-17	440	200	-240	died during recording
9	100	102	+ 2	480	300	-180	killed
10	85	88	+ 3	500	-	-	"
11	92	96	+ 4	490	-	-	"
12	90	89	- 1	460	-	-	"
Duration of experiment - 17 days							

Clinical Behaviour

The characteristic signs of an acute deficiency as described in detail in Part II, Exp.I, were present in all rats on the 17th day, hence the decision to

terminate the experiment then. Rat 8 died during the recordings, all of which were made without resource to a hypnotic. No food was eaten in the last 36 hours by any animal.

Pathological Observations

There was cloudy swelling of the retinal bipolar & ganglion cells.

The optic nerve fibres were slightly more tortuous than normal.

There were no signs of degeneration otherwise in retinas, nerve fibres, myelin sheaths, nor geniculate bodies.

A few of the twigs of the sciatic nerve revealed marked degeneration of myelin, except in Rats 3 and 5 where in the twigs removed for examination no abnormality was discovered.

EXPERIMENT VI - Acute thiamin deficiency plus ariboflavinosis, non-recovered

Diet:- Glucose, the synthetic vitamins less thiamin and riboflavine plus pyrithiamin

Exp.VI Rat	Weights		Diff.	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
13	80	87	+ 7	-	-	-	killed
14	75	70	- 5	500	300	-200	"
15	70	72	+ 2	480	250	-230	"
16	80	77	- 3	-	-	-	"
17	90	79	-11	500	220	-280	"
18	85	95	+10	-	-	-	"
Duration of experiment - 19 days							

Clinical Behaviour

Exactly as for Experiment V, although the onset of anorexia, spasticity, and convulsions occurred 2 days later.

Pathological Observations

No abnormality was found in the visual neurones. The sciatic nerves revealed a similar degree of degeneration to that found in Experiment V.

EXPERIMENT VII - Acute thiamin deficiency, recovered

Diet:- Glucose, the synthetic vitamins less thiamin plus pyrithiamin

Exp.VII Rat	Weights			Heart Rates			Disposal
	Initial	At 15 days	Final	Initial	At 15 days	Final	
19	130	120	165	440	250	440	killed
20	110	111	148	480	300	460	"
21	165	185	235	440	360	420	"
22	110	107	-	500	200	-	died 13th day
23	120	115	155	480	240	480	killed
24	135	121	-	460	-	-	died
Duration of experiment - 60 days							

Clinical Behaviour

Rat 22 died on the 13th day. Rat 24 died suddenly during the recordings, which were performed without a hypnotic. The other rats were all spastic and inco-ordinated on the 15th day when their diet was restored. Rats 19 and 23 were given intraperitoneal thiamin as their heart rates were so low. This was repeated for 3 days; otherwise they might not have lived.

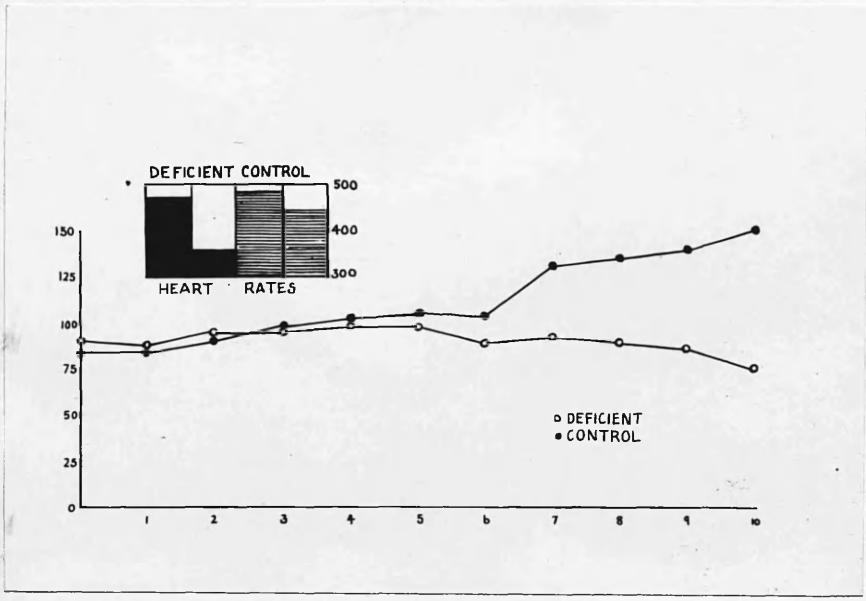
Pathological Observations

No signs of degeneration or repair in the visual pathway, nor sciatic nerves, were seen.

EXPERIMENT VIII - Subacute thiamin deficiency, non-recovered

The controlled administration of thiamin hydrochloride used to induce this deficiency, it was hoped, would greatly prolong the deficiency state. Although it was an improvement on Experiment I, we found later that we must categorise this Experiment as a subacute rather than a chronic deficiency.

THIAMIN PLAN B	
Administration of thiamin hydrochloride	
Week	Daily Dosages
1	5 µg.
2	-
3	5 µg.
4	-
5	5 µg. every second day
6	ditto.
7	3 µg. every second day
8	ditto.
9	-
10	2 µg.
11	-
12	5 µg. every second day



Wt. in gms.

Period in Weeks

PLATE I

Series 1: Starch, autoclaved yeast, and thiamin (Plan B)

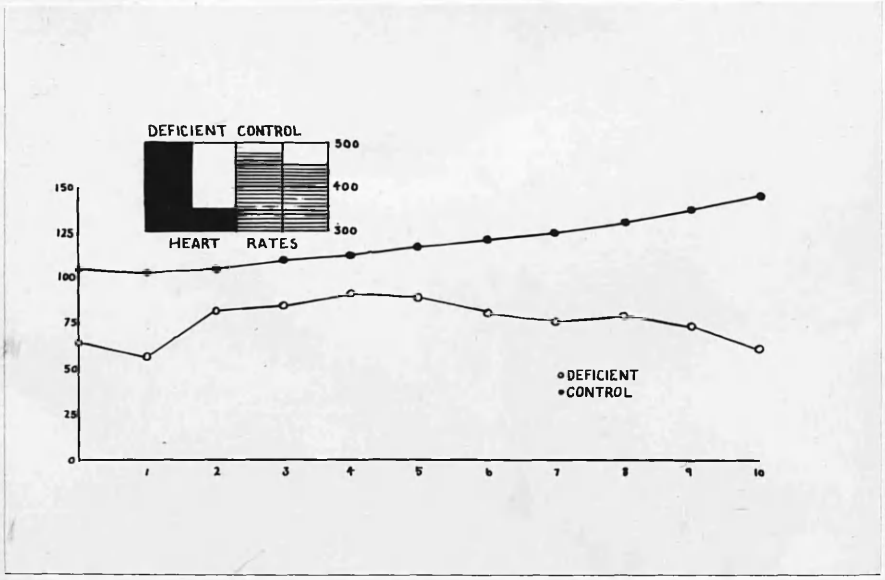
Exp.VIII S.1 Rat	Weights		Diff.after 75 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
25	110	107	- 3	480	360	-120	All
26	96	93	- 3	500	370	-130	killed
27	90	60	-30	480	350	-130	on
28	92	66	-26	500	360	-140	75th
29	72	99	+27	500	400	-100	day.
30	83	60	-23	500	400	-100	
Duration of experiment - 75 days							

Clinical Behaviour (Plate I).

Anorexia first appeared on the 35th day. It remained negligible (less than 5 g. residue) until the 68th day. The rats were sacrificed on the same day as anorexia was seen to be severe, and inco-ordination was present in all the rats. There was no spasticity. The general signs we had come to expect were all present, wasting, lassitude, the crouched position, and 'porcupine' fur. The heart rates, as in each of the following 6 series were taken under a hypnotic.

Pathological Observations

No abnormality was discovered.



Wt. in gms.

Period in weeks

PLATE II

Series 2: Starch, the synthetic vitamins, and thiamin (Plan B)

Exp.VIII S.2 Rat	Weights		Diff.after 73 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
31	70	90	+20	500	380	-120	killed
32	65	60	- 5	500	300	-200	"
33	64	70	+ 6	500	360	-140	"
34	62	65	+ 3	500	-	-	died 63rd day
35	68	92	+24	500	420	- 80	killed
36	65	68	+ 3	500	300	-200	"
Duration of experiment - 73 days							

Clinical Behaviour (Plate II).

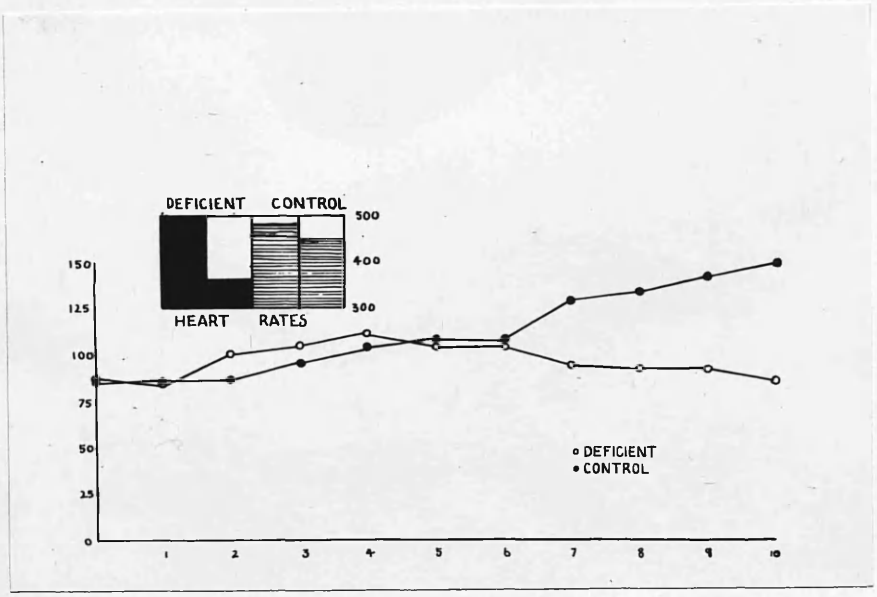
Behaviour paralleled the course of Series 1 up to the 63rd day. At this point Rat 34 was found dead. By the 72nd day, Rats 31, 35, and 36 were spastic, but still able to move about when placed on the examination table. Their gait was inco-ordinated. They were all killed on the next day, when their heart rates had been found markedly reduced.

Pathological Observations

The visual pathway of Rat 32 was the only one exhibiting signs of degeneration. The fibres were moderately varicose, and many long oligodendrocyte chains were found in nerve and tract. Some isotropic granules

could be seen in the myelin sheaths with polarised light, but the number of Marchi bodies was not significant. In the dorsal nucleus there were a few sclerosed hyperchromatic cells which in view of the precautions we took were considered pathological. The retina was normal.

The brain of Rat 34 (which had been found dead) was discarded.



Wt. in gms.

Period in weeks

PLATE III

Series 3: Glucose, autoclaved yeast, and thiamin (Plan B).

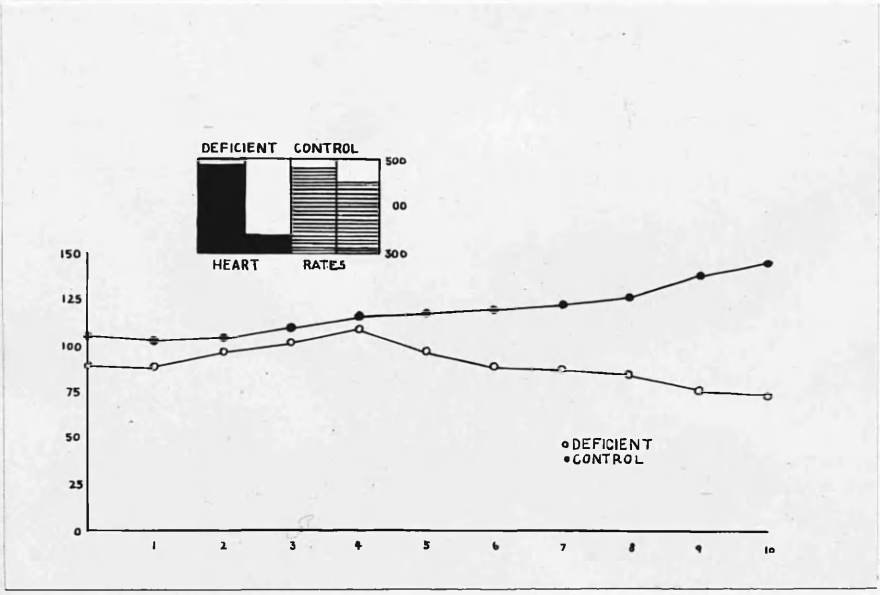
Exp.VIII S.3 Rat	Weights		Diff.after 75 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
37	95	90	- 5	500	420	- 80	killed
38	95	87	- 8	500	360	-140	"
39	95	125	+30	500	420	- 80	"
40	90	63	-27	500	320	-180	"
41	95	82	-13	500	400	-100	"
42	68	82	+14	500	360	-140	"
Duration of experiment - 75 days							

Clinical Behaviour (Plate III)

This Series paralleled Series 1 in all respects.

Pathological Observations

In Rat 40 there was roughly the same number of sclerosed geniculate cells as had been found in Rat 32. The nerve fibres, however, were not so markedly distorted, and the sheaths appeared unaffected. The retina was normal.



Period in weeks

PLATE IV

Series 4: Glucose, the synthetic vitamins, and thiamin (Plan B)*

Exp.VIII S.4 Rat	Weights		Diff.after 75 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
43	95	87	- 8	500	360	-140	killed
44	77	65	-12	500	300	-200	"
45	80	81	+ 1	500	360	-140	"
46	82	72	-10	500	420	- 80	"
47	85	83	- 2	500	300	-200	"
48	107	77	-30	480	360	-120	"
Duration of experiment - 75 days							

Clinical Behaviour (Plate IV)

The only difference in the clinical behaviour was a greater difficulty in controlling the anorexia. 3 µg. of thiamin had to be given each day during the 8th week before the appetite returned. Rats 21 and 24 were spastic at the end of the experiment, and the rest were very shaky.

Pathological Observations

Rats 43 and 47 exhibited signs of degeneration corresponding to those described in Rat 32. In Rat 47, the myelin sheaths as viewed by Marchi and Loyez were somewhat distorted. This is useful, it will be recalled, in

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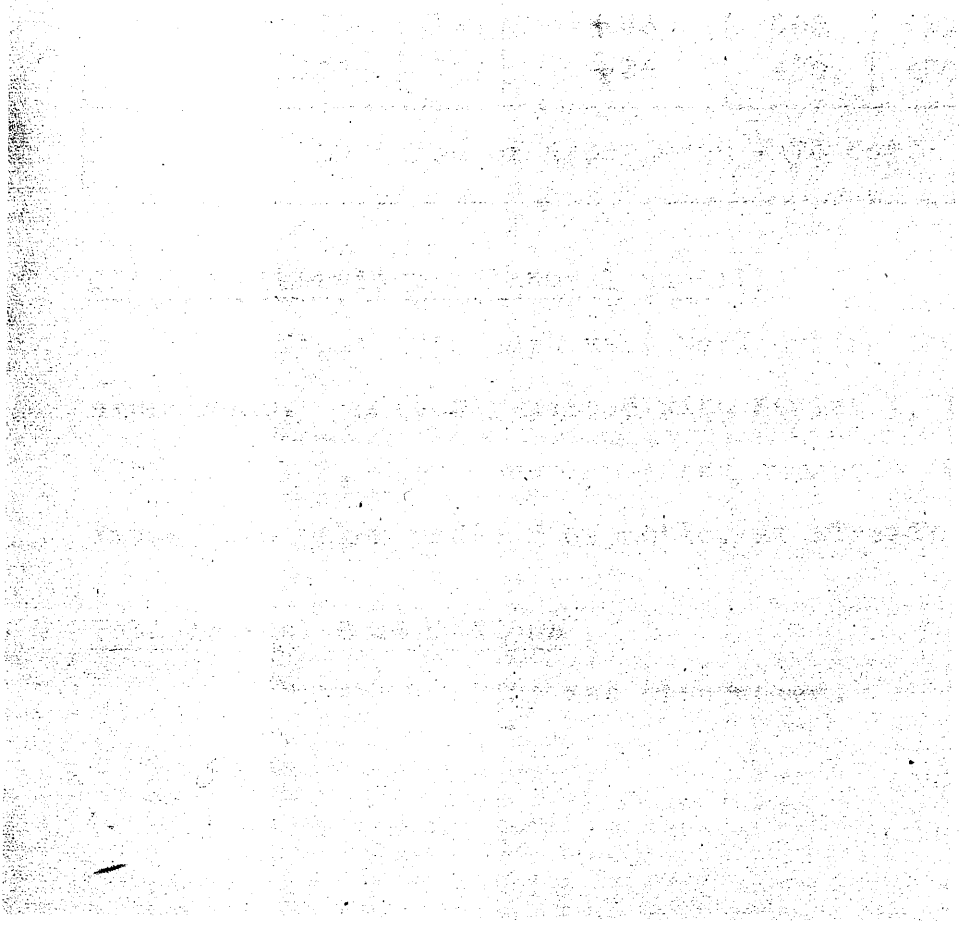
* See text for variation in administration

confirming axis cylinder changes: it does not necessarily imply myelin degeneration. In Rat 47 in fact the myelin appeared healthy as viewed by the polarising microscope.



Series 5: CROSS CONTROL - Starch and fresh dried brewer's yeast

Exp.VIII S.5 Rat	Weights		Diff.after 75 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
49	85	115	+ 30	500	480	-20	killed
50	65	130	+ 65	500	480	-20	"
51	85	128	+ 43	500	480	-20	"
52	95	145	+ 50	500	480	-20	"
53	85	138	+ 53	500	480	-20	"
54	106	142	+ 36	480	450	-30	"
Duration of experiment - 75 days							

Clinical Behaviour (Plates I and III)

These rats kept very well during the entire experiment. Directly paired with Series 1, indirectly with Series 3, they never appeared hungry. The heart rates were taken under a hypnotic, as already mentioned.

Pathological Observations

No abnormality was discovered.

Series 6: CROSS CONTROL - Starch and the synthetic vitamins, including full thiamin

Exp.VIII S.6 Rat	Weights		Diff.after 75 days growth	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
55	108	147	+39	480	460	-20	killed
56	102	122	+20	480	480	0	"
57	112	117	+ 5	480	480	0	"
58	101	155	+54	480	470	-10	"
59	100	123	+23	480	480	0	"
60	103	118	+15	480	480	0	"
Duration of experiment - 75 days							

Clinical Behaviour (Plates II and IV)

These rats also flourished, although they did not appear so well contented as the Series 5 Controls. Directly paired with Series 2, indirectly with Series 4, they were always ready to eat. They kept fit, however.

Pathological Observations

No abnormality was discovered.

EXPERIMENT IX - Chronic thiamin deficiency, recovered

Series 1: Glucose, the synthetic vitamins, and thiamin (Plan B) varied

In an endeavour to prolong the experiment even further, thiamin Plan B was altered. After the 6th week, 2 µg. was given daily up to the 14th week when no thiamin was administered at all until the turn of the experiment.

Exp.IX S.1 Rat	Weights			Heart Rates		Disposal
	Initial	At 14th week	Final	Initial	At 14th week	
61	50	80	-	550	-	Died 63rd day
62	115	110	160	480	340	Killed 140th day
63	50	60	-	550	-	Died 63rd day
64	55	85	100	500	320	Killed 90th day
65	55	92	-	500	360	Died during recording
66	55	75	-	500	-	Died 63rd day
67	70	60	235	500	380	Killed 140th day
68	55	70	-	550	-	Died 63rd day
69	72	125	255	480	390	Killed 140th day
Duration of experiment - 140 days						

Clinical Behaviour

By the 63rd day, marked signs of deficiency were present. Rats 61, 63, 66, and 68 were moribund, and intraperitoneal thiamin had no effect. They died in convulsions. The others were shaky, but otherwise

well, especially Rat 69, and heart rates were taken under light urethane. Rat 65 did not regain consciousness. Rat 62 was spastic next day, so all the survivors, including Rat 69 which was still very active, were given intraperitoneal thiamin, and a full diet instituted. They made an uninterrupted recovery, gaining weight rapidly. Anorexia in this experiment had been remarkably well controlled so that the paired rats (Series 2) seldom had to have their intake reduced. Rat 64 was sacrificed on the 90th day, the others on the 140th. There was no residual palsy.

Pathological Observations

Changes were found in Rats 62, 64, and 67. The visual path and eyeballs of Rat 69 were normal.

There was great evidence of glial proliferation in the retina of Rat 64, including histiocyte aggregation around the retinal vessels. The changes in the retinas of Rats 62 and 67 were not at first so striking, consisting of the abnormal presence of what appeared to be "gemästete cells". These cells approached in size sclerosed ganglion cells, however, and might have been mistaken for them. The number of them present in 7 μ sections outwith the macular region, nevertheless, was suggestive. The ganglion cells themselves appeared

normal, although satellitosis around them was common. The other retinal elements were unaffected.

The cells in the dorsal geniculate nuclei were almost entirely normal. Here and there were a few sclerosed cells, and small aggregations of oligodendrocytes: but no true satellitosis. In the tract of Rat 62 were many long oligodendrocyte chains.

Changes in the nerve fibres were of an equally slight nature. In the tract of Rat 62 there was a moderate degree of distortion.

The nerves and tracts, however, revealed a high degree of patchy demyelinisation, which was also present in the nerve head of Rat 67. None was found in Rat 64.

The sciatic nerves showed circumferential degeneration involving some of the nerve trunks. In others, there was distortion of the myelin sheaths when viewed transversely. Some sheaths exhibited ballooning.

Series 2: PAIRED CONTROL - Glucose, the synthetic vitamins, and thiamin (full)

Exp.IX S.2 Rat	Weights		Heart Rates		Disposal
	Initial	Final	Initial		
70					All killed on 140th day
71	110	220	480	440	
72					
73	55	190	500	480	
74					
75					
76	60	188	500	480	
77					
78	68	200	500	460	
Duration of experiment - 140 days					

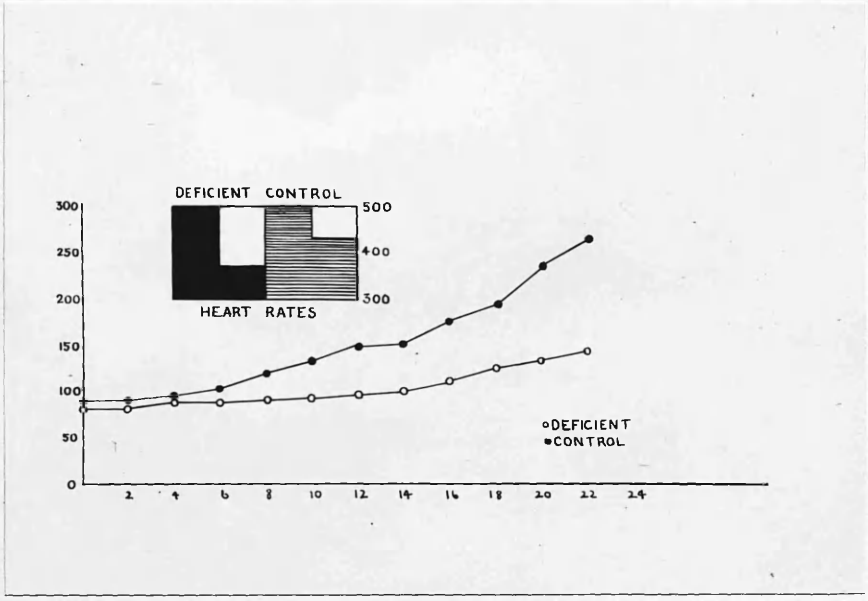
Clinical Behaviour

As anorexia in the experimental rats had been well controlled, these rats flourished, seldom appearing hungry.

Pathological Observations

No abnormality was found.

Wt. in gms.



Period in weeks

PLATE V

EXPERIMENT X - Chronic thiamin deficiency plus ariboflavinosis, non-recovered.

Series 1: Glucose, the synthetic vitamins less riboflavine, and thiamin (Plan B)

Exp.X S.1 Rat	Weights		Diff.after 160 days	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
79	85	145	+ 60	500	360	-140	Killed 160th day
80	75	100	+ 25	500	360	-140	" 130th day
81	85	107	+ 22	500	320	-180	" 100th day
82	80	142	+ 62	500	340	-160	" 160th day
83	75	138	+ 63	500	380	-120	ditto.
84	85	150	+ 65	500	360	-140	ditto.
Duration of experiment - 160 days							

Clinical Behaviour (Plate V)

The rats in this Series to our great surprise continued to live in moderate health and to grow (although at a reduced rate) long after their fellows (thiamin, Plan B, with full riboflavine - Exp.VIII, Series 4) had died.

Their appetites remained moderately good, although they appeared to require less. They never exhibited signs of nervous disorder.

After the 4th week, they became listless, spending most of the day asleep. When roused, nevertheless, they were quite active.

PLATE VI

PLATE VII

At the 8th week, the hair around the eyelids was seen to be lost (Plate VI). A close examination at the 20th week revealed the presence of several other signs characteristic of a mild riboflavine deficiency. These might have been present earlier. The hair over the belly was sparce, the fur contained many small white dry scales (Plate VI) and the mucosa of lips, mouth, and nostrils was erythematous, and swollen (Plate VII).

Such was still the picture on the 160th day when the 4 remaining rats (2 had been sacrificed earlier) were killed.

The characteristic vascularization of the cornea found in riboflavine deficiency was observed with the loupe after death.

Pathological Observations

Changes were found only in those rats killed on the 160th day.

Many "gemästete cells" were present in the ganglion cell layer of the retina. The latter cells were rather grossly vacuolated, and were in some instances distorted in outline: in the latter aggregation of nissl substance could be seen. A few Gitter cells were also present. About a third of the bipolar cells exhibited cloudy swelling. The pigment epithelium and rods and cones were normal.

The geniculate cells to our surprise were unaffected. Glial changes were found only in the nerve and tract, many long oligodendrocyte chains being present.

There was a moderate degree of varicosity and tortuosity in the fibres of nerve and tract. The nerves showed a gross demyelinization in Rats 79, and 82. There were only a few focuses of demyelinisation in the other two animals.

The sciatic nerves revealed extensive demyelinisation.

Series 2: PAIRED CONTROL - Glucose, the synthetic vitamins, and thiamin (full)

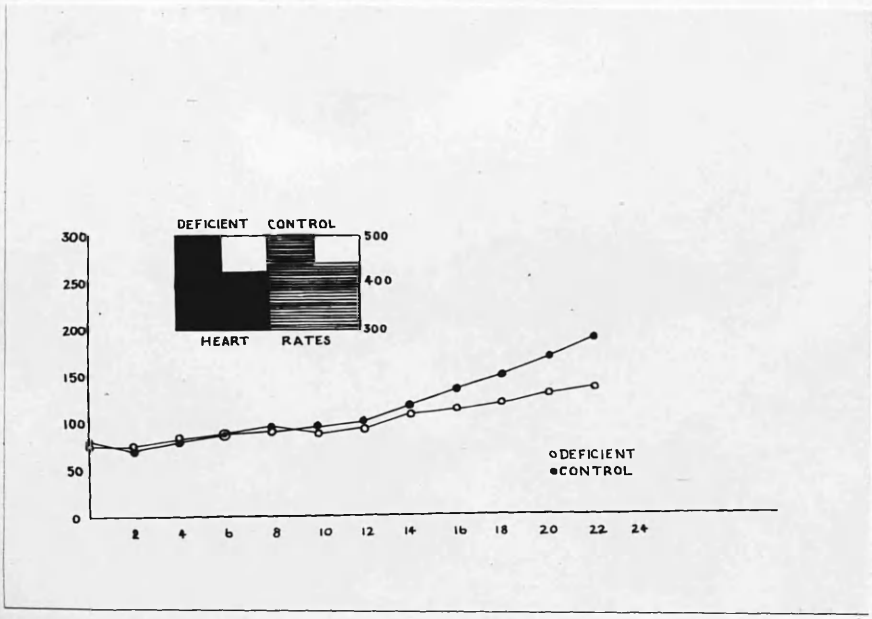
Exp.X S.2 Rat	Weights		Diff.after 160 days	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
85	85	280	+ 195	480	440	-40	killed 160th day
86	70	220	+ 170	500	460	-40	" 130th day
87	92	300	+ 208	480	440	-40	" 100th "
88	90	311	+ 221	460	440	-20	" 160th "
89	75	220	+ 145	500	480	-20	ditto.
90	87	265	+ 178	500	460	-40	ditto.
Duration of experiment - 160 days							

Clinical Behaviour (Plate V)

These rats grew at the normal rate, and kept well.

Pathological Observations

No abnormality in the visual paths.



Wt. in gms.

Period in weeks

PLATE VIII

EXPERIMENT XI - Chronic ariboflavinosis, non-recovered

Series 1 : Glucose, the synthetic vitamins less riboflavine, and thiamin (full)

Exp.XI S.1 Rat	Weights		Diff.after 160 days	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
91	85	130	+ 45	500	400	-100	killed 130th day
92	65	140	+ 75	550	420	-130	" 160th "
93	75	75	0	500	-	-	died 52nd "
94	80	70	-10	500	-	-	" 45th "
95	80	150	+ 70	500	420	- 80	killed 160th "
96	75	65	-10	500	-	-	died 50th "
Duration of experiment - 160 days							

Clinical Behaviour (Plate VIII)

Rats 69, 70, and 72 having gained weight during the first three weeks of the experiment then went steadily downhill. They became very bedraggled, and although continuing to eat moderately well (leaving less than 5 g.) they spent most of the time crouched in the corner. Rat 94 died on the 45th day, 96 on the 50th, and 93 on the 52nd. The corneas were grossly vascularized, and abdominal alopecia was marked.

Rats 91, 92, and 95, however, made steady progress, and although exhibiting a marked disinclination to exercise kept well. On completion of the experiment, they exhibited mild signs of ariboflavinosis similar to those seen in the rats of Experiment X. There was no swelling of the nasobuccal mucous membranes. Dandruff, slight abdominal alopecia, and "Spectacle-rings" around the eyes were present, however. Corneal vascularization was present in only one animal, Rat 91.

Pathological Observations

No abnormality was found in the survivors.

Series 2: PAIRED CONTROL - Glucose, the synthetic vitamins, plus riboflavine and thiamin (full).

Exp.XI, S.2 Rat	Weights		Diff.	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
97	85	249	+164	500	440	-60	killed 130th day
98	67	251	+184	500	440	-60	" 160th "
99							
100							
101	80	260	+180	500	440	-60	killed 160th "
102							
Duration of experiment - 160 days							

Clinical Behaviour (Plate VIII)

Nothing to report.

Pathological Observations

No abnormality.

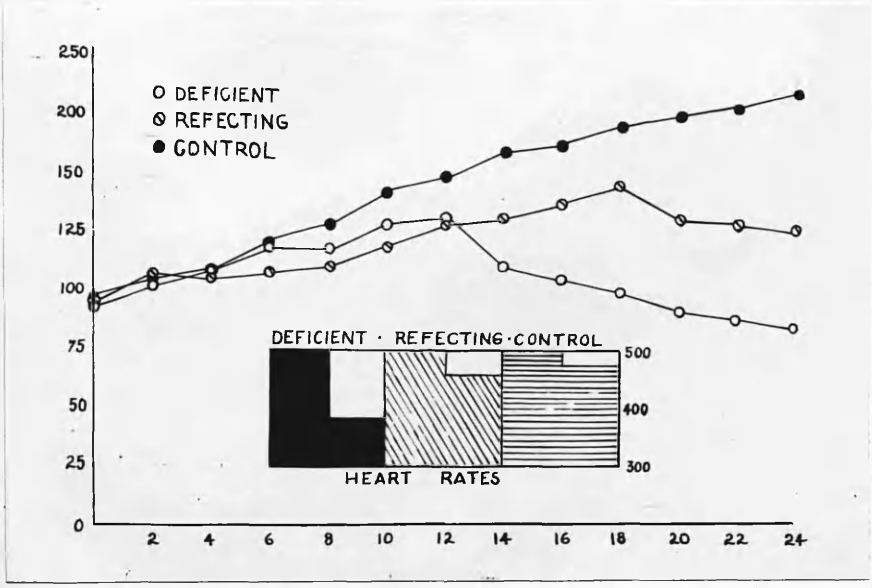
EXPERIMENT XII - Chronic thiamin deficiency (second series), non-recovered

Series 1: Glucose, the synthetic vitamins, and thiamin (as required)

We already had evidence that a thiamin deficiency of prolonged duration could lead to a degeneration of the visual neurones, a degeneration that was irreversible. This second series was run in the hope that if the duration of the reduced thiamin intake be further prolonged these signs might be more dramatic.

The adoption of a preconceived plan for the administration of thiamin to the group was rejected. We decided each animal required individual attention. A daily dose of 2 µg was, therefore, administered individually until the first signs of anorexia. The dose was then increased in that rat to 5 µg. until the appetite returned, when 2 µg. was once again given, ad infinitum.

As can be seen in the table below, Rats 103, 105, and 106 existed quite happily on a daily maintenance dose of 2 µg., although they had gained on the average only about 18 g. in weight at the end of 180 days.



Wt. in gms.

Period in weeks

PLATE IX

Exp.XII S.1 Rat	Weights		Diff.after 180 days	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
103	95	100	+ 5	500	480	- 20	All
104	90	90	0	500	360	-140	killed
105	90	120	+ 30	500	480	- 20	on
106	92	110	+ 18	500	420	- 80	180th
107	90	95	+ 5	500	340	-160	day
108	90	89	- 1	500	360	-140	
Duration of experiment - 180 days							

Clinical Behaviour (Plate IX)

Up to the 12th week all the rats did well. The increase in weight was slow but steady. The animals were thin but fit, and their appetites had been maintained. (Plate X). Hereafter it became increasingly difficult to prevent anorexia in Rats 104, 107, and 108.

By the 160th day they had become bedraggled and shaky. There were no gross signs of inco-ordination, however, and they kept eating up to 2/3rds of their daily ration. We observed a marked ptosis of the upper lids. (Plate XI, a and b). Even when the animals were given a fright, the palpebral fissures remained reduced in size. It appeared to be a true paralysis. The animals, moreover, were seen to be suffering from drop-foot (Plate XII). They still ate, however, and could

PLATES X & XII

Rat 104 at the end of 10 weeks (Plate X) and 24 weeks (Plate XII). In the latter, note the 'porcupine' fur, ptosis, wasting, and foot-drop.

PLATE X

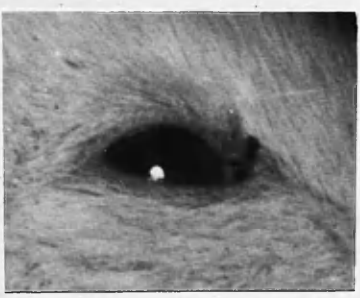


PLATE XI, a.

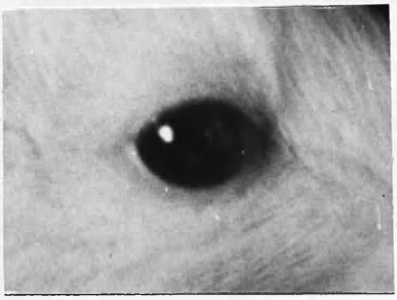


PLATE XI, b.

PLATE XII

sit up to wash themselves. We observed one of them regurgitate while feeding on two occasions. When the heart rates were found to be below 400 beats per minute (restrained), it was decided to terminate the experiment.

The continued good health of the other three rats was considered due to refection. Their records are separately recorded in Plate IX.

Pathological Observations

Rats 103, 105, and 106 revealed no abnormality; Rats 104, 106 and 107 each exhibited identical changes.

The retinal ganglion cells were hyperchromatic and somewhat sclerosed. That was the only change in the retina.

The optic nerve and tract revealed widespread signs of degeneration increasing in intensity as the fibres ascended: the nerve fibres were thickened, distorted, and very tortuous (corkscrew fibres). The terminal branches within the lateral geniculate body were similarly affected; and in addition the larger ones had fragmented. There were many small black patches, although we saw no true ring shapes. We found only a few endings enlarged. The fibres of the third neurone as far as we could see were similarly affected.

Within the dorsal nucleus, the visual cells were nearly all hyperchromatic and sclerosed. The degree of sclerosis was not gross, but it was definite. Both nuclear and cellular outlines were distorted.

Within the nerve and tract, the oligodendrocytes had proliferated considerably into long chains. These cells were of normal size, but the small amount of cytoplasm which is present was hyperchromatic. We did not find any evidence of satellitosis, but within the dorsal nucleus were many rod cells.

These changes were widespread.

The myelin changes, although variable, were more diffuse than in the case of Experiments IX or X. In places the myelin had completely disappeared, many gitter cells and "gemästete" cells being present. Where the myelin sheaths persisted, irregularity and ballooning was common.

Series 2: PAIRED CONTROL - Glucose, the synthetic vitamins, and thiamin (full)

Exp.XII S.2 Rat	Weights		Diff.after 180 days	Heart Rates		Diff.	Disposal
	Initial	Final		Initial	Final		
109	100	265	+ 165	500	480	-20	All
110	90	180	+ 90	500	480	-20	killed
111	102	275	+ 173	500	500	0	on
112	95	290	+ 195	500	460	-40	180th
113	87	190	+ 103	500	460	-40	day
114	90	200	+ 110	500	480	-20	
Duration of experiment - 180 days							

Clinical Behaviour (Plate IX)

These rats did well. Rats 110, 113, and 114, being subjected to the vagaries of appetite in the paired experimental animals, were often hungry. Latterly all were hungry, 15 g. evidently being not sufficient.

Pathological Observations

Absolutely normal visual pathways.

Conclusions

There appeared to be little doubt as the experiments unfolded that the most important distinction which has to be drawn is that between a 'complete deficiency' and a 'reduced intake' of thiamin.

In complete deficiency - as when administering the analogue - rats suddenly exhibit severe signs of neuromuscular weakness and disorder at the end of about 15 days (depending on their age), and will die, if untreated, within 36 hours. During this time they suffer from a marked anorexia. This period of starvation, however, is not of sufficient length to produce cytological changes within the central nervous system. Although, as we saw, inanition accounted for a minor degree of demyelinisation in the sciatic nerves, the changes were much more gross in the acutely-deficient animals. These findings suggest that the peripheral neuropathy of man, known as beriberi, is chiefly due to a thiamin avitaminosis. The beriberi patient, of course, it is well known, is frequently quite well nourished. One must remember, however, that in the starved rats, the peripheral nerve changes, slight though they were, were not prevented by giving full

vitamins, an observation in agreement with that of Vedder (1938).

The gross upset of postural sensibility and the convulsive seizures prior to the death of acutely-deficient rats, on the other hand, we consider to be entirely due to inanition: Experiment IV is surely evidence of that.

In animals depleted less drastically, and over a prolonged period, a progressive weakness slowly develops, characterized by loss of weight, and unsteadiness of gait. The paired control rats with a high intake of thiamin and an isocaloric diet never presented this picture. These 'controls' were under close observation throughout; their behaviour remained perfectly normal, their appetites excellent. Subsequently, the visual pathways revealed no abnormality whatsoever. We presume, then, that the histological changes in the central and peripheral nervous systems which were found in the deficient animals arose as a result of thiamin deficiency.

At first sight there is a remarkable similarity in the pathologies of subacute starvation, and chronic thiamin deficiency.

Although the duration of the diet, however, was prolonged, that is not to say that the cytological changes were of equal long standing. There is every reason to suspect in fact that for many weeks the disorder remains purely functional. We cannot state the exact time when the defect in nutrition leads to structural changes. If, however, the animals are sacrificed shortly after the onset of these changes, the appearances may turn out to be atypical of a "chronic" lesion. Such a sequence of events would explain the confusing similarity in appearance of these two series.

On closer observation, however, we discovered that a slight structural difference exists between the two, subacute inanition, and chronic thiamin deficiency. In the latter the fibres are thicker, and the cells more greatly shrunken, than in the former. In fact, it appears that we have, here, two distinct stages in the evolution of cellular degeneration, very finely distinguished.

Zimmerman (1943) tells us that in the dog twice the length of time is required to produce degeneration of the nervous system by a riboflavine deficiency than by a thiamin deficiency. On these

premises, therefore, the rats of Experiment XI (chronic ariboflavinosis) - whose survival on a riboflavine-free diet we can only presume was due to a high degree of resynthesis - should have been left another 140 days before being killed. We cannot, in short, conclude that a riboflavine deficiency does not lead to degeneration of the nervous system on the grounds that none was found in this group. By that kind of argument too many other authors have committed errors of judgement.

Where the deficiency involved both thiamin and riboflavine (Experiment X) the duration of the experiment was greatly prolonged. It would seem to us that the degeneration found in this group must be due to a lack of thiamin rather than of riboflavine, the former deficiency having reached the necessary degree of chronicity as a result of the withdrawal of the riboflavine. The rats of Experiment XI who exhibited corresponding signs of ariboflavinosis, but who received full thiamin, acted in this respect as a control group. It is because they did not exhibit signs of degeneration that we feel inclined to say that the thiamin deficiency overshadowed the riboflavine in Experiment X.

In the acute deficiencies the changes found in the sciatic nerves were gross, and yet appeared to be recoverable. In the chronic, on the other hand, similar changes were not recoverable. It is possible that the degree of myelin-recovery depends upon the integrity of the nerve cells and their processes for in the acute deficiencies the latter were intact, whereas in the chronic there were residual signs of neuronal disease. On these grounds we conclude that as a general rule the integrity of myelin depends upon the integrity of the axis cylinder, although the reverse, as we saw in Part III, is not the case.

To return to the visual path, even as different rats appear to be more susceptible than others, so different fibres are more readily affected than others. As we do not know the pathway taken by the macular fibres in the rat, we cannot say, even if the central fibres alone had degenerated, that the papillo-macular bundle is more susceptible than any other bundle. Such an argument might be well levelled at the conclusions of Marchesini and Papagno, who, if you remember, described a papillo macular

degeneration in pigeon optic nerves. It is interesting to find, in fact, that the lesions produced in rats by a thiamin deficiency of long duration are disseminated throughout the visual pathway, and increase in severity as it ascends. This fact supports our other finding that the retinal ganglion cells are not greatly affected, a somewhat paradoxical finding in view of their extremely low resistance to chemical poisons.

The appearance of the retina in Experiment XII suggests, however, that if the thiamin deficiency had been even more prolonged, the retinal ganglion cells in the end would have become affected just as much as were the visual cells in the diencephalon. Perhaps the explanation of retinal immunity is to be found in the extremely active drainage afforded by the choriocapillaris.

In no specimen did we find changes affecting the retinal pigment or the bloodvessels. Furthermore, there was a total lack within the visual path of any sign of a haemorrhagic crisis. The Giemsa technique is particularly suited for observations on blood. We cannot say, of course, whether the floor of the

IVth ventricle was affected or not*. It would appear, however, that we were justified in leaving the rat to manufacture its own vitamin K.

In like case, the endogenous supply of vitamin C was adequate for there were no signs of scurvy.

Summary

Using dietary methods described in detail in Part II, and histological techniques described in Part III, the following observations were made:

1. When thiamin deficiency in rats is of moderate degree and prolonged to about 150 days, or over, degeneration of the visual pathway develops in certain susceptible animals, its intensity being greatest centrally. This occurs even when the caloric intake is adequate.

2. The degeneration consists of three distinctive features:

   (1) The visual cells become sclerosed and hyperchromatic - the retinal much later than the geniculate.

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* In the rat, this is the preferential site for such haemorrhages, beneath the ependyma which covers the floor.

(2) The axis cylinders become thickened, tortuous, and varicose, the terminal branches fragmented, and the nerve endings enlarged.

(3) Multiple foci of demyelinisation occur, to be replaced in time by clearly delimited glial plaques.

3. This chronic deficiency is associated with degeneration of the peripheral nerves.

4. In acute deficiency, spasticity may result from a similar degeneration of the peripheral nerves. Postural imbalance, and convulsive seizures are, probably due to impaired function, and not to cellular degeneration: they appear to arise as a result of inanition.

5. The neuronal changes described above are reversible, provided the cell body and its process are not unduly damaged. In some instances normal fibres may be seen in areas of demyelinisation. In others, the fibres despite a long period of recovery are still distorted.

6. The simultaneous withdrawal of riboflavine and thiamin reduces the thiamin requirement. Perhaps

this is due to the preferential use of fat. In such instances, degeneration due to lack of thiamin occurs before degeneration due to lack of riboflavine.

We consider that experimental optic atrophy in rats suffering from a prolonged thiamin deficiency - a finding disputed by so many - is established by these findings.

References

1. Vedder, E.B. 1938, J.A.M.A. 110, 893.

2. Zimmerman, H.M. 1943, Res.Pub.Ass.nerv.ment.Dis. 22, 51.

APPENDIX A - PLATES XIII to XXI

We offer our sincere thanks to Mr. A. Pegg of the Department of Photography, Medical School, King's College, Durham University, for the excellent photomicrographs illustrating this thesis throughout.

PLATE XIII

a. Cloudy swelling and chromatolysis of the glial nuclei in the retina in subacute thiamin deficiency (Rat 62). The appearance simulates the "gemästete" cells of Nissl, which are found characteristically around anoxic infarctations. Triple x 800.

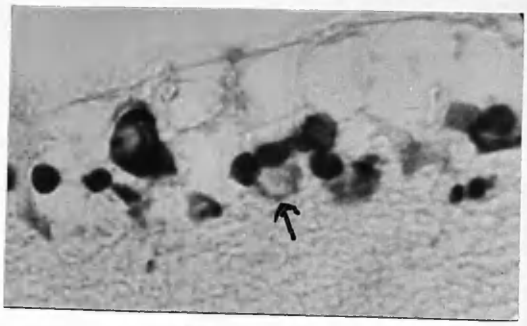
b. Hyperchromatism of the ganglion cells of the retina in chronic thiamin deficiency. A few Gitter cells are also present (Rat 79). Nissl x 760.

c. Normal retina in chronic thiamin deficiency (Rat 105). The disrupted appearance is due to over-embedding. Giemsa x 200.

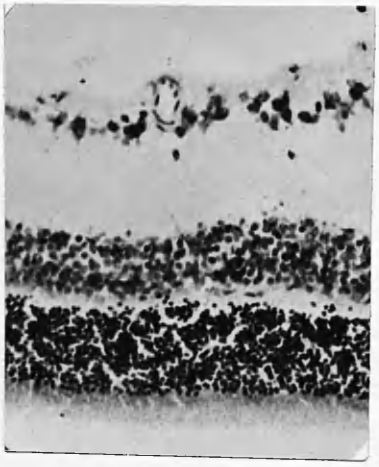
d. Normal rods and cones in subacute thiamin deficiency (Rat 32). Triple x 670.

e. Vacuolation of retinal ganglion cells in chronic thiamin plus riboflavine deficiency. This is considered to be pathological (Rat 83). Giemsa x 1200.

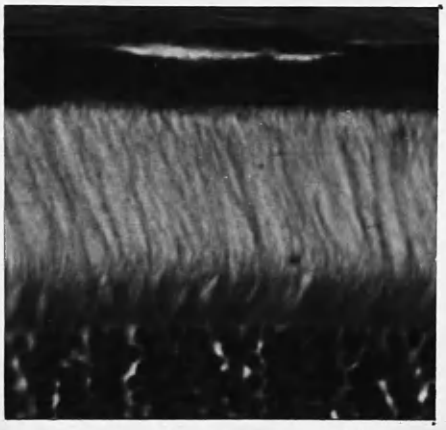
a(x800)



b(x760)



c(x200)



d(x670)

e(x1200)

PLATE XIII

PLATE XIV.

a. Central demyelinisation in recovered chronic thiamin deficiency (Rat 67). Optic nerve. Loyez x 200.

b. The same in another rat of the same group. (Rat 64).

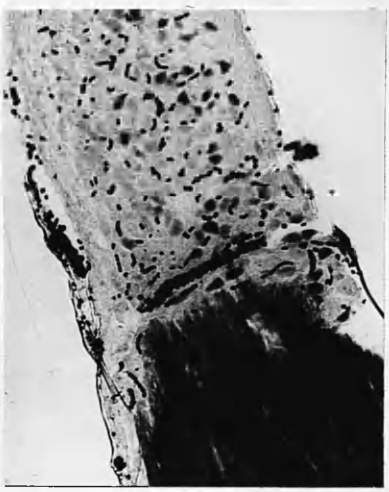
c. Soft area of demyelinisation in the intracranial portion of optic nerve. Chronic thiamin deficiency (Rat 106). Weigert-Pal x 580.

d. Recovered chronic thiamin deficiency. Dense plaque of organised demyelinisation sharply delimited from the proximal portion of the optic nerve, almost at the chiasma (Rat 62). Optic nerve. Loyez x 150.

a(x200)

b(x200)

c(x580)



d(x150)

PLATE XIV

PLATE XV

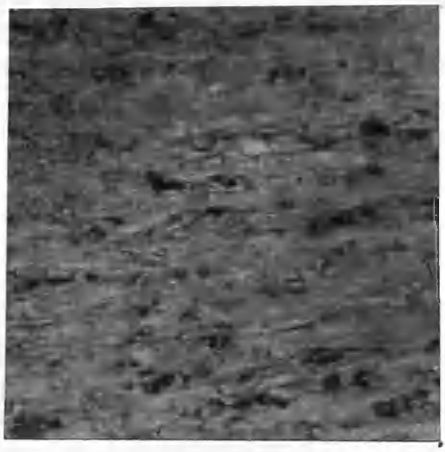
a. Myelin sheaths in optic tract showing irregularity and ballooning - viewed in longitudinal section. Chronic thiamin deficiency (Rat 108). Weigert-Pal x 640.

b. Deep patchy demyelinisation in optic tract, as it passes around the cerebral peduncle towards the diencephalon (Rat 107). As above ~~Weigert-Pal~~ Loyez x 100.

c. Disseminated areas of demyelinisation in recovered chronic thiamin deficiency. Optic tract (Rat 62). Loyez x 80.

d. The same in chronic thiamin and riboflavine deficiency (Rat 84). Loyez x 100.

(App.A)



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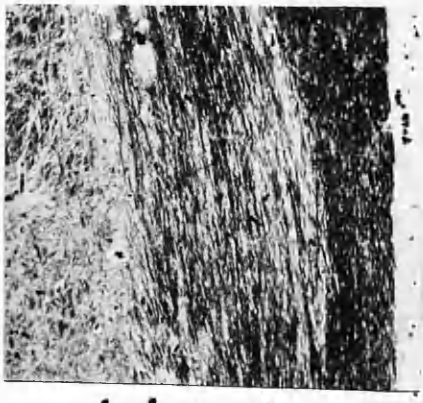
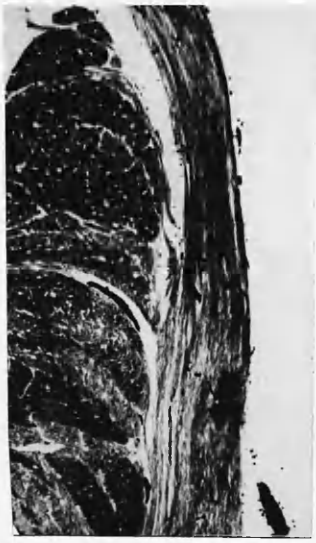


b(x100)



c(x80)

d(x100)

PLATE XV

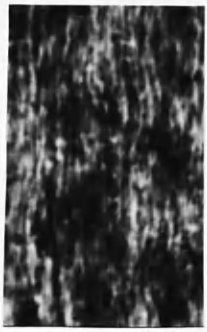
PLATE XVI

a. Granular Isotropism in acute thiamin deficiency. Optic nerve. Polarized light x 250.

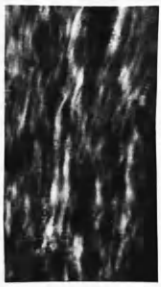
b. The same, plus slight swelling at nodes, in acute thiamin and riboflavine deficiency. Optic nerve. Polarized light x 250.

c. Complete destruction of myelin in chronic thiamin and riboflavine deficiency (Rat 82). Optic nerve. Polarized light x 250.

d. Early changes in chronic thiamin and riboflavine deficiency (Rat 80). Swelling at the nodes, and slight granularity. Optic nerve. Polarized light x 250.



a(x250)



b(x250)

c(x250)

d(x250)

PLATE XVI

PLATE XVII

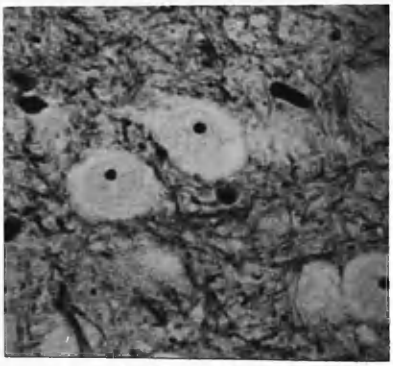
a. Optic tract terminals. Normal appearance of myelin sheaths. Loyez x 500.

b. Slight distortion of myelin in subacute thiamin deficiency (Rat 43). Geniculate terminals. Loyez x 1700.

c. The same (Rat 47). Loyez x 700.

d. Chronic thiamin deficiency. The tortuosity of the axis cylinders can be well seen, although the myelin sheaths in this region of the optic nerve are fairly normal (Rat 107). Loyez x 500.

e. The same animal. Fragmentation and globulation in the optic tract. Weigert-Pal x 640.



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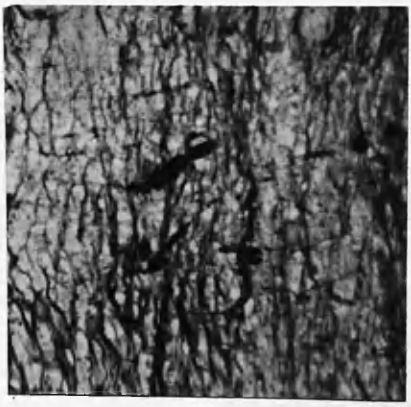
b(x1700)

c(x700)



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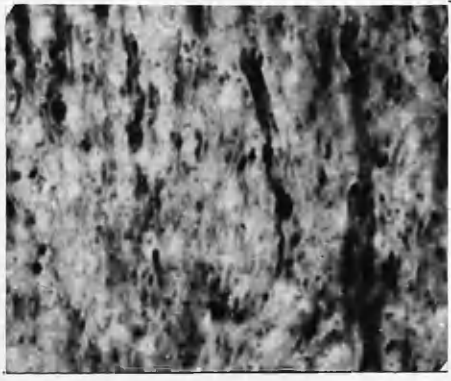


e(x640)

PLATE XVII

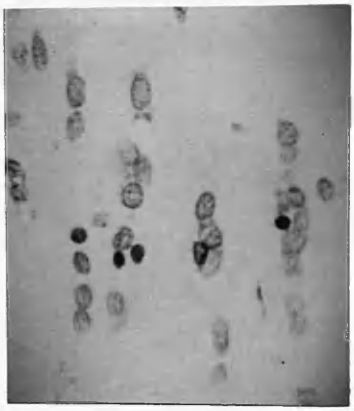
PLATE XVIII

a. Normal distribution of oligodendrocytes and histiocytes in optic nerve and tract. Giemsa x 350.

b. Proliferation of oligodendrocytes into long chains in subacute thiamin deficiency (Rat 32). Giemsa x 300.

c. The same in recovered chronic thiamin deficiency (Rat 62). Giemsa x 300.

d. The same in chronic thiamin and riboflavine deficiency (Rat 84). Author's silver x 600.



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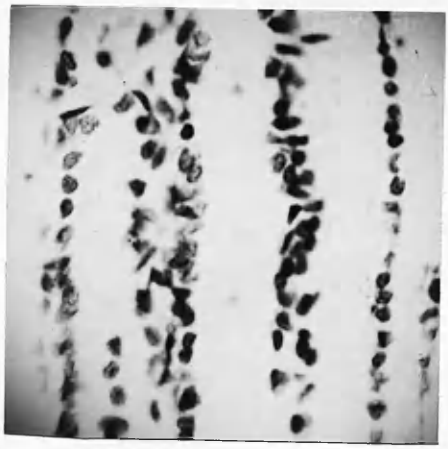

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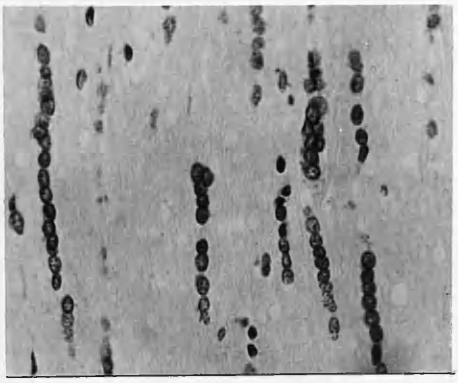


c (x300)

d (x600)

PLATE XVIII

PLATE XIX

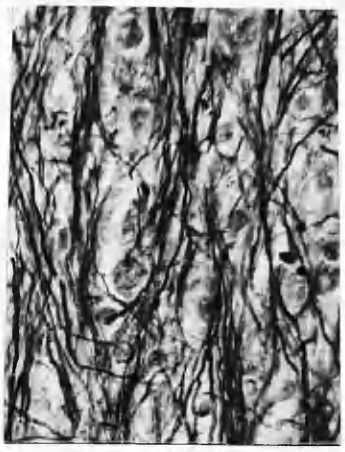
a. Normal visual fibres beginning to separate out from optic tract. Author's silver x 670.

b. Subacute thiamin deficiency (Rat 32). Early degenerative changes. Varicosity and tortuosity. Optic tract. Author's silver x 670.

c. Grosser changes in optic tract in subacute thiamin deficiency (Rat 47). Author's silver x 670.

d. Chronic thiamin deficiency (Rat 107). Marked tortuosity in optic nerve. Author's silver x 670.

e. The optic tract terminals in the same animal, revealing as an additional feature, fragmentation. Author's silver x 670.



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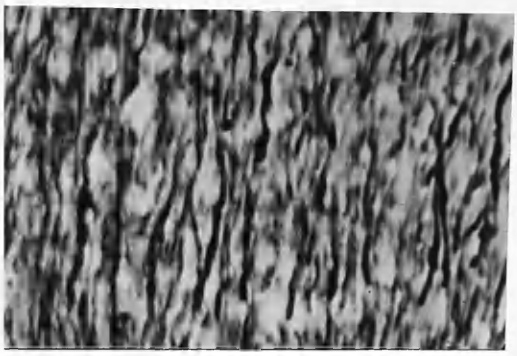

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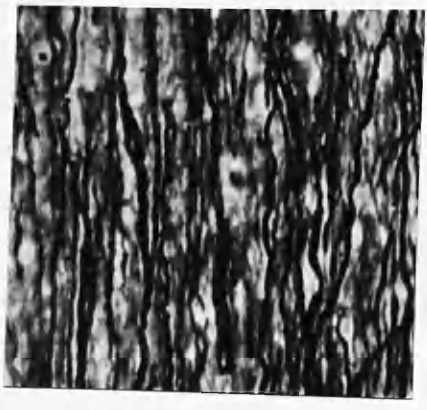

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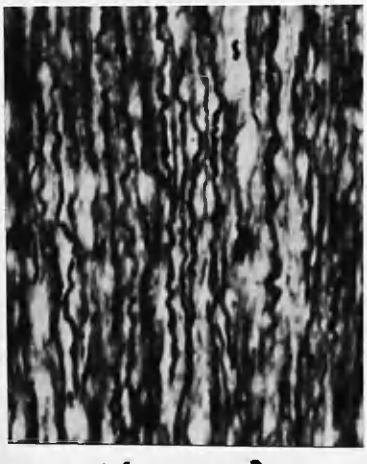
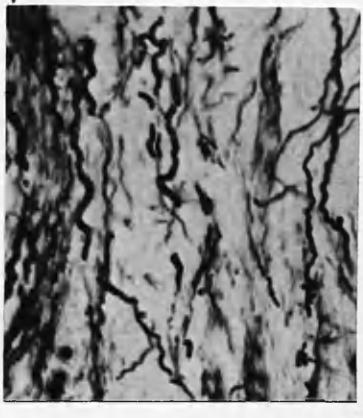


d(x670)



e(x670)

PLATE XIX

PLATE XX

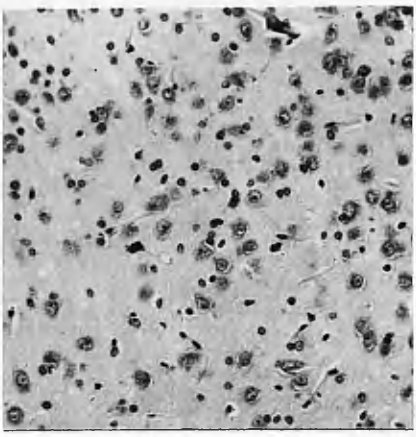
a. Chronic thiamin deficiency (paired control). Normal ganglion and neuroglial cells in the dorsal nucleus of the lateral geniculate body. Giemsa x 175.

b. Chronic thiamin deficiency. Sclerosis and hyperchromatism in lateral geniculate cells. Compare with paired control above. Giemsa x 175.

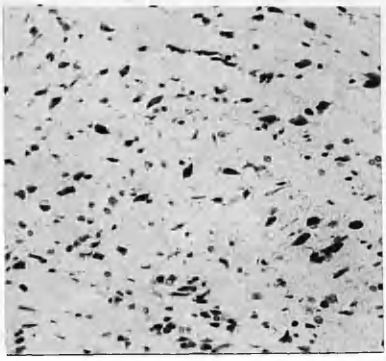
c. Normal cells in paired control (Rat 110). Giemsa x 650.

d. Abnormalities (sclerosis and hyperchromatism) in chronic thiamin deficiency (Rat 104). Giemsa x 650.

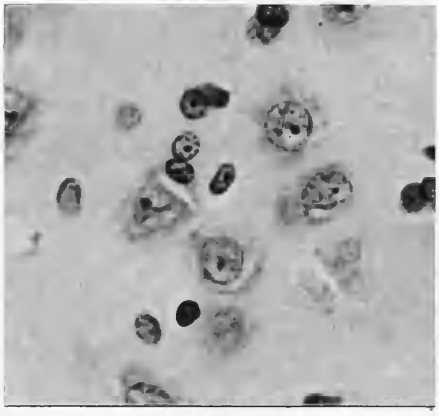
e. Slight satellitosis and glial hyperchromatism in recovered chronic thiamin deficiency (Rat 67). Giemsa x 220.



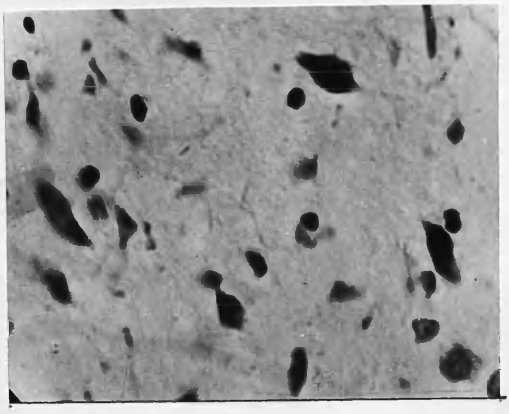
a(x175)



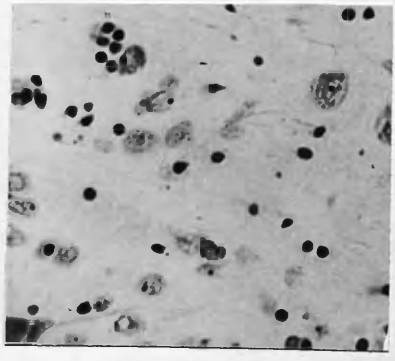
b(x175)



c(x650)



d(x650)



e(x220)

PLATE XX

PLATE XXI

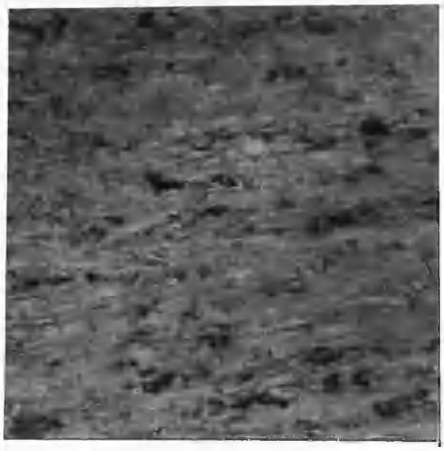
a. Gross destruction of myelin in main branch of sciatic nerve in acute thiamin deficiency* (Rat 9). Marchi x 140.

b. The same in another animal (Rat 15). Marchi x 540.

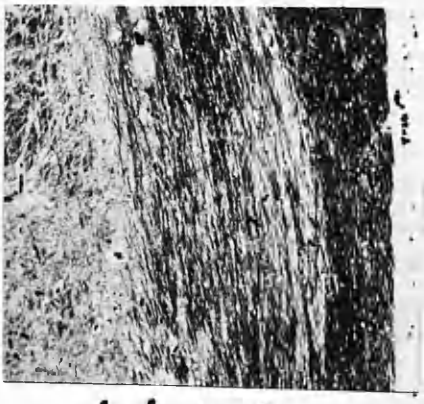
c. Distortion of myelin sheaths in twigs apparently unaffected (Rat 2). Marchi x 670.

d. Destruction of myelin in peripherally placed sciatic nerves in chronic thiamin deficiency (Rat 82). Weigert-Pal x 140.

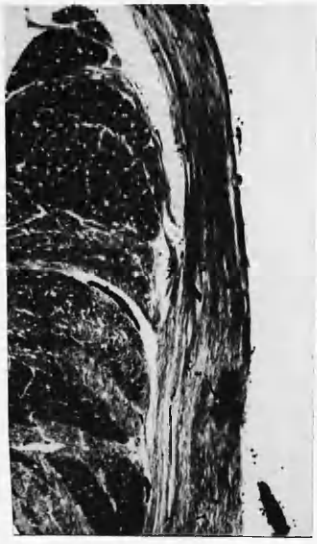
* A portion of an unaffected (normal) twig can be seen on the right.



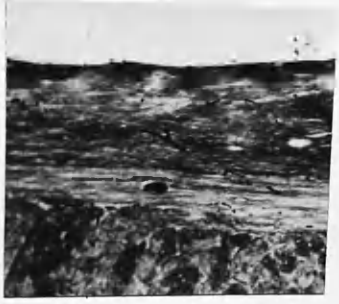
a(x640)



b(x100)



c(x80)



d(x100)

PLATE XV

PART V.

A SHORT REVIEW OF THE CLINICAL EVIDENCE AS TO WHETHER OR NOT THIAMIN DEFICIENCY CAN PRODUCE OPTIC ATROPHY

Clinical beriberi cannot now be considered to be due entirely to a deficiency in thiamin, as in man it is unlikely that uncomplicated thiamin deficiency ever occurs except under experimental conditions. Where it does occur, it is always associated with a coincidental deficiency in the other members of the B complex. Nevertheless, the thiamin-nonfat-calorie ratio (Cowgill's prediction formula) is invariably very close to the danger level, if not actually below it, when beriberi occurs: and that thiamin is deficient there can be no doubt. Hou (1943) found the vitamin consistently absent from the urine. Any association, therefore, of optic atrophy with beriberi implicates B_1. Especially is this so if in addition there be present an alimentary disorder limiting absorption, as it appears man depends on some degree of symbiotic resynthesis, just as does the rat. Najjar and Holt (1943) in experimental human beriberi, for example, found that those of their subjects who did not exhibit signs of the disease excreted large quantities of thiamin in their faeces. They concluded that such immunity resulted from an unusually high degree of intestinal synthesis in these individuals.

In human experiments of this type, however, defective vision has never been reported, although neurological disturbances in most instances were present. This is true of the experiments by Williams et al.(1939, 1940, 1941 and 1943), Jolliffe et al.(1939), Elsom et al.(1935, 1940 and 1942), Egana et al.(1942) and Barborka et al.(1943).

In the literature published prior to 1939, the majority of clinical workers omit to mention visual symptoms in beriberi, or merely suggest that if cranial nerve involvement occurs at all it is very rare. As has been pointed out recently, however, by Spillane (1947) many Japanese authors before this time referred to failing vision as a prominent symptom (Yamamoto, 1903 : Kagoshima, 1918: et al).

On examination, most of these papers proved to be unsatisfactory, especially as they were not being read in the original, nor even quoted first hand. One nevertheless stood out above the others in its promise (Kagawa, 1938), and well repaid further study.

Kagawa describes 200 cases of retro-bulbar neuritis seen at his hospital in Tokio, 61.5% of whom were suffering from beriberi, 30.5% being lactating mothers, and 8.0% whose only abnormality was the visual defect. It is interesting indeed to note that in the

first group (61.5%) loss of vision was the only complaint. Kagawa calls this 'a latent type of beriberi', its most significant sign being absence of the ankle jerks (47%). This observation, if correct, is a very important one, suggesting for one thing that the optic nerve might be especially affected by a low intake of thiamin: but even more important was Kagawa's claim to have cured the visual defect (if of recent origin) by administering thiamin.

Further evidence of the relationship between beriberi and optic atrophy is afforded by Hobbs and Forbes (1946). Writing of their wartime experiences, they described, in a group of 1470 men, 163 with partial optic atrophy. Of this number, 44 gave a description when giving their histories of having suffered from beriberi. In addition, 101 of the optic atrophy cases - including the majority of the beriberi types - developed their visual symptoms immediately after dysentery.

In 33 ex-Ps.W. which we ourselves saw during our duties in the Army*, 20 claimed to have had beriberi at the time of onset of the visual defect, and all of them suffered from chronic diarrhoea.

---

* As Captain (now Lieut.-Colonel) R.A.M.C.

Unfortunately, there is some confusion as to what constitutes the beriberi syndrome. The presence of dysaesthesiae of the lower limbs with or without oedema appears to be the criterion most generally adopted. It must be said, however, that some authors consider pain and numbness of the legs to be pellagrinous. In the human thiamin-deficient experiments of Williams and of Jolliffe mentioned earlier, although no syndrome characteristic of oriental beriberi was induced, neurological symptoms appeared, which subsided rapidly on treatment with thiamin. These symptoms, including tender calves, burning feet, cramps, and numbness and tingling below the knees, are identical with the symptoms experienced by British Ps.W. in the Far East.

According to Dee Shapland (1946a), neuritic beriberi of this type started early on after capture, oedematous beriberi later. The oedema, of course, might have occurred as a result of the deficiency in protein, but Warrack (1946) and Todd (1946) report that it cleared rapidly with intravenous or intramuscular thiamin injections.

The opportunity of providing evidence by therapeutic test of the implication of thiamin in nutritional amblyopia was, of course, never greater than in the Japanese prison camps. Nevertheless not many of these

tests were specific. Treatments naturally had to be of the "blunderbuss" variety, as the condition, unfamiliar though it was, was early on recognized by those ophthalmologists, themselves Ps.W., as being a serious one. Regrettable though this may seem, the decision to treat cases in this way was the only one. A few cases nevertheless are reported who were treated with thiamin alone by force of circumstance.

Houwer (1946), formerly chief ophthalmic surgeon in Batavia, was a P.W. for 3½ years. During this time, he saw many cases of nutritional amblyopia. His clinical descriptions are models of accuracy, and his observations and conclusions must rank very high in the literature on the subject. He noted that the patients nearly all experienced abnormal sensations of feet, legs, hands, and arms. Oedema of the legs was rare. When the visual defect was of recent origin, and when thiamin was available, Houwer administered it liberally in its pure form till the visual defect recovered. It always did recover, and so he concluded that "Camp Eye" (as the Dutch School called nutritional amblyopia) arose primarily as the result of a thiamin deficiency, although he recognized as contributory factors a deficiency in the other members of the B complex,

a high-carbohydrates diet, and the hardships which his patients had to endure. Here, then, appears to be irrefutable evidence.

These views are given support by Warrack, who states that 25% of Officer Ps.W. in a Hong Kong camp suffered from defective vision. Unable to quote actual figures, he remembers, however, that injections of thiamin hydrochloride at the early stage arrested or markedly improved the condition. A significant remark in view of the relationship of thiamin to carbohydrate metabolism is expressed by Claffy, reported by Dee Shapland (1946b) that when the rice ration in the O.Rs. camp in Hong Kong was (later) halved, the incidence of amblyopia dropped almost to nil of its own accord.

This was not the experience of Hazelton (1946), like Claffy an Australian ophthalmologist, and a P.W. in Singapore. Despite the administration of thiamin hydrochloride at the level of 1,000 i.u. intramuscularly daily for 14 days, the visual defect remained unchanged. As a therapeutic test, however, this treatment can scarcely be said to have been adequate.

The most important evidence apparently excluding thiamin as a possible factor in nutritional amblyopia is supplied by Moore (1939, 1940). In

describing a syndrome, first observed in Nigeria by Stannus (1911), and the main symptoms of which are apathy, a sore tongue, angular stomatitis, and a peculiar dry scaly rash of the genitalia, he states that it is usually accompanied by defective central vision, which if untreated becomes permanent. This loss reveals itself clinically as a retrobulbar or optic neuritis to be followed by a partial optic atrophy. In short, here is the characteristic description of nutritional amblyopia.

In a large series of such cases (171), he went on to show how both autoclaved yeast, and autoclaved Marmite were effective in restoring vision, especially when administered early on. He considers as a result that this condition is due to a (Heat-stable) B complex deficiency. If there is some confusion as to which member of the B complex is responsible, there is none, however, over the question whether or not it is thiamin.

In conclusion, it is interesting to note that Bell and O'Neill (1947), after carefully investigating 95 cases of nutritional optic atrophy, suggest that certain optic nerves are more susceptible biologically or congenitally, to the vitamin deficiency, whatever

its nature may be. They would not commit themselves beyond this point, however, as they found no significant relationship in their series between the visual defect and any particular vitamin.

Such, then, briefly are the more important - of the few - references to the subject under discussion. The evidence is no more conclusive than we found the experimental to be. It is known, nevertheless, that a number of cases of nutritional optic neuropathy treated with pure thiamin have been cured. The failures, it would seem, may be explained on the grounds of inadequacy of treatment.

Certain optic nerves, moreover, appear to be more susceptible than others, although the discrepancy in the incidence of amblyopia among men enduring identical conditions of hardship and malnutrition may be due to a variation in the degree of symbiotic vitamin resynthesis in different individuals.

The one serious argument against the implication of thiamin in the disease lies in the work of Moore. Here we are dealing apparently with the same type of amblyopia as was experienced (later) in the Japanese prison camps: and yet the associated symptoms differ somewhat in the two. It may well be, then, we

are dealing with differing although allied syndromes.

It is a pity, however, that Moore did not attempt a cure with the heat-labile member* - nor does he state whether he permitted his cases to continue on their maladjusted diets, the cause of their malady, or (as would seem probable in a District Medical Officer) advised them - or the local authorities - how to select a better balanced diet rich in all the vitamins. Without these assurances, one hesitates to accept his conclusions unconditionally.

---

* Moore did attempt to make a more precise investigation before being forced to give up his work as a result of illness. Three patients were treated at Lagos with nicotinic acid. The results were inconclusive.

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PART VI.

NUTRITIONAL AMBLYOPIA : A STATISTICAL REPORT OF THE COURSE AND PROGRESS OF 238 CASES

The subjects for this research were all ex-Far East Ps.W. As their visual histories dated from 1939 up to the present day, a period of over 10 years is thereby covered.

Whatever the cause of this strange ailment may be - and there may be more than one explanation of it - prior to the last war very few cases were on record to help understand it. A different state of affairs exists now. A wealth of literature has accumulated, some written by those on the spot, in the prison camps, some by those who, like the author, investigated the subjects soon after their release.

While it may be argued that we now know all that is to be known about the clinical manifestations of this disease, the fact remains that few authors agree in every particular. An attempt to clarify the clinical picture should in itself, then, be worth while. The further opportunity of surveying the records of so many Europeans with starvation amblyopia over a ten-year period may of course never happen again: it is, as far as we know, unique in the history of ophthalmology.

Such a study, therefore, as is planned here, should yield some new information - particularly with regard to the progress of nutritional amblyopia - and at the same time put the condition generally into better perspective.

Perhaps the lack of subjective detail in those papers written prior to 1940 (Stannus, 1911; Scott, 1918; Moore, 1939) was due to the illiteracy of the subjects. In our cases this does not apply. Just as important as literacy however are the physical and psychological states of the individuals at the time of examination. In 33 cases of which we had personal experience, these factors were carefully considered. We cannot say whether they were considered by other ophthalmic surgeons. Here and there a note is interpolated suggesting it was. Very few ophthalmologists, however, can afford to neglect these points when assessing the degree of disablement, so that in a series as large as the present one, we feel reasonably certain that the records are for the greater part valid. We have not hesitated to express doubts, nevertheless, when we felt there were reasonable grounds for suspecting the integrity of the subjective findings.

Cookhouse Dietary, Stanley P.W. Camp Hong Kong, 1942-45*

1st Year				2nd Year	3rd Year
Commodity	Average Ration in gms.	Frequency	Remarks		
Rice (occasionally replaced by old white flour)	400	daily	No red rice avail. Poor quality 'coolie' rice, mouldy & full of maggots.	The same; but rice only.	300 gms. rice only.
Meat (pork, horse or bullock)	120	Once a week.	fresh	Once a month.	None
Fish	120	Once or twice a week.	fresh	Twice a week.	Frozen fish only.
Vegetables (spinnach, beet, lettuce, or white cabbage)	60	daily	Green leaves used as well as roots. Fresh.	less frequent but sweet potatoes & pumpkins now available.	As in 1st year. Chrysanth leaves & pressed seaweed occasion.
Soya bean curd	400	Monthly	Bought in canteen (if able).	3 times a year.	1-2 yearly.
Oil (peanut, bean or white cotton seed)	120	Every 10 days.	Bought in canteen.	60-120 every 20 days.	None
Rock salt	various	Every 10 days.	Bought in canteen.	The same.	Rarely
Eggs	None	None	None	1-3 a week late in the year.	The same

TABLE I.

* In addition, 6½ Red Cross parcels were received by each man during his captivity. Two pounds of tea obtained from this source lasted the entire period.

Factors precipitating nutritional amblyopia

The conditions leading to the visual defect are too well known to bear much repetition here. Markowitz (1946) gives an unusually vivid account of them. As one of our patients throughout his period of internment had been in charge of a P.W. camp cookhouse in Hong Kong* we append a detailed dietary to remind the reader quickly of the background (Table I).

The diets varied somewhat in each camp, but every diet was dominantly carbohydrate, and deficient especially in the water soluble vitamins. As the thiamin : non-fat calorie ratio was frequently as low as 0.17, the deficiency in thiamin mattered all the more. The total daily caloric intake averaged around 1,800, only about 50 of which were derived from fat and protein. It is not surprising that under such conditions the survivors were found to have lost from 2-5 stones in weight at the end of 3½ years.

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* Mr. J.W. Hudson, Chief Prison Officer in Hong Kong Jail. We are indebted to his enthusiastic co-operation in compiling this dietary.

Incidence of Beriberi associated with the onset of Nutritional Amblyopia

a. Incidence by Camps

	Cases	Cases associated with beriberi	%
Singapore	118	54	45.8
Hong Kong	46	25	54.3
Islands	74	32	43.2
Total	238	111	46.6

A x^2 test gives $x^2 = 0.79$ with 2 D.F., which is definitely not significant. Therefore, there is no evidence that the incidence varied from camp to camp.

b. Comparison with general incidence of Beriberi (8.7%)

The following test shows that the incidence of Beriberi associated with the onset of Nutritional Amblyopia is very significantly greater than the total incidence of beriberi to be expected among 238 Ps.W.

Cases of Nutritional Amblyopia	238
Cases associated with beriberi	111
Expectation	20.706

therefore $x^2 = 393.75$ with 1 D.F.

TABLE II.

Most of the Ps.W. suffered from a variety of ailments, malaria, dysentery, and beriberi*. According to the 238 cases we surveyed statistically**, a very close relationship exists between the latter disease and the visual defect. This is markedly illustrated by the fact that the proportion of amblyopia cases in which the onset of the defect was closely associated with beriberi is significantly much higher than is the total incidence of beriberi among all the repatriated Ps.W. (Table II,b).

The total incidence of nutritional amblyopia in those who were repatriated from the Far East was 6.8%. No figures are available giving the incidence of those who recovered during emprisonment or of those suffering from it who died before repatriation.

The 238 repatriated survivors came from three regions, Singapore, Hong Kong, and various camps, dominantly "islands" (Ambon, Java, Borneo, Formosa, Saigon, etc.). There is no statistically

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* The probability of a mistaken diagnosis in the case of beriberi is discussed on page 170.

** Without the helpful guidance of Mr.J.M.Runcie, we would have found the statistical problems very much harder.

Incidence of Nutritional Amblyopia by Camps

	Singapore	Hong Kong	Islands	Total
Ps.W.	32,124	2,299	4,613	39,036
Cases observed	118	46	74	238
Cases expected	195.9	14.0	28.1	238
x^2	30.98	73.14	78.98	179.10

($x^2 = 179.10$ with 2 D.F. which is very highly significant. The expected number of cases is calculated from the total Ps.W. and the average incidence in each camp. The Null hypothesis is that the incidence is the same in all camps.)

TABLE III.

significant difference in the incidence between the latter two (Hong Kong and the islands), but there is a very highly significant discrepancy between them and Singapore, where the incidence is much lower (Table III). We cannot be certain of the explanation of this.

Such, then, briefly, is the background. We shall now describe and discuss the course and progress of the disease, having arbitrarily sub-divided it into 3 stages, acute, subacute, and chronic. It may be argued that the latter corresponds to a period when sequelae are observed. We hope to show, however, that this is not necessarily the case.

1. The Acute Stage

The present writer never had the opportunity of observing the disease in its acute stage. Houwer (1946) and Hazelton (1946) are two ex-Ps.W. authors giving good descriptions of it: and there are many others, among which we prefer the accounts of Churchill (1945), Spillane and Scott (1945), Ridley (1945), Claffy (1946), and Bloom, Merz and Taylor (1946). From such contemporary records,

Average characteristics of Age and Time to onset*

Camp	Age on Capture		Time to onset (months)	
	Cases	Average	Cases	Average
Singapore	114	26.8	103	11.4
Hong Kong	45	27.0	42	10.7
Islands	72	28.	66	11.1
All Camps	231	27.4	211	11.2

TABLE IV.

* The numbers of cases vary from characteristic to characteristic throughout owing to missing data.

by the careful questioning of our own 33 subjects (personal series), and from the records of the 238 cases (major series) a fairly accurate picture may be formed.

(a) The onset of the visual defect

The time of onset of the amblyopia varies considerably, as at first sight does the mode of onset.

Some authors state it ensues after 3 months, others after 3 years: in our major series the average was 11.2 months (Table IV).

In similar fashion, some authors say the onset was acute, others insidious. Houwer writes "... at a certain day the patients (they all had the same story) perceived that they could not recognize persons at a moderate distance because smaller or larger parts of the face had 'dropped out'. The complaint got rapidly worse, and then after 1 or 2 weeks became stationary". Bloom on the other hand says "... blurring of vision began insidiously, described by most of the patients as an intermittent fading. This process was gradual and went unnoticed by many". This was a common history

The relationship between Time to Onset and Age

T* / Age	0-	5-	10-	15-	20 & over	Totals
19-	3	15	35	4	1	58
24-	-	18	47	7	2	74
29-	-	17	27	3	3	50
34-	2	6	11	-	1	20
39 & over	-	3	4	-	-	7
Totals	5	59	124	14	7	209

TABLE V.

* Time to onset in months.

with us also. Most of our major-series subjects described prodromata of a similar nature; retrobulbar pain, frontal headache, lacrimation, photophobia, heaviness in the lids, a burning sensation in the eyes, intermittent dimming of vision, especially when reading, and teichopsia.

Hazelton deserves much credit for recording that he was able to cure these symptoms with thiamin, with the exception of the teichopsia, and the central acuity defect. Divorced thus from the amblyopia, it seems very likely that the insidious blurring of vision is evidence only of ciliary fatigue, and that the onset of the amblyopia is sudden, being preceded a few weeks only, by retinal teichopsia.

In that respect, the condition would correspond to the toxic amblyopias.

We found no significant relationship between the time to onset and the age of the subject (Table V), perhaps because the range of age was too limited.

(b) The visual acuity and fields

The visual acuity in the untreated acute case is invariably markedly depressed, the defects

ranging from 6/24 to 5/60 or less. After some weeks the defect becomes stable. The disease may now be said to have entered into its subacute phase. In many instances, of course, it was not possible to record the acuity, test charts being unavailable, or of an inaccurate make-shift nature.

The same lack of equipment handicapped the P.W. ophthalmologist when it came to plotting the visual fields. Nevertheless, Houwer states that central or paracentral scotomata were the rule. Bloom describes in addition peripheral constriction: this may have resulted of course from the difficulty in fixation, and have no special meaning. That there was a central defect is supported by the almost constantly reported improvement in acuity by night. The scotopic mechanism seldom seems to have been affected. Night blindness is reported in only 1 of our major series. This is surprising in view of the low intake of β-carotene at some of the camps.

Unfortunately none of our major-series cases has any record of the field defects at the outset of the malady, otherwise the nature of the lesion might have been elicited by observing the subsequent behaviour.

If, however, Houwer is correct in stating that nearly all the scotomata were connected with the blind spot, in the circumstances under which the condition occurred such a defect would in itself suggest a toxic lesion affecting the ganglion cells, or their processes, between the foveal and caecal regions. The first result of such a lesion would be an impaired conductivity leading to a diminution in the perception of colours. Houwer had no means of ascertaining whether this existed or not, but Hazelton found that his patients confused greens with blues, and, as do Claffy and Bloom, reports disproportion between the red and white fields. Such defective perception does not of course determine the precise cause of the conduction impairment, but it helps us to interpret the pathology. Haemorrhages or oedema of the nerve head (as we shall hear shortly) have both been observed, and instanced by some authors (Dekking, 1946) as the probable cause of the defective acuity. By themselves, however, retinal oedema and haemorrhage do not influence

conduction, or there would be no good vision ever present in papilloedema. The defect, therefore, on the evidence of the centrocaecal shape of the scotomata and the impaired conductivity, would appear to be due to a primary lesion of the ganglion cells, or the intraretinal portion of the optic nerve.

Spillane (1947) also found several centrocaecal scotomata in cases occurring under less severe conditions in the Middle East. He supplies valuable detailed information as to their size and shape (Plate II). It would appear that in the initial stages the central scotomata were larger than in the later (10^{o} as against 5^{o}). This alteration in the shape is in keeping with the belief that the visual defect is the expression of a defective nutrition involving especially the central regions*. In the end, in face of the prolongation of adverse conditions, it is the more highly developed ganglion cells at the macula which perish. The others recover. Such a pathology would explain adequately why so few centrocaecal scotomata persisted after repatriation.

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* By 'defective nutrition' we do not intend to infer the lesion is haemorrhagic.

(c) The fundal appearances

Ophthalmoscopes and/or batteries were rare in the P.W. camps, and as a result reports of the fundal appearances in the acute stage are meagre.

Most observors describe papillary hyperaemia only. Houwer notes the presence of a slight macular granularity, and in some, even the young, paramacular exudates, corresponding to drusen. In a few, there were retinal haemorrhages, lying in the inner layers. Dumoulin (1946) and Churchill were exceptional in describing an abnormally red retina with small macular exudates, a hyperaemic oedematous papilla, and tortuous dilated vessels. One wonders if gross lesions like these would have been found in the absence of the many types of complicating systemic disease. They appear somewhat out of character. Cases in the Middle East, seldom complicated by uncontrolled fevers or dysenteries, never exhibited them. Zimmer and his colleagues (1944), Spillane and Scott, and many others state the fundi

they saw were normal, or at the most exhibited a mild hyperaemia of the disc. In our opinion a fundus of this kind is more in keeping with the nature of the condition. It would appear in all probability the characteristic picture in the acute stage of an uncomplicated nutritional amblyopia.

2. The Subacute Stage

This stage corresponded in point of time to the remaining months of emprisonment. A period of tolerance appears to have set in, which was maintained up to the time of the first examination on release, despite the fact that conditions became progressively worse. As it was at the end of this phase that really effective ocular examinations were made, we are much more familiar with the clinical picture. It was at this time that we personally obtained our first sight of the disease, and that the visual histories of the major-series cases restarted.

We must stress at this point that the descriptions which follow refer only to those cases which did not undergo spontaneous remission, nor benefit to any marked extent from treatment, nor

Survey of the Visual Acuity in 33 cases of Nutritional Amblyopia over a 10year period

	V.A.before Capture 1939	V.A. on Release 1945	V.A.after 1 recovery-year 1946	V.A.after 5 recovery-years 1950
1	6/6 -6/6	6/36-6/36	6/36-6/36	6/36-6/36
2	6/6 -6/6	6/60-6/36	6/60-6/36	3/60-3/60
3	6/6 -6/6	6/18-6/9	6/60-6/18	6/60-6/18
4	6/6 -6/6	6/60-6/36	6/60-6/36	6/24-6/24
5	6/9 -6/9	6/36-6/36	6/36-6/36	6/60-6/60
6	6/6 -6/6*	6/36-6/60	6/36-6/36	6/18-6/18
7	6/5 -6/36	6/60-6/60	6/60-6/60	6/36-6/60
8	6/6 -6/6	6/24-4/60	6/36-5/60	6/60-6/60
9	6/6 -6/6	6/60-6/60	6/60-6/60	6/60-6/60
10	6/6 -6/6	6/24-6/24	6/24-6/24	6/24-6/24
11	6/6 -6/6	6/12-6/9	6/12-6/9	6/12-6/9
12	6/6 -6/6	6/36-6/36	6/36-6/36	6/24-6/18
13	6/6 -6/6	6/60-6/18	6/60-6/18	6/60-6/36
14	6/6 -6/6	6/36-6/36	6/36-6/36	6/36-6/36
15	6/6 -6/6	6/36-6/36	6/36-6/36	6/60-6/36
16	6/9 -6/9	6/60-6/60	6/60-6/60	3/60-6/24
17	6/6 -6/9	6/36-6/60	6/36-6/60	6/24-6/36
18	6/6 -6/6	6/36-6/36	6/36-6/36	6/24-6/18
19	6/6 -6/6	4/60-4/60	2/60-2/60	1/60-1/60
20	6/9 -6/6	6/24-6/36	6/24-6/36	6/18-6/18
21	6/6 -6/6	5/60-5/60	5/60-5/60	No PL
22	6/6 -6/6*	6/18-6/18	6/18-6/12	6/12-6/12
23	6/12-6/9	6/36-3/60	6/60-3/60	3/60-3/60
24	6/6 -6/6	6/36-6/36	6/36-6/36	6/60-3/60
25	6/6 -6/6*	6/12-6/12	6/24-6/24	6/36-6/36
26	6/6 -6/6	6/18-6/18	6/12-6/18	6/12-6/24
27	6/6 -6/6	6/18-6/24	6/18-6/24	6/18-6/24
28	6/9 -6/6	6/24-6/36	6/24-6/36	6/24-6/36
29	6/6 -6/6*	6/60-6/60	6/24-6/24	6/12-6/12
30	6/6 -6/6	6/24-6/24	6/60-6/60	3/60-3/60
31	6/6 -6/6	6/24-6/18	6/24-6/24	6/24-6/36
32	6/6 -6/6	6/24-6/36	6/24-6/24	6/18-6/18

changes in the degree of enforced labour, prior to their release. They may in short be a self-selected sample.

It is interesting to note in this connection that amid the increased hardships of slave-labour on the Mulmein Railway, and with a grossly reduced rice intake, many of the nutritional amblyopias underwent permanent remission or improved considerably (Reid and Gibson, 1947). According to Dee Shapland (1946), Claffy with a good supply of multivite tablets cured many of his cases.

(a) The visual acuity

Some measure of the state of the acuity on release may be obtained by consulting Table VI. These figures are taken from our personal-series records. They correspond closely to what we found in the major series, ranging from 6/12 to 2/60.

Houwer suggests that the severity of the affliction becomes greater with increasing age. We found no statistically significant relationship, however, between the age of the subjects and the degree of the defect (Table VII over page).

The relationship between Age and the severity of the visual defect

VAR* / Age	0-	10-	20-	30-	40-	50-	60 & over	Totals
19-	1	19	26	8	2	2	1	59
24-	10	35	24	6	2	2	5	84
29-	3	12	21	11	4	1	3	55
34-	2	8	6	2	1	-	2	21
39-	1	3	4	-	-	-	-	8
Totals	17	77	81	27	9	5	11	227

TABLE VII.

* Visual acuity on release

As Table VII is the first of several correlating visual acuity to various factors, it is necessary to explain our system of acuity scoring. To simplify the work, a combined binocular index has been used. The Snellen fraction for each eye is converted to a common denominator of 60, and the two numerators then added. The index is thus a score out of 120, e.g. R.6/6 L.6/6 = 60/60 + 60/60 = 120; R.6/24 L.5/60 = 15/60 + 5/60 = 20.

Classification of Field Defects in 238 cases of Nutritional Amblyopia

1. Bilateral and equal	
Pericentral (i.e. central) Scotomata	151
Paracentral Scotomata	23
Centrocaecal Scotomata	4
Peripheral Contraction*	9
2. Unilateral	
Pericentral Scotomata	13
Paracentral Scotomata	8
Centrocaecal Scotomata	0
Peripheral Contraction	0
3. Atypical (See Table IX)	19
4. No Record	11

TABLE VIII.

* Except in 1 case, all associated with central defects.

This disagreement may be accounted for by the restricted range of age in the subjects studied by us (20-40). It seems reasonable to believe that Houwer's findings may hold good in the higher range of age of his subjects, who were civilian internees (40-60). Investigation over a very wide range might reveal a critical age beyond which the severity and incidence of the defect changes sharply.

(b) The visual fields

Table VIII lists the various field defects found on termination of the subacute stage.

Undoubtedly the characteristic defect, bilateral and equal, consisted of a pericentral scotoma absolute in nature, steep edged, never breaking through to the periphery, averaging up to 5 degrees in extent, and in shape approximately circular (63.4%). Centrocaecal scotomata surprisingly were very rare (1.6%) at this stage. We have already given an explanation of this finding.

The problem presented, therefore, at this time is one of explaining a static scotomatous field defect, frequently unassociated with any macular disturbance (as we shall see shortly); and of course it is

Classification of Atypical Fields* in 238 cases of Nutritional Amblyopia

Defect in Field	No.
Pericaecal Scotomata (i.e. enlargement of blind spot)	12
Insular Scotomata	2
Annular Scotomata	2
Homonymous Quadrantopic Scotomata	3
Total	19

TABLE IX.

* All associated with central defects, except 2 cases with pericaecal scotomata.

difficult to learn much from fields of such a nature other than the rather obvious fact that the lesions are usually infrachiasmal, and exert a peculiar selectivity for the ganglion cells, or their axis cylinders, in the central areas. We might, however, be able to gain further information by studying the atypical fields (Table IX).

Apart from 2 cases where the defect was not associated with central depression, enlargement of the blind spot would appear to be a valid finding. The remaining 10 cases suggest that at some time in the course of the disease there has been a local optic nerve lesion causing oedema of the nerve head, especially as the histories show that central vision in these cases was involved from the beginning. The observations of those authors who observed occasional blurring of the discs are as a result given added credence.

The annular defects (enclosing central scotomata) are more difficult to fit into the general picture. Such scotomata in the absence of myopia are generally due to local pressure as by a neoplasm. They might possibly arise if oedema in the nerve led

to a localised venous congestion and arterial ischaemia. In night blindness ring scotomata have been found, but their validity has not been thoroughly established. Moreover, neither of these cases suffered from night blindness. Multiple concentric rings have been described as functional in nature, but we have no knowledge of bilateral solitary rings being so categorised.

The 2 homonymous quandrantopic and the 2 insular scotomata are extremely important. In our opinion their presence favours the belief that the main site of choice of the lesion does not necessarily lie within the retina. They suggest that in nutritional amblyopia there may be a patchy degeneration anywhere in the visual path, similar to what we know occurs in multiple sclerosis. That there was no sparing of the macula suggests the lesions are probably subgeniculate.

The peripheral contraction, as we suggested earlier, has probably no significance in view of the difficulty in fixation. It was never associated with the higher degrees of optic atrophy. Only 1 case was not associated with a central defect. Perhaps if a visual angle smaller than the one used (3/2000) had been presented, the central scotoma,

typical of the rest, would have been revealed in this instance too.

(c) The fundal appearances

Ophthalmoscopic examination reveals characteristically bitemporal pallor. This is variously reported as being 'probable', or 'marked', or 'within normal limits', by different observors. Our own impressions are echoed in an unpublished report by Somerville-Large, who wrote "the pallor is never very definite, and might almost be considered within normal limits, although I consider it to be pathological. This pallor of the discs is the temporal pallor that is such a rare clinical entity, although so commonly described by the physician armed with an electric ophthalmoscope"*.

As the complaint is always bilateral, it is extremely difficult of course to be certain that temporal pallor exists at this time. It is interesting to consider what its cause may be. In a previous paper (Rodger, 1943) several conjectures were made in this connection in the case of the traumatic atrophies, a condition presenting an almost identical picture.

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* At that time Lieut.Col. L.B. Sommerville-Large, R.A.M.C Advisor in Ophthalmology, Central India, whose cases are included in the major series under discussion. His ebullient teaching during the war is gratefully acknowledged.

The pallor might be due, we suggested, to an accompanying gliosis of the nerve head, or to an atresia of the tiny nutrient papillary vessels leading to localised ischaemia.

The disc margin, however, is always remarkably well outlined, so it is unlikely that the colour change is due to the proliferation of glial tissue in this region. On the other hand, the disc capillaries were invariably seen to have disappeared in the worst-affected cases of our personal series. Whether this is the cause of the atrophy, or the effect, it is impossible to say. At any rate several of those exhibiting the most definite signs of pallor had the least upset in visual acuity, so that the degree of pallor bears no relationship whatsoever to the functional loss.

There is an even greater difference of opinion concerning the presence of macular changes in the subacute phase. Dekking (1947) found mottling of the macula in 20% of his cases, Moorees (1947) in 53%. This was not our experience. In none of our personal series of 33 cases did we observe any such change, although in 2 cases there was pigment scattered at the disc margin outwith the choroidal-ring region. One could have been inflammatory,

the other was associated with a high degree of unilateral myopia. In our major series, 19 cases exhibited macular changes. These on the whole corresponded to the changes described by Houwer in the acute stage of the disease, namely, a granularity in and around the macula with a few dot-like exudates interspersed here and there. All these cases were in the lower age groups.

An observation of ours made at this time has not been generally noted: the fundi are markedly anaemic, a factor which tends to negative the degree of pallor.

3. The Chronic Stage

This period begins after restoration of normal conditions of diet and environment. Reference may be made again to the state of the visual acuity at the end of this stage as detailed in Table VI.

(a) The visual acuity

A survey of the state of the visual defect at the end of the first and at the end of the fifth recovery year reveals the interesting information that the course of nutritional amblyopia is by no means ended on restitution of a full diet. The

Progress of Visual Acuity after 5 recovery years

Camps	DETERIORATED		STATIC		IMPROVED		Totals
	Cases	%	Cases	%	Cases	%	
Singapore	39	37.5	35	33.7	30	28.8	104
Hong Kong	18	46.2	14	35.9	7	17.9	39
Islands	23	33.8	20	29.4	25	36.8	68
Totals	80	37.9	69	32.7	62	29.4	211

TABLE X.

progress of the acuity is roughly in the proportions of one-third deterioration, one-third improvement, and one-third remaining stationary (Table X).

In the absence of objective evidence indicating a gliosis of the optic nerve head we must presume either that the retina is not involved or that some other process is at work. It might of course depend upon a biochemical factor, the worst possible treatment during the first important year of recovery being that afforded by an uncompromisingly full diet, but there is no proof of this. If the lesion is retrobulbar it is surprising that the atrophy is always seen to be primary in type. For these reasons, we consider that the probable explanation is that the condition (after release of the Ps.W.) enters into a chronic stage.

The age of the subject does not affect the course of these changes (Table XI over page): nor can we give a prognosis as to how the acuity is likely to change by considering its state at the end of the subacute phase (Table XII over two pages). One might think that the better the tolerance had been, the greater the likelihood of an improvement. This is not so. It seems

The relationship of Age to the progress of the Visual Acuity after 5 recovery-years

Progress* / Age	DETERIORATED			Static	IMPROVED		
	-20	-10 to -19	0 to -9		0 to 9	10 to 19	20
19-	2	5	9	19	8	8	5
24-	3	9	23	24	9	7	5
29-	1	7	8	18	6	3	5
34-	-	4	5	6	2	1	-
39 & over	1	1	2	2	-	3	-
Totals	7	26	47	69	25	22	15

TABLE XI.

* Measured by binocular index.

The relationship of Visual Acuity on Release to its subsequent progress

Progress*	DETERIORATED			Static	IMPROVED		
VAR**	-20	-10 to -19	0 to -9		0 to 9	10 to 19	20
0-	-	-	2	8	5	1	-
10-	-	4	25	23	11	5	2
20-	1	8	18	22	6	14	4
30-	1	10	2	9	1	1	2
40-	1	2	-	1	2	-	4
50-	1	1	-	1	-	-	2
60 & over	3	1	-	5	-	1	1
Totals	7	26	47	69	25	22	15

TABLE XII.

* Measured by binocular index.

** Visual Acuity on release.

the prognosis must remain as indeterminate as is that of the toxic amblyopias when the toxin has been removed. Traquair (1946) notes how many of these toxic cases show little or no recovery, being frequently succeeded later on by a second and permanent loss of vision. Nutritional amblyopia, we now can see, corresponds exactly.

(b) The visual fields

The importance of following up the behaviour of field changes has already been pointed out, as such changes may provide valuable information indicating the nature of the lesion.

At the outset, and despite the disappointing inexactitude of many of the later perimetrical examinations, we can say that there are remarkably few changes in the fields despite the extreme changes which occurred in the visual acuity.

In 13 of the major series there were records revealing that the scotoma completely, or partially, cleared. These improvements, however, bore no relationship to the acuity. This is of course not surprising. A scotoma may partially clear, and yet the vital foveal area be still affected: nor is there any reason to expect an improved acuity

simply because a dense nucleus becomes less dense. It is still a scotoma. The behaviour of these 13 cases does not help us.

The fact, however, that so many did not change is suggestive. Almost certainly no inflammatory element exists, so that the frequently applied term "retrobulbar neuritis" is a misnomer. The lack of change also suggests that the disease is probably not vascular in nature, and that the changes in acuity are unlikely to be due to glial proliferation in the region of the nerve head.

This leaves us pretty well with only one case to make, and that is that the vitality of the affected nerve cell or fibre (which must be extremely high) persists at a reduced level over a period of several years, until finally it recovers in whole or in part, or dies*.

Such a view favours the belief that the causative agent is a toxin, a view held by Dee Shapland (1946) who considers that the degree of impairment varies directly with the concentration of an endogenous toxin in the blood.

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* This will be discussed further in the concluding part in the light of our experimental findings.

(c) The fundal appearances

The chronic phase is characterised ophthalmoscopically by the gradual development of a very definite clear-cut partial or complete optic atrophy. Where doubt existed as to the presence of pallor previously, it now no longer exists. Every one of our personal series exhibited such atrophy. This is the only sign, even as central amblyopia is the only symptom, in the chronic stage*.

We are, therefore, faced once again with the problem as to why the disc becomes white, as it is certain it bears as little relationship to the degree of functional loss as did the initial doubtful temporal pallor. Particularly hard is it to understand why 26 out of 238 cases progressed to total atrophy. Atrophy of the disc, of course, is only a subjective term, as it is possible for its appearance to be associated with a very minor defect in vision.

Two explanations suggest themselves. One is that some, not all, of the small disc vessels

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* Narrowing of the retinal arteries is not a feature of the fundal appearance. We observed it in only 1 of our personal series, and it is seldom mentioned (3 times) in the major series of 238 cases.

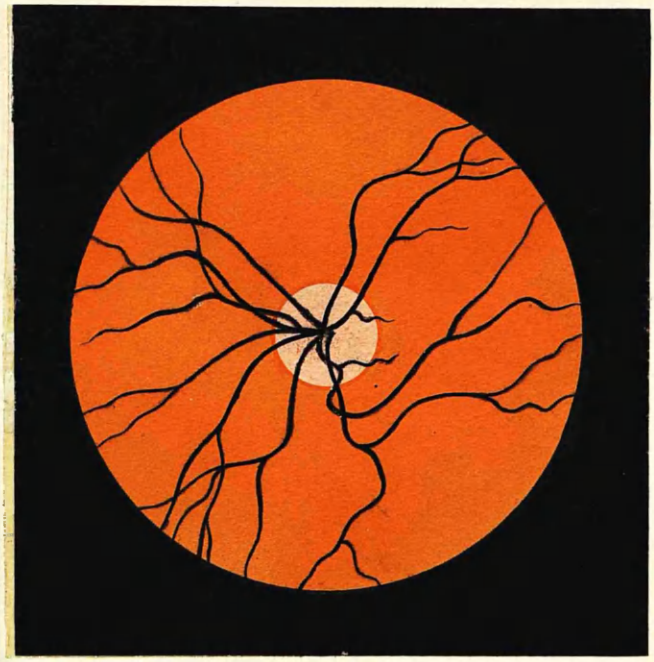
become compressed by a slowly developing astrocyte gliosis. It would not require very much in the way of cicatricial interference so to occlude them. It is well known that in an acute traumatic atrophy the disc vessels are conspicuous by their absence, and that gliosis is even more likely to occur in a chronic degeneration. These tiny vessels arise from two sources, the central artery, and the circle of Zinn. Those derived from the central artery are the more likely to be affected by a process originating within the optic nerve. The absence of the central branches would not necessarily lead to a complete ischaemia of the nerve head in view of the alternative circulation. Thus, such a pathology might very well explain the varied progress of the acuity, although, as previously suggested, the lack of change in the behaviour of the fields is rather against such a view.

The disc may or may not lose its pinkish colour in this way. The second explanation which we put forward, however, is less easy to revoke. The phenomenon could be a physiological one, based on the juxtaposition of an already white atrophic papilla (at the subacute stage) with the red fundus.

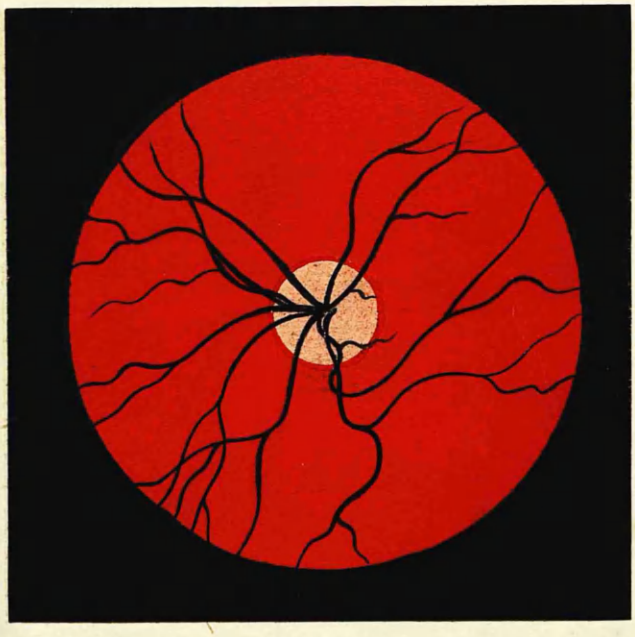
PLATE I

a. Representation of fundal appearance in a case of nutritional amblyopia (subacute stage). It is extremely anaemic.

b. The same fundus 3 years after repatriation. The optic disc is the same colour in each case, but by simultaneous contrast appears atrophic in this second picture, especially when viewed with the ophthalmoscope. The patient is no longer anaemic.



a.



b.

PLATE I

As the subject's general health improves in the terminal phase, so what was once a pale anaemic fundus improves in colour, thereby permitting a better contrast to be affected (Plate I, a and b)*. When viewed with the ophthalmoscope, the plates more effectively illustrate this phenomenon. Obvious though such a process may seem, it is one that is seldom taken into account in clinical descriptions.

* * * *

We have given a clinical description of nutritional amblyopia at some length, discussing the symptoms and signs as they arose. We realise, however, that the figures from which we formed our opinions may not be accurate. The Relative Sick Discharge rate on a first or second diagnosis of amblyopia (retrobulbar or optic neuritis, and optic atrophy) may be greater than 6.8%. If the sample for this reason is not exhaustive, then any conclusions based on totals and incidence rates will not be correct. In our opinion, nevertheless, the sample is sufficiently large to warrant the assumption that it is representative of all possible cases. The internal evidence (on which we concentrated

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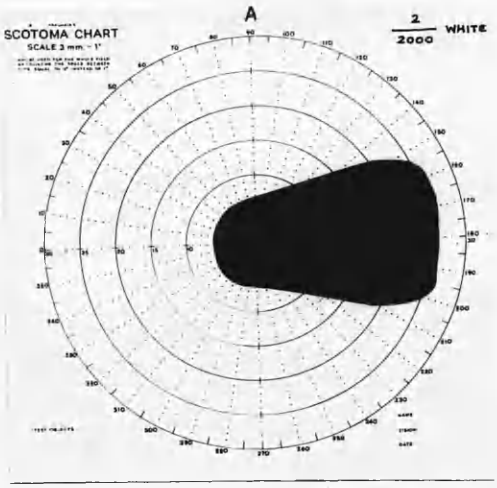
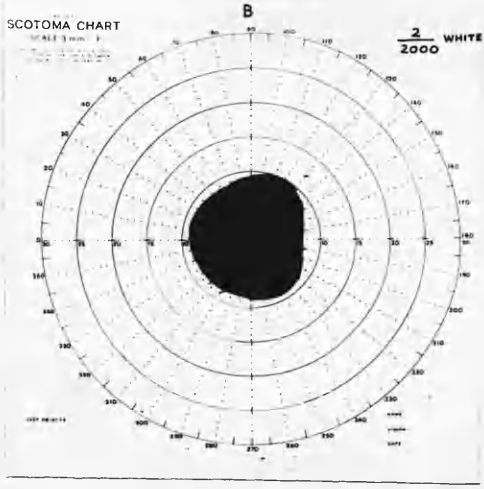
* We are grateful to Mr. D.P. Hammersley for these paintings.

our attentions) in that event should be reasonably accurate.

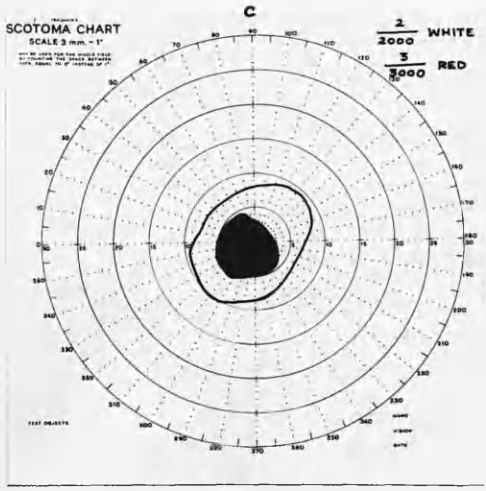
Scotomata found in Nutritional Amblyopia.



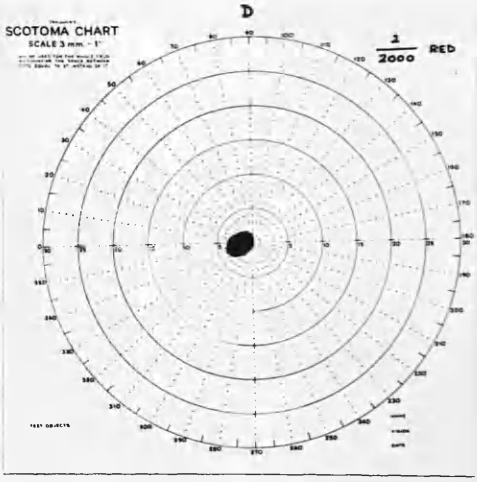
Centrocaecal (white)



Pericentral (white)



Paracentral (white) and pericentral (red)



Paracentral (white)

PLATE II

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PART VII.

A FINAL DISCUSSION IN WHICH ARE INCORPORATED PERSONAL OBSERVATIONS ON CERTAIN ALLIED CLINICAL CONDITIONS.

We have now made many experimental and clinical observations on the subject of our thesis, as well as critically reviewing a great deal of the relevant literature.

That there may be fallacies in an argument which applies to human beings conclusions formed as a result of animal experimentation has been underlined by more than one author. We shall not, then, unduly stress the experimental results we obtained. Even as reactions vary from species to species, so it may differ in man. It has been often suggested in fact that man is much less susceptible to dietary insufficiencies than are most of the lower mammals. This is a somewhat rash generalisation, however, for it depends upon which vitamin he is made deficient in. We have shown in the rat that a deficiency in vitamin C or vitamin K does not produce any ill effects, whereas in man such deficiencies do. There is every reason to suspect, however, that both are equally susceptible to a deficiency in the B complex, provided the diet favours such a deficiency.

In man, nevertheless, as in the rat, there is a considerable variation in the degree of susceptibility to a B complex deficiency. The divergent views as to the human requirements of the various members of the complex supports this belief. The usual explanation of such variations in susceptibility is that the resynthesis of the vitamins in the intestine varies. It is unwise to assume, however, that this is the prime factor. The intestinal flora of man do synthesize the vitamins: but that is not to say they are available to the host. As far as thiamin is concerned, it is possible that very little is absorbed in the large intestine; present in the body tissues as co-carboxylase, it must before absorption be dephosphorylated by enzymes which may not be present in this situation (Prescott, 1946). It will be dangerous, therefore, to draw any fierce conclusions until we learn just what vitamins, synthesized in man's intestine, are made available by absorption.

* * * *

Whether the above argument be true or false, beriberi and pellagra in temperate climates

secondary to disorders of the gastrointestinal tract have been described by more than one author (Simpson, 1935: Yudkin, Hawksley, and Drummond, 1938: Ungley, 1939). Similarly, the chronic alcoholic, imbibing most of his calories, suffers characteristically from a digestive disorder greatly interfering with absorption. The amblyopia of chronic alcoholism is as a result thought to be avitaminotic in origin (Carrol, 1936: Fine and Lachman, 1937). The condition in fact has been cured by the administration of large doses of thiamin, even although addiction was allowed to persist (Carroll, 1944).

Tobacco Amblyopia has not been shown thus to improve with thiamin administration. Contrary to the American point of view, this condition frequently occurs in the absence of heavy drinking. Whether tobacco interferes with the absorption, or the utilization, of any vitamin is still open to question. Recently we obtained some interesting data in this connection. Tobacco Amblyopia we found had been diagnosed 5 years previously in 2 out of 6 cases of optic atrophy associated with pernicious anaemia. We were encouraged as a result to

investigate 3 recent cases of **Tobacco Amblyopia** in the clinical laboratory*. Each of them had complete achlorhydria, although the blood pictures were normal. The field defects were characteristic of this condition (centrocaecal scotomata), and the macular areas exhibited the peculiar granularity found by Houwer in his "Camp Eye" cases. The discs were atrophic**.

The finding of achlorhydria, of course, need not suggest any more than that a diagnosis of Tobacco Amblyopia should never be made until a blood examination and test meal is performed, for the ocular syndrome (macular changes apart) in each case, Tobacco and P.A. Amblyopia, are identical (Turner, 1940).

It seems unlikely, however, that in these Achlorhydria-Tobacco cases we have stumbled upon early cases of Pernicious Anaemia, although according to Cohen (1936), amblyopia may be (rarely) the presenting sign in the latter disease.

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* For arranging the laboratory investigations and for his constant interest, we are grateful to Dr.C.C.Ungley Royal Victoria Infirmary, Newcastle/Tyne. Mr.V.Ingram very kindly collected the Tobacco Amblyopia cases for us, and gave us permission to use the Eye Department for our examinations.

** For details, see Appendix A.

It may be justifiably said that the age of these cases was of an order where histamine-fast achlorhydria is on the increase. In the circumstances, however, the association of it with Tobacco Amblyopia in every case examined must be ranked as a somewhat remarkable coincidence. We feel in short that this observation of ours supports the belief that Tobacco Amblyopia is a deficiency disease. Deficient absorption frequently results from achlorhydria, and Ungley (1938) has pointed out that it is believed to accentuate avitaminosis. This careful statement is as far as we can go, even today. Suggestive as these findings are, however, they will require further examination.

A second clinical condition believed to be related to thiamin deficiency, and caused by a gastrointestinal disorder is the amblyopia associated with Hyperemesis Gravidarum. It is an extremely rare condition, of course, to which only scant reference is made in modern obstetrical literature: but that it can and does occur there is no doubt (Ungley, 1938: Strauss, 1938: Laurent and Sinclair, 1938: Ballantyne, 1941).

It is interesting to find that in 4 out of 6 acute cases described by Ballantyne the fields

corresponded to those of Nutritional Amblyopia. Despite gross loss of vision at the height of the illness, Ballantyne notes that no complaint may be made by the patient. This encouraged us to investigate the ocular state of 16 cases (now recovered)*. We examined them with a view to finding some points of similarity to the Nutritional Amblyopia cases.

Only 1 of the 16 was over 30 years of age: and in only 1 had there been a complaint of defective vision during the pernicious vomiting. The most recent had been delivered six months previously.

To our surprise in 4 of the 16 cases there were small relative scotomata present. In 3, they were bilateral and central; in 1, homonymous and quadrantic. Two of these patients were low grade myopes (below -1.75 D.S.). Of the other two, 1 was slightly hypermetropic, the other emmetropic.

We could not be certain as to the presence of temporal pallor except in 1 case. We were certain, however, in all 4 subjects that there were

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* Our thanks are due to Mr. Linton Snaith, Newcastle General Hospital, for permitting us to examine these cases.

macular changes, which corresponded exactly with those found in our Achlorhydria-Tobacco cases. While we may have felt in the latter instance, that we were dealing with a central senile macular degeneration, in view of the age of the subjects now under review no such complication should be expected*.

We cannot draw even a broad conclusion from such a small series, but here surely is further suggestive evidence that a central degeneration (in 3 cases, in the optic nerve; in 1, within the tract) may arise as a result of defective intestinal absorption. In the hospital in which these 16 cases had been treated, the administration of vitamin B after admission has become the rule since the War, and has proved greatly beneficial. Wagener and Weir (1937), cured a case of Hyperemesis Amblyopia by administering vitamins B and C.

In view of what we have been saying, the importance of concurrent gastrointestinal disease in the P.W. nutritional amblyopias must be obvious.

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* For further details, see Appendix B,& Plate I.

Any interference with the absorption of the small amount of exogenous vitamin available would undoubtedly aggravate the deficiency, and perhaps precipitate the symptoms.

* * * *

This brings us to the question of which vitamins were deficient in the P.W. diets.

In the absence of night blindness and scurvy, we can assume that the supplies of A and C were adequate. There were no signs of a deficiency in D, which is not surprising as there was unending sunlight. The B complex, however, was markedly deficient. <u>There is in our opinion no doubt that we need only look for the cause of Nutritional Amblyopia in an insufficiency of one or other of the B group.</u> The question is which one.

Here we appear to be up against an insoluble problem. When we come to analyse the relationship between the B complex members and the various nutritional disorders of the nervous system we find there is a considerable overlap between the symptomatology of beriberi, pellagra, and ariboflavinosis. Almost certainly the nutritional amblyopia cases

suffered from each one of these diseases, although in many instances the symptoms may have been sub-clinical. The correlation of a particular disease (as we found in the case of beriberi) with the onset of amblyopia does not, therefore, really help us to decide which vitamin is responsible for the latter.

However, the realisation that the requirement of thiamin depends to a very large extent on the carbohydrate intake is of considerable value. If we are to protect the individual against the neurological manifestations designated as beriberi, the thiamin:non-fat calorie ratio must be very close to 0.30. In the nutritional amblyopia cases it was usually around 0.18. That the incidence of beriberi was not higher is in fact surprising. We might explain this of course on the grounds that beriberi was the most frequent cause of death, and that in many others who survived the symptoms were not severe enough to make the patient report sick. When, in addition to these considerations, however, we recall that many of the subjects recovered in Thailand, where the intake of rice was less, and the energy output more - factors diminishing the need for B_1 - the deficiency in thiamin comes to occupy

a highly significant position. This greatly influenced us in our choice of experiment.

* * * *

In considering the biochemical actions of the vitamins involved in beriberi, pellagra and ariboflavinosis, a most significant fact emerges. Thiamin, riboflavine, and nicotinic acid are all components of inter-related respiratory-enzyme systems.

It is well known from the experiments of Peters and his colleagues (1934 to 1940) that thiamin largely controls the metabolism of the cells of the central nervous system. It may well be that riboflavine and nicotinic acid also exert a direct influence in the same direction. That the nervous system is primarily involved in a thiamin deficiency is, of course, because thiamin is concerned with the metabolism of carbohydrate, which is exclusively used by the nervous system as its source of energy. It is doubtful however, whether a primary deficiency in thiamin can be made solely responsible in view of the possibility of inter-action between the various respiratory-enzyme vitamins. A characteristic

feature of the members of the B complex in fact is their capability of forming different enzyme systems with different specific proteins. Inter-action and interdependence between the three would appear then to be quite a probability.

Such a relationship is given support by the experiment we performed where both thiamin and riboflavine were excluded from the diet. The animals lived twice as long as the control group on a similar degree of thiamin deficiency unassociated with ariboflavinosis. From this it would seem that riboflavine deficiency diminishes the requirement for thiamin, although in the end a state of chronic thiamin deficiency ensues, as illustrated by the reduction in heart rate in the experimental animals of this group. Perhaps, as we have stated elsewhere, the metabolism is made to depend much more upon fat in the absence of riboflavine.

We have not unfortunately been able to obtain any literature in support of this observation of **ours**, but, if true, it would explain a number of things: why in the P.W. camps signs of thiamin deficiency were so seldom dramatic, the onset of tolerance, and the remission of symptoms when conditions

involving a reduction in carbohydrate and riboflavine became worse.

We must not expound further, however, on these biochemical possibilities, as it is a branch in which a great deal remains to be discovered. Theoretically, nevertheless, it appears probable that a deficiency in any one of the respiratory-enzyme vitamins may lead in the end to the death of the visual neurones, although to thiamin seems to obtain the greatest responsibility in maintaining their integrity. A deficiency in all three on the other hand may prolong the adverse conditions over a longer period at a less drastic level*.

* * * *

As an alternative explanation of the cause of nutritional amblyopia, it has been suggested that certain foodstuffs, containing an analogue destructive to thiamin, are responsible. There is apparently a fish diet factor which increases the need for thiamin

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* It may well be asked why we did not investigate a deficiency in nicotinic acid. Changes in the nervous systems of experimental animals made deficient in this vitamin, however, are much more equivocal than are those found either in aribo-flavinosis, or thiamin deficiency. We did not as a result digress further from our main theme.

in this manner, and we know fresh fish was supplied to the Ps.W. by the Japanese. This factor if present is not destroyed by grilling, which in Hong Kong was the popular method of cooking the fish. Later when the incidence of amblyopia began to fall, the Japanese forbade grilling and frying, and insisted all fish was boiled without giving any reason.

The work of Clark (1936) suggests another vector for an antivitamin. He states that pellagroid diseases are produced by the inhibition of the respiratory mechanisms of the body by cyanic glucosides, having extracted such substances from a variety of West African foodstuffs, including rice. Later, he produced the classical signs of thiamin deficiency in rats by feeding them on a high carbohydrate diet, adulterated with the crude cyanogenetic cereals.

Whether the presence of an antivitamin in the diet is the dominant factor in the aetiology of nutritional amblyopia, or not, we do not know, but it is a possibility, and further implicates thiamin. In Japan, for example, classical beriberi, which is very common, occurs predominantly among fishermen, who consume an abundant raw fish diet. When the

natural foodstuffs which constituted the P.W. dietary were lacking in the members of the B complex, they were absent collectively rather than singly. A specific antivitamin, such as the one just described might tilt the balance, therefore, and lead to a dominating deficiency in one factor only.

* * * *

An important point, relevant to nutritional amblyopia in man emerges from our experimental studies on a combined deficiency of thiamin and riboflavine, and that is the longer the duration of the deficiency, the greater the severity of the lesions. Of the two, the deficiency in thiamin appears the more likely to lead first to degeneration. Where both are deficient, as in the P.W. cases, it is probable that lesions due to lack of thiamin will precede those due to the other members of the B complex.

Zimmermann (1943), gives support to this belief of ours (argued from the experimental results) when he reminds us that the neuritic complications of human pellagra in its early stages are not influenced by nicotinic acid, although often responding to thiamin.

We can say, then, that in the P.W. camps, although there was a deficiency in all three of the respiratory-enzyme vitamins, and one of chronic duration, it is most likely that the ill effect of the thiamin deficiency would be felt first, and would dominate the picture thereafter.

* * * *

We come now to the question of where the anatomical site of the lesion may be. Here we run into difficulties, for it would appear that the experimental findings contradict the clinical. We have already discussed our reasons for concluding on clinical grounds that in man the lesion lies subchiasmally. In the rat on the other hand we have seen that the lesion consists of multiple foci of degeneration disseminated throughout the sub-geniculate pathway. Now, if applicable to man, this finding would explain a number of the anomalous features of the disease: the absence of clinical evidence that the nerve head, the nerve fibre layer, or the retinal ganglion cells are involved*, and the variable course, and progress of the disease.

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* Macular disturbance, as we have shown, has been described, (Houwer, Dekking, Moorees) but only by a few out of a score of observors.

We do not know of course whether the changes in experimental thiamin deficiency and in nutritional amblyopia are due to the lack of a vital nutritive substance, or to the toxic effect of an intermediate metabolic product*.

Demyelinisation, however, is likely to occur in either instance, and the presence of degenerated myelin is an exacting stimulus to the astrocytes, which respond characteristically by forming dense glial scars. These we found (in an early stage) in our chronic experimental animals: and they have been described in those cases of human nutritional amblyopia which have come to biopsy (Scott, 1918). Why, then, are there no signs of it on ophthalmoscopic examination? The answer must be that the primary lesion lies outwith the eyeball in the nerve proper. If we accept this point of view the variable progress of the visual acuity in the chronic stage is readily explained, much better than the theory we promulgated in Part VI can possibly do**. There can be no doubt that glial proliferation is the more reasonable of the two arguments.

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* In either case a state of relative tissue anoxia is bound to develop within the visual pathway.

** That slowly developing sequelae occurring in damaged nerve cells are responsible for the acuity changes.

The visual fields, however, do not suggest that the defect is one of disseminated foci of demyelinisation. We could argue, of course, that small scattered scotomata might easily be missed during the period of emprisonment. The equipment was poor, and it is reasonable to expect that in the dire circumstances under which the Ps.W. lived, they would not complain until central vision was affected. Dekking in fact describes the regular presence during the acute phase of such small scattered insular scotomata, although his observations have not been generally accepted to date. If these scotomata existed, however, they would be unlikely to disappear without leaving a trace, or be missed by so many observors at the examinations in this country after repatriation. Yet, very few insular or homonymous scotomata were found.

* * * *

As we are not of the opinion that lesions of the kind observed experimentally would recover in the tract, and persist in the nerve, we are forced to conclude that in man it is the nerve which is more usually affected.

Behr (1935), has previously pointed out that in the intracranial portion of the nerve the septal vessels are smaller, and supply a greater number of nerve fibres. We have not observed such differences in the optic nerve of the rat. In man, however, a defect in nutrition would be likely to affect the papillo macular fibres at this level more markedly than anywhere else. This anatomical factor, and the absence of ophthalmoscopic changes, causes us to believe that the lesions are generally to be found in the intracranial portion of the nerve.

The pigmentary disturbances at the macula, to which we have referred on more than one occasion, need not be evidence that the macula is primarily involved. Such regressive changes occur as secondary phenomena in senile macular degeneration subsequent to the sclerosis and obliteration of the choriocapillaris in the central retinal areas. In multiple sclerosis, moreover, thickening and hyalinization of the related arteries occurs in the chronic stage, although in the acute stage they are perfectly normal. There is no known explanation of these vascular changes, but in view of the fact that the retrobulbar neuropathy, described as nutritional amblyopia, presents a pathological picture

identical with the retrobulbar neuropathy of disseminated sclerosis, they may occur in the former condition also. Such an occurrence would explain the macular changes, and the ischaemia of the nerve head seen in the later stages of the disease.

Summary of Conclusions

1. We believe that in man a deficiency in exogenous thiamin will produce a state of relative anoxia within the visual pathway, the severity of which will vary according to the composition of the diet, and the degree of resynthesis and availability of endogenous thiamin.

2. As a result of the defective nutrition, the papillo-macular fibres in the intracranial portion of the optic nerve are most likely to suffer. Any part of the visual pathway, may, however, be affected, if the condition be severe enough, and of long duration.

3. The subsequent course of the defect will depend upon the extent of glial repair. There is no rule nor reason here, any more than there is in the wayward course taken by nutritional amblyopia.

4. In the rat, where the papillomacular fibres are less highly developed, and where the septal vessels appear more equally distributed throughout their course, the lesions are not so precisely localised, but may be found in any part of the visual pathway.

5. On continuation of the deficiency in thiamin, the small vessels at the nerve head, and foveal choriocapillaris, start to regress. As a result the nerve head becomes ischaemic, presenting the characteristic appearance of an optic atrophy, and the pigment epithelium responds by withdrawing its processes: subsequently it exhibits proliferation with a dispersal or heaping-up of the contained fuscin. Bruch's membrane may be wrinkled in the process; hence the occasional appearance of 'drusen' is not surprising.

6. The integrity of the visual pathway depends upon the maintenance of an adequate supply of the respiratory-enzyme vitamins, thiamin in particular.

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CASE HISTORIES

APPENDIX A.

Case 1: Mr. S. W. aged 59

Failing sight 4 weeks

R.V. 6/60 unimproved

L.V. 6/18

Holth positive

V.Fs: show large bilateral centrocaecal scotomata for white (2/2000).

O.E: scattered granularity in macular areas: a few paramacular exudates (drusen). Discs clear cut and pale. Blood vessels normal.

Blood: Reds 4.65 mills/c.mm.

Whites 6,600/c.mm.

Hb. 85%

P.C.V. 42%

M.C.V. 90.3 μ.ccs.

M.C.Hb. 27.1 μμg.

M.C.Hb.conc. 30%

Test Meal: Histamine-fast achlorhydria, complete after 120 minutes.

Case 2: Mr. N. H. aged 53

Failing sight 5 weeks

R.V. 1/60 unimproved

L.V. 4/6

Holth positive

V.Fs: show large bilateral centrocaecal scotomata for white (3/2000).

O.E: Tigroid fundi. Wide region (2.5 mms. diam.) around maculae exhibit characteristic granularity. Foveal reflexes large. No drusen. Discs not unduly pale. Blood-vessels normal.

Blood: Reds 5.50 mills/c.mm.

Whites 6,300/c.mm.

Hb. 78%

P.C.V. 40%

M.C.V. 72.7 μ.ccs.

M.C.Hb. 20.0 μμg.

M.C.Hb.concn. 28.8%

Test Meal: Histamine-fast achlorhydria, complete after 120 minutes.

Case 3: Mr. A. M. aged 59

Failing sight 5 weeks

R.V. 6/24 unimproved

L.V. 6/36

Holth positive

V.Fs: show moderately large bilateral centrocaecal scotomata for white (3/2000).

O.E: Macular granularity. No drusen.

Blood: Reds 4.30 mills/c.mm.

Whites 6,000/c.mm.

Hb. 82%

P.C.V. 45%

M.C.V. 70.5 μ.ccs.

M.C.Hb. 22.0 μμg.

M.C.Hb.concn. 30.0%

Test Meal: Histamine-fast achlorhydria, complete after 120 minutes.

APPENDIX B.

Case A: Mrs. B. aged 38

Admitted 2 years ago, severe hyperemesis. Did not notice D.V.

Present R.V. 6/6 without gl.

L.V. 6/6

V.Fs: show small bilateral central scotomata for red and white (2/2000).

O.E: Macular granularity. Discs normal. In L.E. appears to be small paramacular haemorrhage (old) at 12 o'clock.

Case B: Mrs. C. aged 28

Admitted $2\frac{1}{2}$ years ago, severe hyperemesis. On about 6 occasions remembers objects blurred. No residua.

Present R.V. 6/6 with 0.75 D.S. both eyes.

L.V. 6/6

V.Fs: show small bilateral central scotomata for red (2/2000).

O.E: Broadening of foveal reflex. Macular granularity. Bitemporal pallor.

Case C: Mrs. H. aged 27

Admitted 10 months ago, severe hyperemesis. Did not notice D.V. "Could not have cared less at the time".

Present R.V. 6/9 with -1.75 D.S. and -0.50 Cyl. at 80

L.V. 6/9 and -1.00 D.S. and -0.50 Cyl. at 160

V.Fs: show elongated bilateral central scotomata of moderate extent for red (2/2000).

O.E: No sign of myopic degeneration. Discs clear cut. Broadening of foveal reflex, and macular granularity. A few drusen.

Case D: Mrs. S. aged 26

Admitted 1½ years ago, severe hyperemesis. Did not notice D.V.

Present R.V. 6/6 with -0.25 D.S. and 1.00 Cyl. at 65

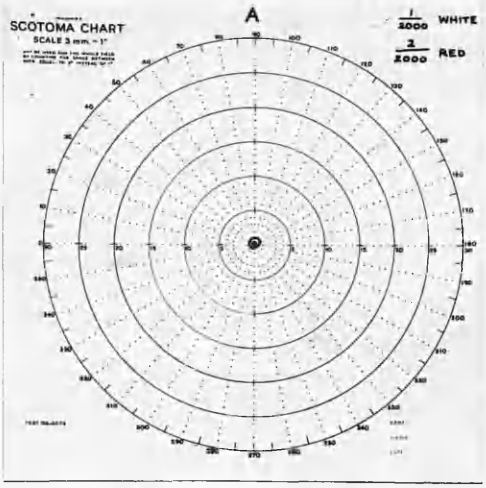
L.V. 6/5 and -0.25 D.S. and 1.25 Cyl. at 130

V.Fs: show small homonymous quadrantic scotomata for red (2/2000).

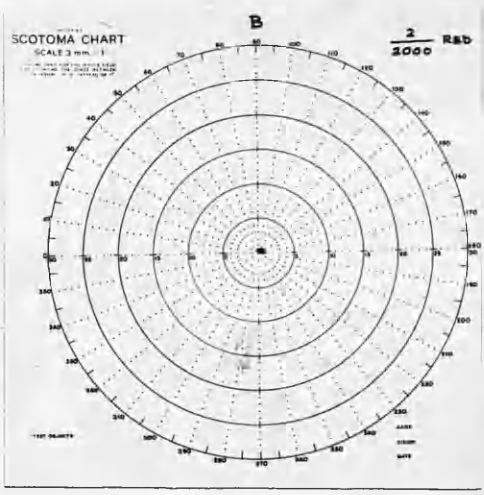
O.E: no abnormality.

APPENDIX B

Scotomata found in (old) Hyperemesis Gravidarum.



Pericentral



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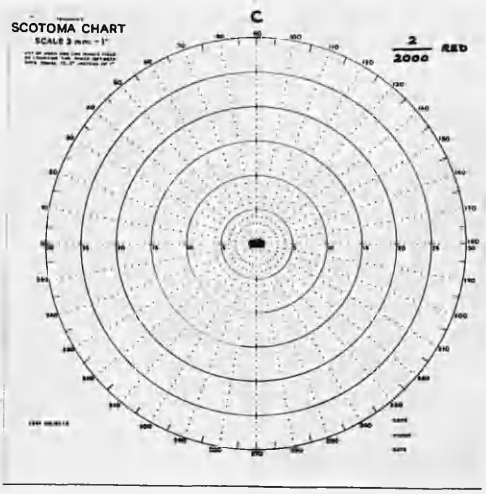
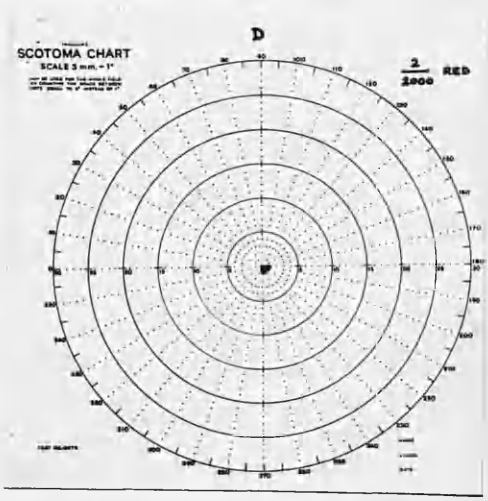


Pericentral



Homonymous Quadrantic

PLATE I

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* Not read in the original.