The experimental production and prevention of degeneration in the spinal cord / by Edward Mellanby.

THE EXPERIMENTAL PRODUCTION AND PREVENTION OF DEGENERATION IN THE SPINAL CORD.

BY EDWARD MELLANBY.

(Department of Pharmacology, University of Sheffield.)

In 1926, E. Mellanby described before the Physiological Society experimental work in which degenerative changes of nerves in the spinal cord of dogs were produced and prevented under certain dietetic conditions [1]. Since then developments of this work, especially in relation to ergot, have been from time to time described in lectures before medical and scientific bodies [2 and 3].[1] The investigations have made but slow progress and even now the subject is in an elementary stage. The experimental procedure as at present used is prolonged and the unravelling of the factors involved has necessitated the examination and the elimination of many food elements. A sufficient number of facts have, however, come to light to warrant their description, and it is hoped that this publication will hasten the further elucidation of a physiological and pathological problem which may have a wide bearing on nervous disease in man. Some of the experimental symptoms and lesions produced in the dogs, and the manner in which these are produced and prevented, are so reminiscent of nervous ergotism, pellagra and lathyrism, found in man, especially in times of dietetic restriction, that the experiments seem certain to throw light on the ætiology of these human illnesses. Their significance may, however, be of wider interest, for demyelination of central nervous system fibres is a characteristic change, although it is found in such varied diseases as pernicious anæmia, tabes dorsalis, disseminated sclerosis, general paralysis, post-vaccinal encephalitis and

[1] In addition to the occasions referred to, the facts mentioned below, except those concerning the action of carotene, were described before the Manchester Medical Society, October, 1928, and also in a series of lectures given at University College, London, in May, 1929.

cancer. The experimental work may also have some bearing on the causation and control of these conditions. A discussion on its possible significance will be given at the end of the report.

Since Eijkman's classical work published in 1897 [4] in which he produced polyneuritis gallinarum by means of diet, it has been recognized that the structure and function of peripheral nerves are controlled by a specific dietetic ingredient, namely vitamin B_1. Eijkman's discovery formed the starting point of that fruitful field of study which embraces the group of pathological conditions known as " deficiency diseases," a group which includes scurvy, rickets, infantile tetany, dental hypoplasia and caries, periodontal disease (pyorrhœa alveolaris), pellagra and probably certain types of bacterial and worm infection. Of course there are, in some of these at least, other factors at work than the specific deficiency, but generally speaking, the other factors are harmless provided there is or has been no specific dietetic deficiency. Evidence will be given in this paper to show that demyelination of spinal cord fibres is yet another instance which illustrates, among other things, the importance of specific dietetic factors in physiological development and function.

Apart from the work of Orr and Rows [5] who studied spinal cord degeneration produced by bacteria and bacterial toxins, experimental investigation on this aspect of the central nervous system has not been very fruitful. In 1916, Hart, Miller and McCollum [6] described changes in the anterior horn cells of pigs' cords produced by means of dieting. The changes described by these authors included shrinkage of the motor cells especially those of the ventro-medial and ventro-lateral columns, crowding of Nissl's granules, the near obliteration of nucleus and nucleolus, and degeneration of the processes. No changes in the conducting fibres of the spinal cord were described. The authors decided they were dealing with a form of beri-beri, not, however, due to a deficiency of vitamin B, which was present in abundance in the wheat germ portion of the diet. Indeed the pathological condition was ascribed to a toxic action of the wheat germ. By supplementing the diet with alfalfa meal and meat scraps the cell changes were prevented. They ascribed the protective effects of these foodstuffs to a combined action of additional proteins, fat-soluble vitamin and a better salt mixture, but did not consider that any one of these substances could in itself afford protection. As far as I know, they did not follow up this work.

More recently an account of an investigation bearing closely on the

present work has been published by Hughes, Lienhardt and Aubel [7], in which is described the experimental production of degenerated fibres in the spinal cord and peripheral nerves of animals by means of diet. Young pigs were fed from the time of weaning on white corn, tankage and bone ash and in six to ten months developed blindness, inco-ordination and spasms. On post-mortem examination degenerative changes were found by these workers in the spinal cord and in the optic, sciatic and femoral nerves. The addition of cod-liver oil, butter fat, yellow corn and alfalfa leaf meal prevented or relieved the condition, and thus the preventive factor was probably vitamin A.

Finally, there remain for reference some interesting results obtained by Castle. For an account of this work I am indebted to Dr. Castle and for the privilege of seeing his unpublished manuscript. He fed a number of dogs on diets deficient respectively in vitamins B_1 (anti-neuritic) and B_2 (pellagra-preventing), and some with a combined deficiency of both vitamins. In all the animals deprived of the anti-neuritic vitamin he found post mortem a diffuse, irregular loss of myelin chiefly in the white matter of the cord and some in the cerebral cortex. In nine out of these fourteen animals slight lesions of the peripheral nerves were also found. Early changes were found post mortem in the nervous system of four dogs fed on the diet deficient in vitamin B_2, but in life the animals did not show any significant neurological symptoms. It is of interest to note that the nervous symptoms in the animals deprived of vitamin B_1 could always be improved within a few hours by the administration of a source rich in this substance. This particular observation of Castle's is reminiscent of the rapid results seen in avian beri-beri following the administration of vitamin B_1 and suggests that the problem here is a similar one to that of avian beri-beri rather than to that of combined system disease seen in human beings.

The history of the present investigation dates back to the time when experiments were made on puppies with the object of determining the ætiology of rickets (E. Mellanby) [8]. The rachitic animals often developed great weakness, especially noticeable in the hind legs; the weakness was sometimes associated with spasticity. At first it was thought that the muscular weakness was one of the manifestations of rickets and this idea was supported by the fact that substances, such as cod-liver oil and milk, which cured or prevented rickets cured or prevented the muscular weakness. At a later stage in the work (1923) it was noticed that some of the experimental animals developed severe inco-ordination when they attempted to walk, and it was this inco-ordinated

gait which led to the examination of the spinal cord by histological methods. It was soon realized that there was more in the problem than rickets, for although some rachitic animals showed degenerative changes in the spinal cord, others did not. Indeed, there was no definite relationship between the pathological changes in the bones and the nervous system. Throughout much of the original work on rickets, which led to the establishment of the anti-rachitic vitamin, there was no clear differentiation between the anti-rachitic vitamin (vitamin D) and vitamin A, and this fact, as will be seen later, resulted in confusion of the explanation of the nerve degeneration results. Indeed, it was

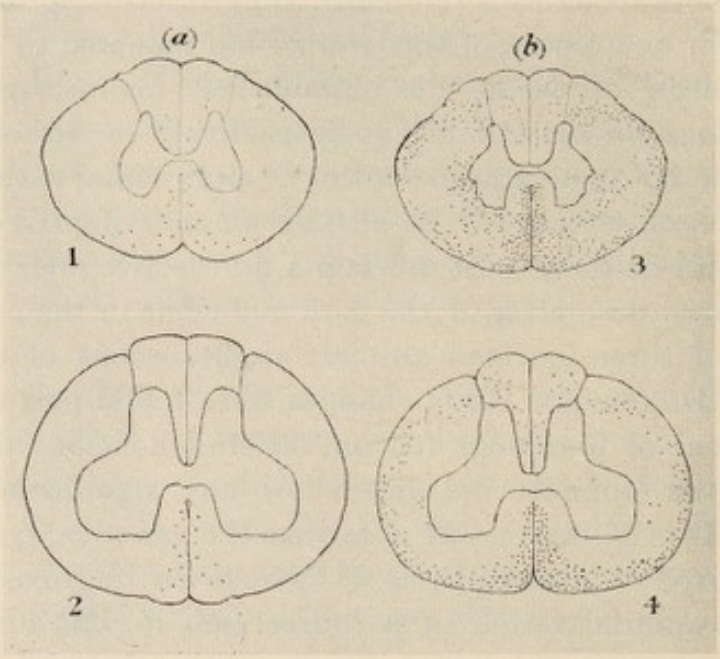

FIG. 1.—Drawings representing the distribution of degenerating nerve fibres as shown by the Marchi technique in the spinal cords of (*a*) and (*b*). (Experiment I.) Drawings 1 and 2 represent sections through the 2nd cervical and 6th cervical regions respectively of (*a*) (white flour). Drawings 3 and 4 are corresponding sections of (*b*) (white flour plus wheat germ). Note the greater amount of degeneration in the cord of (*b*), and also that the degenerating fibres are more scattered in the higher region.

only possible in this case, as in the problem of susceptibility to bacterial infection and its relation to diet, to begin to sort out the various experimental phenomena observed when the differentiation between vitamins A and D was finally established.

Although it soon became obvious that a fat-soluble vitamin played a part in preventing the cord degeneration, attention was first focused on the positive aspect of the phenomenon, for it seemed probable that just as cereals had an interfering action on bone calcification [9] so also they would under certain conditions exert a toxic effect on the nervous system. Difficulties were, however, encountered in the variable results

obtained in different litters of puppies. In some litters the spinal cord degeneration could be easily produced when the diet contained much cereal and was deficient in fat-soluble vitamins. On the other hand, some litters were refractory and showed no cord changes during the experimental period. In those litters giving a positive result, although the addition of wheat germ usually made the cord changes more extensive, yet those eating white flour alone as the cereal part of the diet sometimes developed fairly marked degenerative changes in the spinal cord.

Some of these discrepancies are now easier to understand in the light of further experimental work.

It may be well before describing later developments to illustrate some of the positive results obtained with diets containing white flour, oatmeal and wheat embryo as cereals because it is important that it should be recognized that the pathological changes can often be produced by natural foodstuffs.

Experiment I.

Production of Spinal Cord Degeneration by Diets which include White Flour and Wheat Germ.

Age beginning: 10 weeks.

Duration of experiment: About 4 months.

Basal diet: Separated milk powder, 20-30 grm.; lean meat, 20-10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5-5·0 grm.; orange juice, 3-6 c.c.; sodium chloride, 1-2 grm.

(*a*) had white flour.

(*b*) had 60 per cent. white flour, 40 per cent. wheat germ.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	—	Little degeneration	Active during much of experiment. Fairly steady on legs but weak
(*b*)	Wheat germ	Much degeneration	Very inco-ordinate. Puts legs down slowly and unsteadily: later paralysed

The rate of growth of the two animals was similar.

The degenerative changes of the cords of these animals when stained by Marchi's method are represented in fig. 1.

In this experiment, degeneration developed in the spinal cords of both animals, but in (*b*), with wheat germ added to the diet, it is more extensive.

Another experiment giving similar results may be quoted.

Experiment II.

Production of Spinal Cord Degeneration by Diets which include Oatmeal and Wheat Germ.

Age beginning: 7 weeks.

Duration of experiment: About 3 months.

Basal diet: Separated milk powder, 20 grm.; lean meat, 20-10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt, 3 grm.

(*a*) had oatmeal.

(*b*) had 80 per cent. oatmeal, 20 per cent. wheat germ.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	—	Little degeneration	Nearly normal .. .. ..
(*b*)	Wheat germ	Fair amount of degeneration especially in Cii, in anterior, antero-lateral, and direct cerebellar tracts	Hind legs stiff; later dragged hind legs when walking; later still inco-ordinate

The rate of growth of the two animals was good and comparable during the greater part of the experiment, but towards the end both lost weight, especially (*b*).

These two experiments illustrate the fact that degenerative changes in the cord can be produced in young dogs by diets rich in cereals when the fat-soluble vitamin intake is deficient.

In the following experiments it is demonstrated that the degenerative changes can be prevented by adding to the food a source of fat-soluble vitamins. In the first of these experiments olive oil and butter are compared; in the second, butter fat is used with its fat-soluble vitamin content respectively intact and destroyed by heat and oxidation.

Experiment III.

Production of Spinal Cord Degeneration by Diets which include Bread, Wheat Germ and Varying Quantities of Fat-soluble Vitamins.

Age beginning: 7 weeks.

Duration of experiment: About 2 months.

Basal diet: Bread (80 per cent.) and wheat germ (20 per cent.); separated milk powder, 20-25 grm.; lean meat, 10-20 grm.; yeast, 3-5 grm.; orange juice, 3 c.c.; salt, 1 grm.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	Olive oil, 10 c.c.	Much degeneration	Could not walk straight; had fits occasionally. Inco-ordinate, and finally paralysed. Eyes ulcerated
(*b*)	Butter, 10 grm.	No degeneration	Normal

Both animals grew well for the greater part of the experiment.

Experiment IV.

Production of Spinal Cord Degeneration by Diets which include Bread, Wheat Germ, Treated and Untreated Butter Fat.

Age beginning: 8 weeks.

Duration of experiment: About 4 months.

Basal diet: Bread (80 per cent.) and wheat germ (20 per cent.); separated milk powder, 20 grm.; lean meat, 20-10 grm.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt 1 grm.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	Olive oil ..	Much degeneration	Looked dazed; abnormal gait; later paralysed
(*b*)	Butter fat ..	No degeneration	Nearly normal
(*c*)	Butter fat heated and oxygenated 12 hours	Much degeneration	Looked dazed; inco-ordinate; back legs weak; later paralysed

The rate of growth of the three puppies was fairly comparable during the greater part of the experimental period.

Although under the experimental conditions described it is generally possible to obtain the degeneration with cereals, this is not always the case, and the variability made it necessary to seek other means of pursuing the investigation.

It was thought possible that the varying results might depend not only on the early history of the puppies but also on variations in the samples of cereals and especially of the wheat germ. Now the cord degenerations, and to some extent the clinical symptoms, called to mind the pathological conditions of the nervous system associated with human convulsive ergotism. It seemed possible, therefore, that some batches of the wheat germ were ergotized, i.e., infected with the fungus *Claviceps purpurea*, although, since white flour was sometimes associated with cord changes, this explanation was not very likely. Microscopic examination of the wheat germ also gave no support to this supposition. Attempts were, nevertheless, made to produce cord degeneration by adding ergot of rye to the diets of some animals. It seemed indeed probable that if, by the use of ergot, a more certain method of producing demyelination of the spinal cord could be obtained, the problem would be more amenable to study and possibly lead to a clearer understanding of the difficulties.

Reference to the literature dealing with experimental nervous

ergotism in animals did not give much prospect of success. Tuczek [10] not only had much experience of convulsive ergotism in man but also made many attempts to produce cord degeneration in animals by adding ergot to the diet. He described the degenerative changes in the cords of patients deceased of nervous ergotism, but never succeeded in producing similar changes in the cords of animals, although he refers to the paralysis, stiffness, weakness and staggering gait produced in dogs which had received 4 to 6 grm. of ergot daily. Writing in 1882, at a time when convulsive ergotism in Germany was an important practical problem, he says of animal experiments, "Positive Sectionsergebnisse in Centralnervensystem bei Ergotismus finde ich in der Literatur nirgends!" Of course, the technical methods used for preparing nervous tissue for microscopic examination were cruder in those days and this may account for the negative results. Whether spinal degeneration in experimental animals such as is described below has been produced by ergot and demonstrated since Tuczek's time, I have not been able to discover.

The following experiments show that nervous ergotism can be induced in dogs under certain dietetic conditions, and that slight alterations in the diet of a specific nature completely prevent the cord degenerative changes even when the same or larger amounts of ergot are eaten.

Spinal Cord Degeneration produced by Ergot of Rye.

In the early experiments, ergot in small quantities (2 to 5 grm. daily) was added to diets deficient in fat-soluble vitamins, but otherwise, so far as was known, complete, and given to puppies of the same litter. Previous experiments in which the cord changes had developed when cereals formed a large part of the diet suggested that the absence of fat-soluble vitamins would also play an important rôle in controlling any influence on the nervous system that ergot might exert.

Before describing the experimental results it may be well to say that, although it is usually an easy matter to feed puppies on diets deficient in fat-soluble vitamins for some months, it is by no means easy to get them to take ergot under these conditions. They may eat up their food containing the ergot for a few days or weeks and then either refuse it or eat their ration incompletely. To meet this situation different devices are necessary varying with the animal in question. Sometimes the ergot is mixed with a small portion of the food and given as a bolus after some of the food has been eaten, sometimes the amount of ergot is reduced,

and sometimes it is necessary to suspend giving the ergot for at least a few days. In all cases care and discretion are needed, especially as it is advisable that the animals should not lose weight before the symptoms of nervous ergotism are produced.

The following experiment illustrates the intensification of cord degenerative changes when ergot is added to a diet rich in cereal and deficient in fat-soluble vitamins.

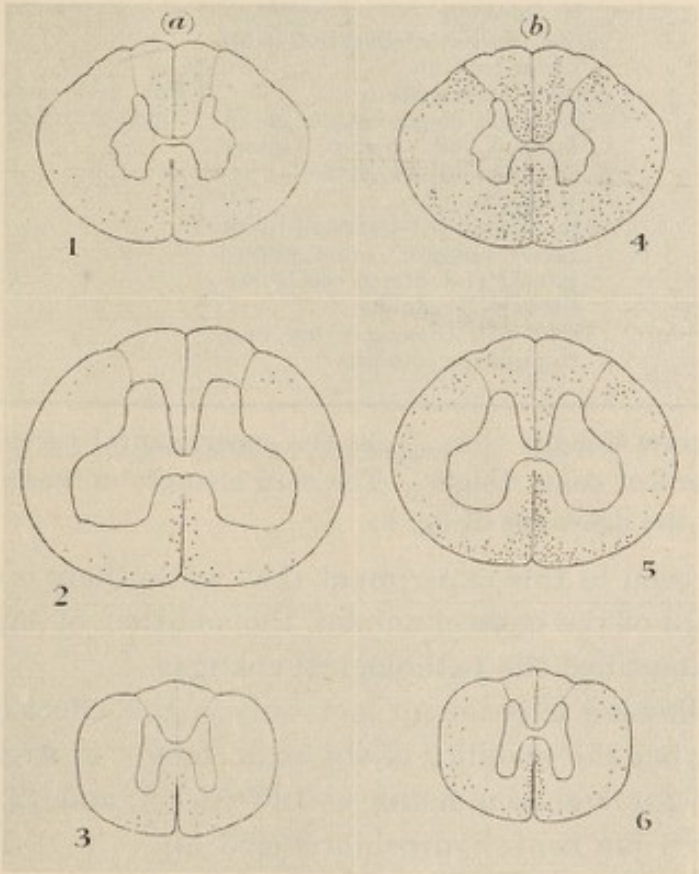

FIG. 2.—Drawings representing the distribution of degenerating nerve fibres as shown by the Marchi technique in the spinal cords of (*a*) and (*b*). (Experiment V.) Drawings 1, 2 and 3 represent sections through the 2nd cervical, 6th cervical and 11th dorsal regions respectively of (*a*) (control diet). Drawings, 4, 5 and 6 represent corresponding sections of (*b*) (ergot). Note the greater amount of degeneration in the cord of (*b*) (ergot), and that there is comparatively little degeneration in the dorsal region.

EXPERIMENT V.

The Production of Degeneration by Ergot.

Age beginning : 9 weeks.

Duration of experiment : About 2 months.

Basal diet : Bread ; separated milk powder, 20 grm. ; lean meat, 20-10 grm. ; peanut oil, 10 grm. ; dried yeast, 2·5 grm. ; orange juice, 3 c.c. ; salt 1 grm.

Animal	Diet variable	Cord degeneration as shown by Marchi staining	Some clinical notes
(*a*)	—	A little degeneration *Ci. A little* degeneration in anterior, antero-lateral columns. A few degenerated fibres in Burdach *Cvi. A little* in anterior and antero-lateral columns. Very little in direct cerebellar, none in Burdach *Dxii. Little* degeneration in anterior column	Fairly active but rather unsteady out of cage
(*b*)	Ergot, 2 g. ..	Much degeneration *Ci. Much* degeneration in anterior and antero-lateral columns, direct cerebellar and Burdach *Cvi. Much* degeneration in anterior column, some antero-lateral and direct cerebellar; little in Burdach *Dxii. Little* degeneration, most in anterior columns	Not very active, and decidedly inco-ordinate

Puppy (*b*) grew steadily throughout the experimental period; (*a*) grew well at first but later lost some weight. The cord changes in these two animals are represented by the drawings of fig. 2.

It will be seen in this experiment that while there is some degeneration in the cord of the control animal, the addition of ergot to the diet has greatly intensified the pathological changes.

In the following experiment not only is the effect of adding ergot examined (*b*), but the stability of the toxic factor in ergot is tested by (1) heating it for eighteen hours at 120° C. (*c*), and (2) boiling it for one hour with 1 per cent. hydrochloric acid (*d*).

Experiment VI.

The Production of Degeneration by Treated and Untreated Ergot.

Age beginning: 8 weeks.

Duration of experiment: About 4½ months.

Basal diet: Bread, 180 grm.; separated milk powder, 20 grm.; lean meat, 20 grm.; peanut oil, 10 c.c.: orange juice, 3 c.c.; dried yeast, 2 grm.; sodium chloride, 1 grm.

(*a*) had basal diet only.

(*b*) had in addition untreated ergot (2-5 grm. daily).

(*c*) ,, ,, ,, ergot treated 18 hours at 120° C. (2-5 grm. daily).

(*d*) ,, ,, ,, ergot boiled with 1 per cent. HCl for 1 hour (2-5 grm. daily).

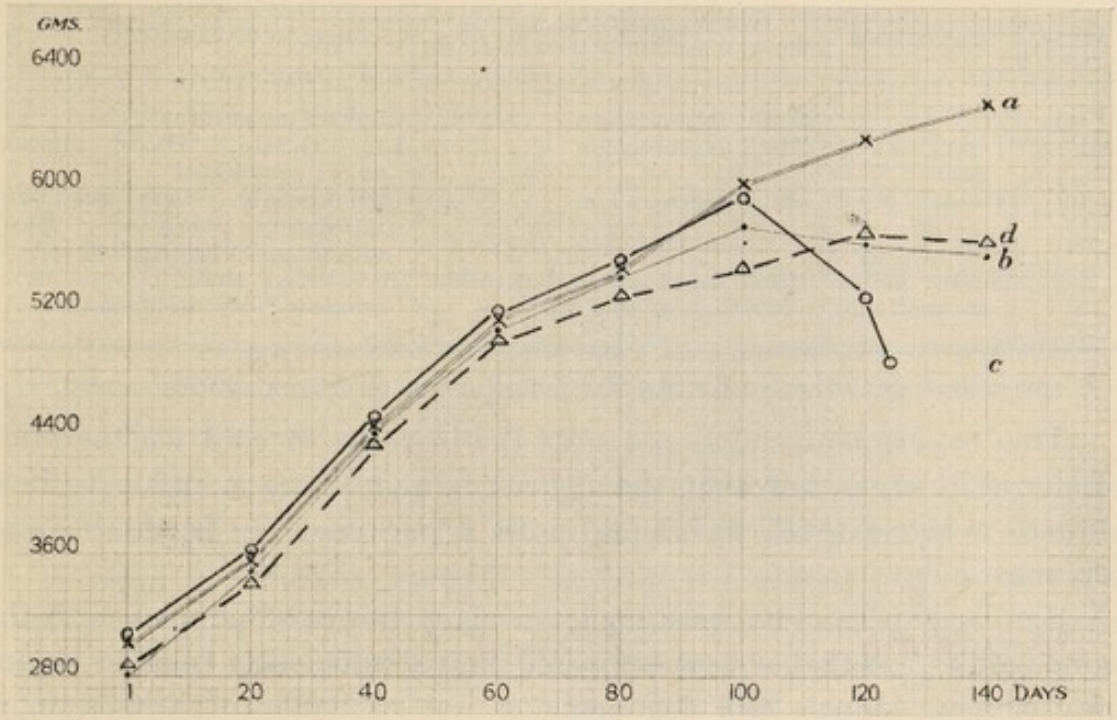

FIG 3.—Weight curve for Experiment VI. *See also* Fig. 4.

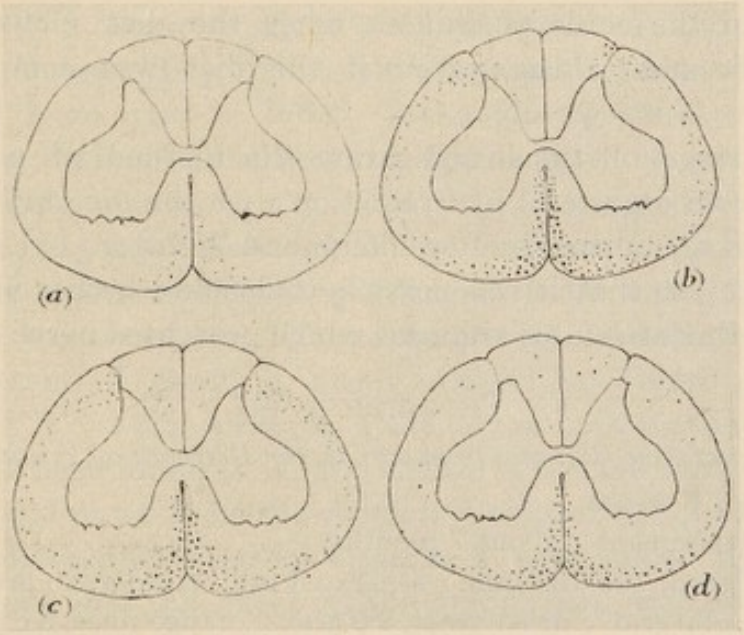

FIG. 4.--Drawings representing the distribution of degenerating nerve fibres as shown by the Marchi technique in the spinal cords at the level of the 6th cervical in dogs (*a*), (*b*), (*c*) and (*d*) of Experiment VI. (*a*) Control; (*b*) ergot; (*c*) heated ergot; (*d*) ergot boiled with dilute acid. Note the increased degeneration produced by ergot whether untreated, heated, or after being boiled with acid. In the latter case the increase is not so great.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	—	Little degeneration	Slightly inco-ordinate
(*b*)	Untreated ergot	Much degeneration	Inco-ordinate. Dazed appearance; several fits
(*c*)	Heated ergot	Much degeneration	Inco-ordinate. Very weak and ultimately paralysed. Head movements uncontrolled
(*d*)	Ergot boiled in weak acid	Much degeneration, but rather less than (*b*) and (*c*)	More active than (*c*), but inco-ordinate; dazed appearance

For rate of growth, see fig. 3 ; for distribution of degeneration, see fig. 4.

This experiment shows not only the increase in cord degeneration produced by ergot, but that the neuro-toxin in ergot is stable to heat and, to a less extent, to boiling with 1 per cent. hydrochloric acid for one hour.

Similar experiments were made on many litters of puppies with the same results,[1] so that it now appeared that a sufficiently reliable means of producing spinal cord degeneration was at hand to allow a more extended study of the problem.

The first point which required investigation was to see whether the cord changes produced by ergot could be prevented by fat-soluble vitamins in the food, for, as mentioned above, demyelination had never been seen in the cords of animals when the diet included cod-liver oil, egg yolk and cabbage, even if the diet was composed largely of cereals.

At this stage of the investigation the method of preparation of vitamin D from ergosterol by irradiation with the mercury vapour lamp had just been announced by Rosenheim and Webster [11], and Windaus and Hess [12], so that it was possible to decide whether vitamin D was the factor in the fat-soluble vitamins which prevented nerve degeneration.

Experiment VII.

Degenerative Changes produced in the Presence of Vitamin D.

Age beginning: 8 weeks.

Duration of experiment: About 7 months.

Basal diet: Bread, 100-160 grm.: separated milk powder, 20 grm.; lean meat, 20 grm.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt, 1 grm.

Each animal also received on the average 3 grm. ergot daily.

[1] Since this was written, failure to produce the cord changes with ergot has been experienced in one litter of puppies. Although it is the only exception out of about forty experiments, this negative result may be of significance and may indicate that some unknown factor or factors have still to be elucidated.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	Olive oil, 10 c.c.	Much degeneration	Head position abnormal. Incoordinate. Discharge from eyes: later ulcer on one eye
(*b*)	Cod-liver oil, 10 c.c.	No degeneration	Normal
(*c*)	Irradiated ergosterol (Vitamin D)	Much degeneration; slightly less than (*a*)	Head position abnormal. Incoordinate. Discharge of pus from eyes

Advanced degeneration, as shown by Weigert's method of preparation, is seen in both (*a*) and (*c*).

This experiment makes it clear that (1) cod-liver oil prevents the neuro-toxic effect of ergot; (2) vitamin D is not the preventive agent.

A casual observation made during this and similar experiments also confirmed the ineffectiveness of vitamin D as a protective agent against ergot. It was noticed that when dogs received ergot, even when eating a diet deficient in fat-soluble vitamins, they did not develop the same intensity of rickets as the animals not getting ergot. It appeared, in other words, that ergot itself contained vitamin D, and so exerted an antirachitic action. This point was followed up, and ergot was shown to contain an appreciable amount of vitamin D (Mellanby, Surie and Harrison [13]), a discovery of interesting significance since, as will be remembered, Tanret [14] first isolated ergosterol, the parent substance of vitamin D, from ergot in 1889. Although ergosterol is very widely distributed in nature, it is rare to find any of it in the activated (vitamin D) form.

Now, since ergot produces spinal cord degeneration, and since it also contains vitamin D as a normal constituent, it did not seem likely that this vitamin in quantities sufficient to prevent rickets would inhibit the toxic action of ergot, a result which has been confirmed in Experiment VII. Since vitamins A and D are both present in cod-liver oil, which has been found to be preventive, it seemed fairly certain that vitamin A would prove to be the protective substance. In the following experiment, different sources of vitamin A were added to the diets in addition to ergot and the effect on the spinal cord studied. It may be stated that the beneficial effects of adding these sources of vitamin A were at once apparent in the general condition of the animals. The difficulty of feeding the ergotized diets greatly diminished, and the animals remained active and healthy looking throughout the experimental period.

Experiment VIII.

Degeneration Changes resulting from Ergot prevented by Sources of Vitamin A.

Age beginning: 8 weeks.
Duration of experiment: About 4½ months.
Basal diet: Bread; separated milk powder, 20 grm.; lean meat, 20 grm.; peanut oil, 10 c.c.; orange juice, 3 c.c.; dried yeast, 2 grm.; sodium chloride, 1 grm.
In addition all had on the average 2·5 grm. ergot daily.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Some clinical notes
(*a*)	—	Much degeneration	Inco-ordinate. Several fits; dazed appearance
(*b*)	Butter, 10 g., replacing peanut oil	No degeneration	Active
(*c*)	Mammalian liver oil (vitamin A), 1 c.c.	No degeneration	Active
(*d*)	Cabbage boiled 30 min., 30-50 grm.	No degeneration	Active, but nervous and jumpy
(*e*)	1 egg yolk	No degeneration	Very active

The weight curves of these animals during the experimental period are shown in fig. 5.

Inference from these results :—

(1) Foods rich in vitamin A prevent the ergot from producing spinal cord degeneration.

(2) With vitamin A in the diet, the animals remain active, eat their food well, develop no nervous symptoms and remain in good health.

It may be added that the animals whose diet included vitamin A often had more ergot than other members of the litter because on diets deficient in vitamin A the ergot had more frequently to be omitted from the daily ration.

Rye Germ and Degenerative Changes in the Cord.

Ergot of rye develops as the result of the growth of a fungus, *Claviceps purpurea*, in the embryo of rye. The embryo under these conditions becomes bigger and is ultimately filled with mycelia of the fungus. It was of interest therefore to see whether rye germ

itself, free from the fungus, had any action on the spinal cord. It may be noted that, after ergot of rye was found to contain vitamin D (Mellanby, Surie and Harrison) [13], it was discovered that the rye embryo itself also contained some of this vitamin, although in smaller quantities than ergot. In other words, the mycelial invasion of the rye embryo did not produce *de novo* vitamin D but only exaggerated the development of a vitamin originally present in the rye germ. It

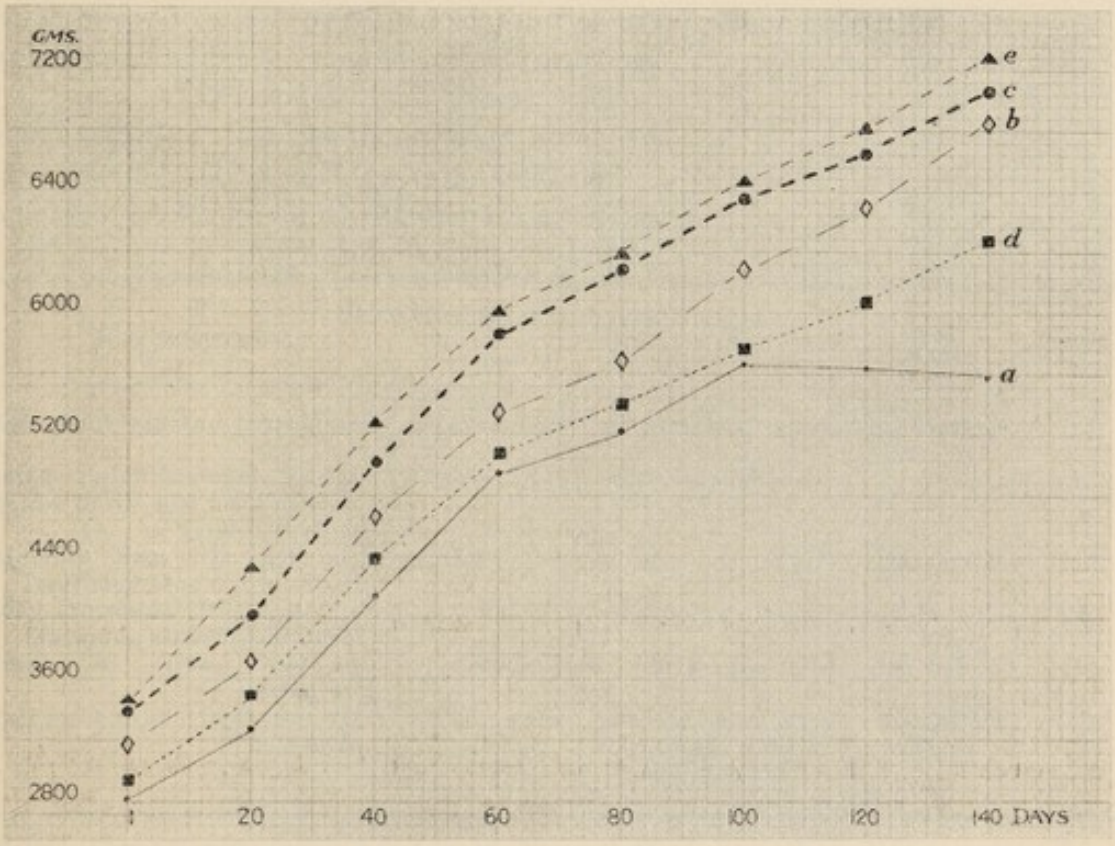

FIG. 5.—Weight curve for Experiment VIII.

seemed likely, especially in view of the earlier experiments with wheat germ, that rye germ would also afford evidence of containing the agent abundantly present in ergot of rye which hastens the cord degenerative changes. If this proved to be the case, it would show that the ergotizing of the rye embryo only results in the production of increased amounts of a toxic agent originally present in the rye germ itself. Experiments carried out to test the action of rye germ on the spinal cord proved that this substance does indeed contain the nerve toxin. The following series of tests made on a litter of puppies illustrates the point.

Experiment IX.

The Production of Cord Degeneration by Rye Germ.

Age beginning: 6 weeks.

Duration of experiment: About 5 months.

Basal diet: white flour; separated milk powder, 25 grm.; lean meat, 20 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; sodium chloride, 2·4 grm.

(*a*) had in addition petrol ether extract of rye germ equivalent to 20 per cent. of white flour.

(*b*) had basal diet only.

(*c*) had in addition rye germ replacing 20 per cent. of white flour.

(*d*) ,, ,, rye germ heated 36 hours at 120° C., replacing 20 per cent. of white flour.

(*e*) ,, ,, rye germ replacing 20 per cent. of white flour, and 20-50 grm. cabbage boiled ½ hour.

(*f*) ,, ,, rye germ extracted with petrol ether, replacing 20 per cent. of white flour.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Condition as regards rickets	Some clinical notes
(*a*)	Extract of rye germ	Some degeneration	Some rickets	Not normal but ran fairly well
(*b*)	—	Some degeneration	Bad rickets	Slightly inco-ordinate; paralysis in hind legs, from which recovered later
(*c*)	Rye germ	Much degeneration	Some rickets	Hind legs stiff, gait difficult. Head movements abnormal
(*d*)	Heated rye germ	Much degeneration	Rickets ..	Hind legs stiff, abnormal gait. Head movements abnormal
(*e*)	Boiled cabbage	No degeneration ..	Rickets ..	Ran fairly well. No inco-ordination
(*f*)	Extracted rye germ	Very much degeneration (also in Goll's column)	Very bad rickets	Gait abnormal; later hind legs paralysed

The degenerative changes in the cords of these animals are represented in fig. 7, and the changes in weight during the experimental period in fig. 6.

These experiments show that:—

(1) Rye germ contains an agent producing spinal cord degeneration, but probably to a less degree than ergot of rye, since much more rye germ was eaten in these experiments than ergot in similar experiments.

(2) This action of rye germ, like that of ergot, is inhibited by cabbage.

(3) Heating the rye germ for thirty-six hours at 120° C. has not destroyed the substance responsible for this action.

(4) The active agent in rye germ is not removed by petrol ether.

(5) There is no relationship between the amount of cord degeneration and rickets.

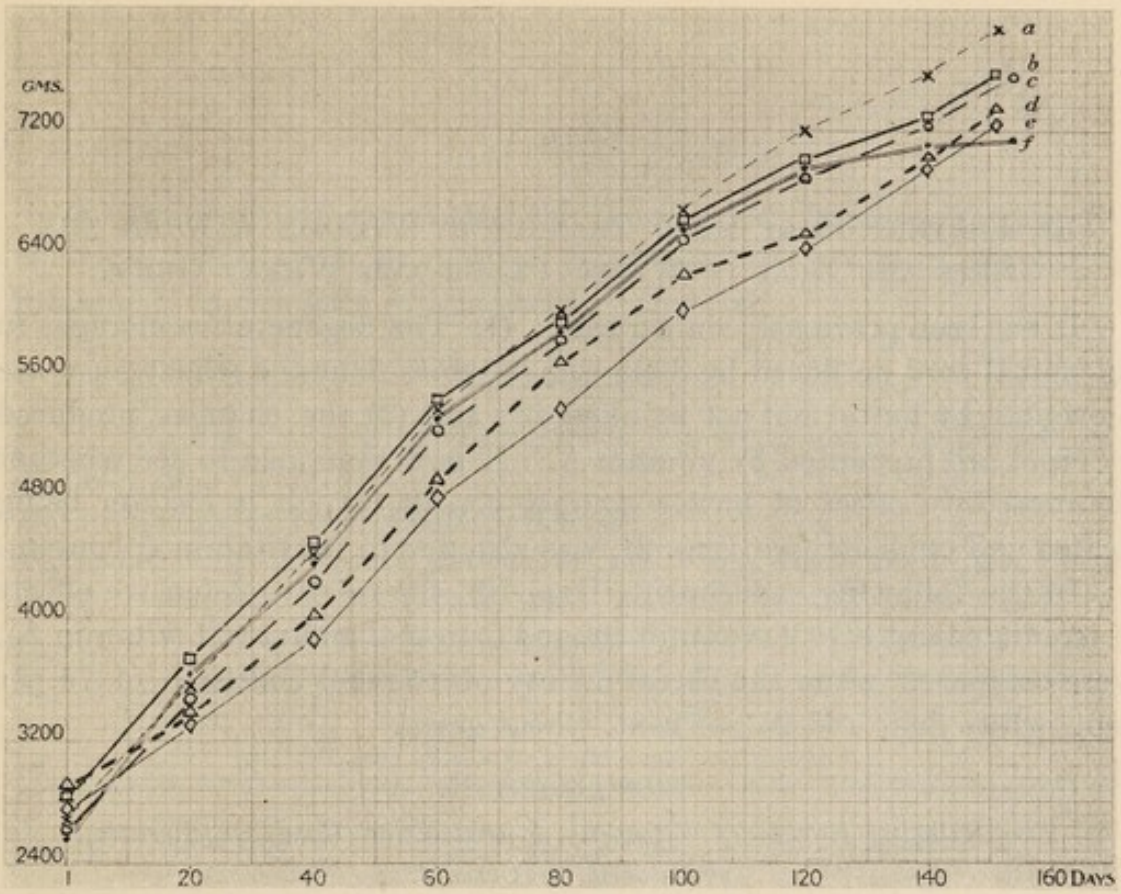

FIG. 6.—Weight curve for Experiment IX. *See also* Fig. 7.

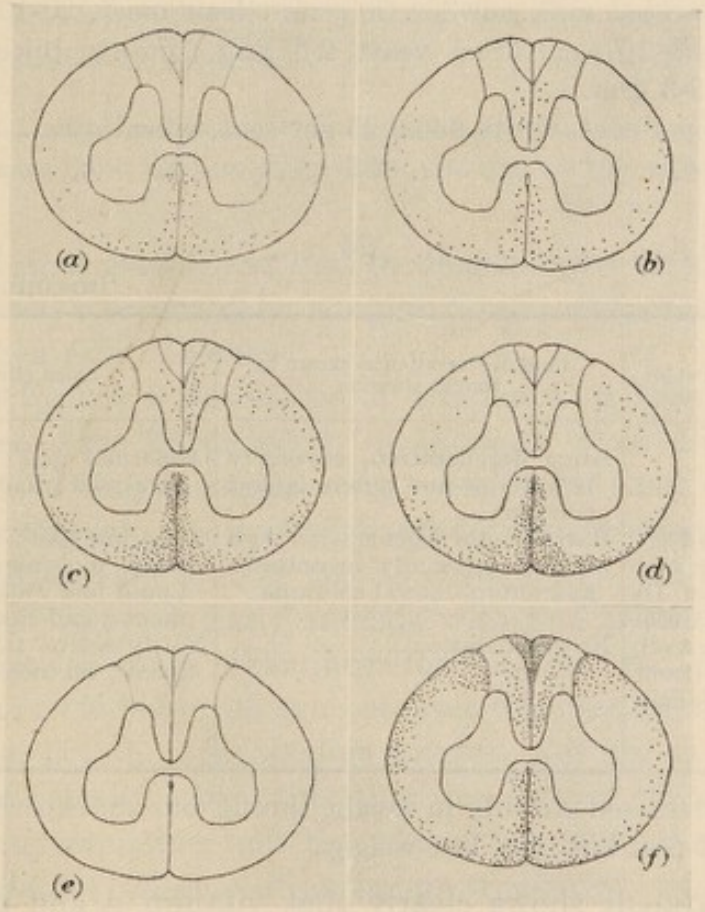

FIG. 7.—Drawings representing the distribution of degenerating fibres as shown by the Marchi technique in the spinal cords at the level of the 5th cervical in (*a*), (*b*), (*c*), (*d*), (*e*) and (*f*) of Experiment IX. (*a*) Petrol ether extract of rye germ; (*b*) control; (*c*) rye germ; (*d*) heated rye germ; (*e*) cabbage (boiled); (*f*) rye germ extracted with petrol ether. Note the greater degeneration produced by rye germ (*c*), heated rye germ (*d*), and extracted rye germ (*f*), while the petrol ether extract of rye germ (*a*) is no worse than the control. The addition of cabbage (*e*) has prevented all degeneration.

The Prevention of Cord Changes by Vitamin A when the Diets are Rich in White Flour and Wheat Germ.

It has been previously shown that: (1) The degenerative changes in the spinal cord produced by diets rich in white flour and wheat germ are prevented by butter but not by olive oil; and (2) the changes produced by ergot are prevented by vitamin A. It now remained to see whether the protective effect of butter against cereals, which it lost on being heated and oxidized (see Exp. 2), was also due to its vitamin A content.

In the following experiment (Exp. 10), the relative defensive effects of irradiated ergosterol (vitamin D), and mammalian liver oil (vitamin A), were compared when the cereal moiety of the diet consisted of 75 per cent. white flour and 25 per cent. wheat germ.

Experiment X.

The Relative Effects of Vitamins A and D on Cord Degeneration produced by Cereals.

Age beginning: 7 weeks.

Duration of experiment: About 4 months.

Basal diet: Separated milk powder, 20 grm.; lean meat, 20-10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt, 2-3 grm.

(*a*) had 75 per cent. white flour, 25 per cent. wheat germ.

(*b*) ,, 75 ,, ,, ,, 25 ,, ,, ,, and irradiated ergosterol.

(*c*) ,, 75 ,, ,, ,, 25 ,, ,, ,, and vitamin A (mammalian liver oil

Animal	Diet variable	Cord degeneration as shown by Marchi staining	Some clinical notes
(*a*)	—	Some degeneration, especially in anterior and antero-lateral columns	Abnormal gait; later hind legs paralysed; had fits
(*b*)	Irradiated ergosterol (vitamin D) 0·05-0·1 c.c.	Rather more degeneration than in (*a*), especially in anterior and antero-lateral columns	Abnormal gait; hind legs stiff and head movements uncontrolled. Could just walk at end of experiment; had fits
(*c*)	Vitamin A oil, 1 c.c. (from mammalian liver)	No degeneration	Fairly active throughout experiment; no inco-ordination

(*b*) and (*c*) increased steadily in weight throughout the experimental period; (*a*) grew well at first but later lost weight.

This experiment shows clearly that vitamin A and not vitamin D prevents the cord degeneration when white flour and wheat germ are the cereals eaten.

Experiment XI shows that the cereal effect can be antagonized by cabbage. Both animals received oatmeal as the cereal of the diet and vitamin D in the form of irradiated ergosterol.

EXPERIMENT XI.

The Prevention by Cabbage of Cord Changes produced by Cereals.

Age beginning: 7 weeks.

Duration of experiment: About 3 months.

Basal diet: Separated milk powder, 20 grm.; lean meat, 20·10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt, 3 grm.; irradiated ergosterol (vitamin D), 0·1 c.c.

(*a*) had 80 per cent. oatmeal and 20 per cent. ground wheat germ.

(*b*) „ „ „ „ „ „ 20 „ „ „ „ and 50 grm. cabbage boiled half an hour.

Animal	Diet variable	Cord degeneration as shown by Marchi staining	Clinical notes
(*a*)	—	Fair amount of degeneration, especially in Cii, anterior, antero-lateral and direct cerebellar regions	Hind legs stiff; later gait incoordinate
(*b*)	Cabbage boiled ½ hour	No degeneration	Gait normal

Both animals grew well until towards the end of the experiment and then lost weight.

It now seemed established that the degenerative changes in the cord could be entirely prevented by vitamin A both when the diet contained ergot and when rich in cereals.

Since the liver is a storehouse of vitamin A, it became a matter of interest to see what happened to these stores when diets respectively rich and poor in this substance were eaten. Did they disappear when the diet was deficient in this respect and did their absence from the liver determine the development of the cord changes? In order to decide this point, the livers of many animals with and without symptoms of cord degeneration were examined for vitamin A. The technique employed was as follows: 20 grm. of liver were heated on a water bath with alcoholic potash; the alkaline mixture was thrice extracted in a separating funnel with ether; the ether was distilled off, care being taken to drive off any moisture, and the colour test of Carr and Price [15], using a chloroform solution of antimony trichloride, was applied to the residue. The intensity of the blue reaction was estimated by means of a Lovibond tintometer (Rosenheim and Schuster pattern), and recorded

in blue units. When this method is used under standard conditions, it is generally believed to give a fairly accurate indication of the amount of vitamin A present in the liver. In the figures recorded in this paper a blue unit is that amount of substance which, dissolved in a 1-cm. cube and treated with antimony trichloride, produces a tint equivalent to one Lovibond unit when viewed through a depth of 1 cm. To convert this figure into the blue units which will probably be officially adopted by the British Pharmacopœia, it should be multiplied by 2·2.

No degenerative changes were found in the cords of these experimental animals except when the liver was denuded of vitamin A. This does not mean that absence of vitamin A from the liver always results in cord degeneration, for, as will be seen later (p. 21), there are cases when, for instance, carotene is a constituent of the diet, where there may be no vitamin A in the liver and yet no degenerative changes in the cord. There are also a few exceptional unexplained cases when there is little or no vitamin A or carotene in the food, no vitamin A in the liver, and yet no cord degeneration. The point that seems clear is that, if there is a store of vitamin A in the liver in these animals, cord changes are not found, and if cord changes are found there is no vitamin A in the liver unless this is given after the changes have developed.

Having shown that vitamin A prevents not only cereals but also ergot from exerting its neurotoxic action, it was necessary to see whether this preventative effect could also be obtained with carotene. Parenthetically it may be stated that when the above-described experiments were made the fact that the vitamin A action of cabbage was due to carotene had not been suggested. The relation of vitamin A to carotene has been under discussion for many years, since Steenbock and Boutwell [16] drew attention to the fact that the colouring matter of foods was often related to their vitamin A content. It was at first thought that carotene might actually be vitamin A. This was soon shown not to be the case, for preparations made from cod-liver oil, although containing abundant vitamin A according to biological tests, were devoid of carotene. The problem was brought to the fore again in 1928, by Euler, Euler and Hellström [17], who found that for growth in young animals carotene could be substituted for vitamin A. This was denied at first by Dulière, Morton and Drummond [18], but confirmed by Moore [19], and by Collison, Hume, Smedley-Maclean and Smith [20]. A further development took place in 1929, when Green and E. Mellanby [21] found that the anti-infective action of vitamin A could be similarly exerted by carotene. Of course, none of these investigations

settled the problem as to whether pure carotene was the active agent and the experience of investigators on the relation of cholesterol, ergosterol and ultra-violet irradiation to vitamin D, has made many hesitate to declare that carotene itself acts like vitamin A and that its effect is not due to some vitamin A impurity. The general trend of results, however, especially those of Euler and his associates [22], suggests that carotene, a chemical substance associated with vegetable life and especially with green vegetables and carrots, has specifically the biological properties of vitamin A. T. Moore [23] has indeed brought forward evidence which suggests that it is the parent substance of vitamin A, and that on giving carotene to animals vitamin A is produced and stored in the liver. In order, therefore, to test the relationship between vitamin A and carotene from quite a different angle, experiments were made to see whether carotene could also prevent the degenerative changes in the spinal cord produced by cereals and ergot.

In the following and other similar experiments, carotene, supplied by the British Drug Houses, was tested to see whether it could prevent the degeneration produced by ergot. Carrots, the richest known source of carotene among vegetable substances, were also tested.

Experiment XII.

The Prevention of Cord Degeneration by Carotene.

Age beginning: 9 weeks.
Duration of experiment: About 2 months.
Basal diet: White flour, 130 grm.; separated milk powder, 20 grm.; lean meat, 10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; sodium chloride, 3 grm.; irradiated ergosterol (vitamin D), 0·1 c.c.

Animal	Diet variable	Cord degeneration as shown by Marchi staining	Vitamin A in liver at P.M. as tested by $SbCl_3$	Some clinical notes
(*a*)	Carotene, 5 mg... Ergot, 2 grm. ..	No degeneration ..	No vitamin A ..	Fairly active. No inco-ordination
(*b*)	Carrots, 25 30 grm. Ergot, 2 grm.	No degeneration ..	No vitamin A ..	Fairly active
(*c*)	Butter, 10 grm... Ergot, 2 grm.	No degeneration ..	Some vitamin A (1 5 Blue Units per grm.	Fairly active. Normal gait
(*d*)	—	No degeneration ..	No vitamin A ..	Fairly active
(*e*)	Ergot, 2 grm. ..	Much degeneration.	No vitamin A ..	Very inco-ordinate. Later lost weight and became weak. Eyes not quite normal

The rate of growth was good in all cases, but (*e*) lost weight later in the experiment. Fig. 8 represents drawings of sections of different levels of the spinal cord

of (*e*), the only animal of this experiment developing cord degeneration. From these drawings the relative distribution in different parts of the spinal cord can be seen.

It is evident that in animals receiving ergot, cord degeneration was prevented by carotene itself and by carrots which contain carotene, and

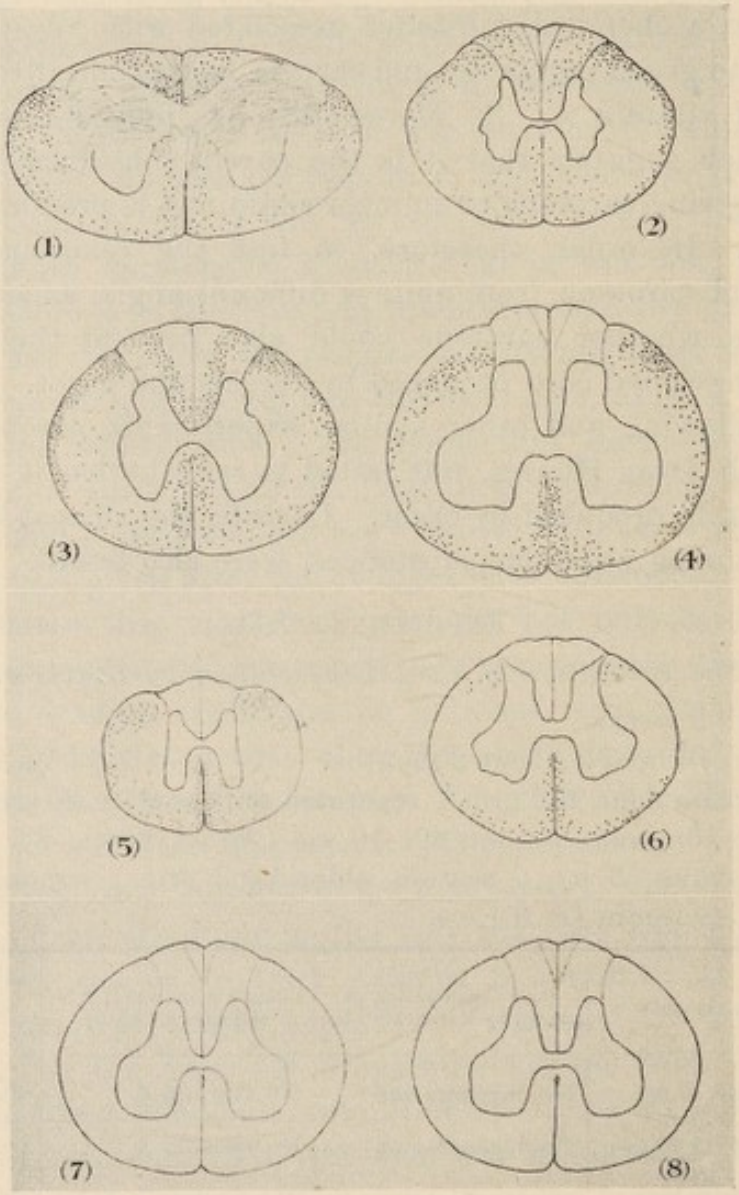

FIG. 8.—Drawings representing the distribution of degenerating fibres as shown by the Marchi technique in the spinal cord at the levels : (1) low medulla ; (2) 1st cervical ; (3) 3rd cervical ; (4) 6th cervical ; (5) 12th dorsal ; and (6) 3rd lumbar, of animal (*e*) (ergot) of Experiment XII. Sections (7) and (8) show no degeneration in animals (*a*) and (*c*) (cervical region), which received carotene and butter respectively.

by butter, which contains both vitamin A and carotene. No degeneration was found in (*d*), the control animal, which was given neither ergot nor a protective agent. In addition to these facts, there are several other

points of interest about this series of animals. The first is the rapidity with which the cord degeneration can be brought about by ergot under favourable conditions. The feeding period only lasted seven and a half weeks and yet in (*e*), where there was no protective agent in the diet, extensive nerve degeneration developed in all the regions examined, including the medulla.

The second point of interest concerns the vitamin A stores in the liver. These were probably very small at the beginning, otherwise (*e*) would not have reacted so quickly to ergot. It will be further noticed that even when carotene and carrots were ingested there was no vitamin A observable in the liver by the colour reaction of Carr and Price. This fact opens up several questions of interest which, however, can only be touched upon here. It was stated earlier that the store of vitamin A in the liver of the experimental dogs has apparently to be used up before spinal cord degeneration develops. It might be inferred, therefore, that when the vitamin A stores are used up, especially when ergot is in the diet, the nerve degeneration would be evident. This does not seem to happen when carotene is in the diet. It may then be asked why, if carotene is the precursor of vitamin A in the liver, there was no vitamin A in the livers of (*a*) and (*b*), which received carotene and carrots respectively. This question cannot yet be answered. Possibly, if larger doses of carotene or carotene-containing substances had been given to these animals, a store of vitamin A would have been produced in the liver. Apparently carotene can act immediately after absorption from the alimentary canal either, as seems probable, directly, or after conversion to vitamin A, and thereby protects the nervous system from the toxic action of ergot. An analogous phenomenon is seen when carotene acts either prophylactically or curatively in bacterial infection in animals (Green and Mellanby [21]). Animals may be fed on diets deficient in vitamin A and so lose their stores. If carotene in sufficient quantity is now given, no infective lesions will develop, or if they have developed, will disappear, and yet the livers will often be found post-mortem to contain no vitamin A. Here, as in the degeneration experiments, although no vitamin A can be found in the liver, carotene exerts its anti-infective action and prevents epithelial hyperplasia.

Moore's results [23], in which he got evidence of the change of carotene to vitamin A in rats, have already been referred to above. The curious thing about experiments of this nature, as my colleague Green and I [21] have previously pointed out, is that surprisingly large quantities of carotene have to be given to these animals before vitamin A can be

found in the liver—much more, in fact, than is necessary to clear up all signs of vitamin A deficiency.

The Antagonism between the Nerve Toxin and Vitamin A and Carotene.

The foregoing results and others of a similar nature show that demyelination of the cord fibres is controlled by at least two factors—a positive harmful influence and the absence of a defending chemical mechanism—and this clarifies the problem to some extent, although of course it leaves much obscure.

The double mechanism may well explain, for instance, the difficulties earlier workers had in producing demonstrable cord changes in nervous ergotism, for at that time not only were vitamins unknown but the protective action of specific food elements was unheard of.

A second difficulty, which is also at least partially explained, is that referred to above (p. 5) in connection with the original experiments in which inconsistent results were obtained in different litters when cereals alone were employed. It appears that the variation in the results must have depended to some extent on the initial stores of vitamin A in the puppies of the different litters at the beginning of the feeding period. The liver is the main organ in which vitamin A is stored and the amount stored in this organ in different animals varies greatly. Sometimes it may be as high as 1,900 blue units per gramme of liver and sometimes it is almost negligible in amount or even absent. The amount in the liver depends on the amount of vitamin A and carotene included in the animal's food. If, for instance, puppies have been fed during the pre-experimental periods on diets containing cod-liver oil, butter and egg-yolk, their livers are very rich in the substance. The relation of the storage of vitamin A and the development of nervous lesions was investigated and it was found that when the pre-experimental diet was rich in vitamin A the time required for the production of nervous symptoms was lengthened. Thus the development of symptoms depends not only on the deficiency of vitamin A and carotene from the diet but also on the time it takes for the stores of the vitamin in the body to be used up. As might be expected the time of denudation varies greatly according to the original stores. In some cases this period may be several months and in others a few weeks. It is advisable, therefore, in experiments of this type to control the pre-experimental diet in order to ensure as far as possible similar reactions to the experimental feeding in point of time and intensity. There is also some evidence which

suggests that good feeding in the sense described, if continued long enough, may confer immunity against the neurotoxins for a long time after the liver vitamin A stores are completely denuded.

The protective action of carotene on the nervous system supports the suggestion that this substance and vitamin A are physiologically similar although not identical chemically. The action of carotene must also be taken into account in considering the effect of cereals of which it is sometimes a constituent. For instance, apart from the influence of the varying stores of vitamin A in the livers of different litters of pups, referred to above, it seemed possible that the irregularity of results in the cereal experiments might be influenced by (1) the presence of traces of carotene in the samples of wheat germ, or by (2) varying amounts of toxic factor in the different samples, or both. Examination of wheat germ for carotene revealed that there is a certain amount of this substance present and it is probable that it prevents in some degree at least the spinal degeneration. My colleague H. N. Green has estimated that 100 grm. of wheat germ contained about 1 mg. of carotene. Whether this amount varies in different specimens, and whether the variation is of significance in explaining the results obtained, is not known. One milligram increase in carotene intake daily would certainly modify the experimental results. It is of interest to note that rye germ was found by Green to contain only half the carotene found in wheat germ.

Although the cereals have not been systematically investigated in this respect, experiments were made in which yellow and white maize formed the cereal element of the diet. Yellow maize has for many years been given in diets as a source of vitamin A (Steenbock and Boutwell [16]), but this quality is almost certainly due to its carotene content, which is large for a cereal. White maize, on the other hand, contains little or no carotene. It would be expected, therefore, that it would be more difficult to produce cord degeneration in dogs when the cereal of the diet consisted of yellow rather than white maize. This is certainly the case, and in preliminary experiments it has not only been impossible to produce the degenerative changes with yellow maize, but even where ergot was added to the diet there was sufficient carotene in the yellow maize to prevent the ergot neurotoxic action (see Exp. XIII below). The difference in the condition of animals when yellow or white maize is added respectively to diets otherwise deficient in vitamin A and carotene is great and, so far as is known, this difference is due entirely to the carotene in the yellow maize. This point will be

referred to again in the discussion on the possible bearing of these results on pellagra.

The following experiment illustrates the protective effect of yellow maize against ergot and, in addition, shows that when white maize forms the cereal basis of a diet deficient in vitamin A and carotene, spinal cord degeneration develops:—

Experiment XIII.

The Relative Effects of White and Yellow Maize.

Age beginning: 10 weeks.
Duration of experiment: About 3 months.
Basal diet: Separated milk powder, 20 grm.; lean meat, 20-10 grm.; peanut oil, 10 c.c.; dried yeast, 2·5 grm.; orange juice, 3 c.c.; salt, 3 grm.

Animal	Diet variable	Cord degeneration as shown by Marchi staining	Some clinical notes
(*a*)	White maize. Irradiated ergosterol.	Some degeneration	Quiet and dazed; stupid appearance. Gait not normal. Ulcer on eye
(*b*)	White maize. Irradiated ergosterol. Ergot, 2 grm.	Much degeneration; little anterior and antero-lateral; big patch in Burdach	Legs spastic; inco-ordinate. Head positions abnormal; looks stupid
(*c*)	Yellow maize, Irradiated ergosterol. Ergot, 2 grm.	No degeneration	Normal

"Curative" Experiments with Substances containing Vitamin A and Carotene.

It has been shown that substances containing vitamin A, such as butter, egg yolk, mammalian liver oil and also carotene and carotene-containing foods including cabbage and carrots, prevent ergot and other toxins from attacking the nervous system and from producing nerve degeneration in the spinal cord. The problem then arises as to what happens if the animals are given ergot with a diet deficient in vitamin A and carotene until signs of nerve degeneration develop and are then given these substances in abundance? In other words, are vitamin A and carotene curative as well as protective in their action? There is definite evidence that the clinical condition tends to clear up rapidly under these conditions, though the degree of improvement naturally depends on the extent of the initial lesion. The immediate effect is often astonishing even when the animal has been paralysed for a long time. In a week or two animals with weak or even paralysed hind legs

become active once more. At first the activity brings out more prominently the inco-ordination and the animals run about with an intoxicated gait. Usually this diminishes greatly and in some cases the animals may later appear almost normal. Presumably further degeneration ceases on giving the additional vitamin A or carotene, and the fibres already showing degeneration are gradually absorbed.

One animal which, although it had been unable to stand for some months, and showed great spasticity especially when held in the standing position, was given vitamin A prepared from mammalian liver. After three weeks the animal could stand but was very inco-ordinate when it attempted to walk. Two months of vitamin A feeding resulted in a very active animal but there was still some inco-ordination which, however, further diminished by the end of the experiment three months later, but, as might be expected, gait never became normal. Examination of the spinal cord by Marchi's method showed complete absence of fibres with demyelination. Areas of sclerosis were found by Weigert's method of staining, indicating that the degenerating fibres had disappeared and had been replaced by neuroglia. Presumably other tracts took on the function of the injured ones and the animal relearnt to walk more or less normally.

The cord of another animal which had received 5 mg. of carotene daily for seventeen days after the development of severe ergot symptoms was kindly examined histologically by Dr. Perdrau. In this period the animal made great clinical improvement but the cord still showed a large number of degenerating fibres when stained by Marchi's method, and areas of more complete degeneration when prepared by Weigert's method.

The first rapid improvement which follows the giving of vitamin A or carotene, especially as regards activity, may depend upon some action of these substances on the muscle itself, but the gradual and continuous improvement which develops later is undoubtedly due to a better functioning of the central nervous system itself. It is clear that vitamin A and carotene not only prevent the cord changes produced by ergot but that they exert a " curative " effect after development of these changes in the sense that great clinical improvement with increased activity and diminished inco-ordination takes place after their administration. In course of time all active degeneration of the nerve fibres in the cord disappears and demyelination demonstrable by Marchi's technique can no longer be found.

There are few more striking effects in animal nutrition than the

improvement brought about by the daily addition of 5 mg. of carotene to an animal suffering from severe nervous ergotism.

Clinical Condition and Degeneration Changes in the Cord.

The main clinical symptoms in the affected dogs are spasticity, especially of the hind limbs, and inco-ordination in walking and running, which may, however, especially if the experiment is prolonged, be masked by weakness. Often the head is held on one side and the ears are awry, giving the animal a weird appearance. In the early stages

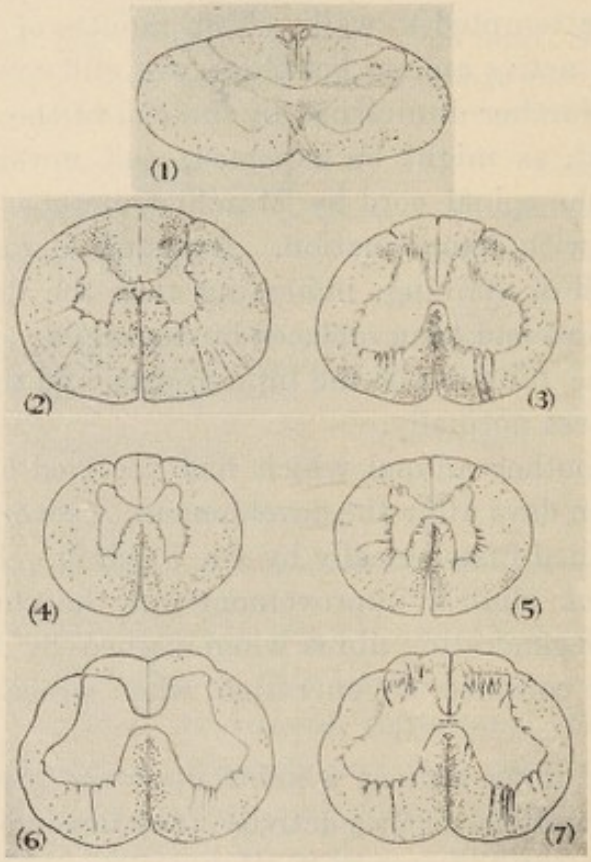

Fig. 9.—Drawings representing the distribution of degenerating fibres as shown by the Marchi technique at different levels of the spinal cord of an animal which had received ergot and no vitamin A or carotene. The levels represented are: (1) medulla; (2) 2nd cervical; (3) 6th cervical; (4) 2nd dorsal; (5) 11th dorsal; (6) 3rd lumbar; (7) 4th lumbar. Note the differences in distribution of degenerating nerve fibres at the different levels, especially the variations found in the posterior columns.

the animal usually has a pica and runs about apparently looking for suitable food and in so doing attempts to eat anything it comes across. At a later stage it seems incapable of fixing its attention for more than a moment and lurches about with no apparent object, and looks stupid. At a still later stage weakness, especially of the hind legs, becomes more apparent and the animal remains standing, often with legs apart and

spastic. After passing through a stage when it prefers to sit and make no effort to move, it may become paralysed. The eyes often develop pathological changes and the inco-ordination then appears more prominent owing to defective vision. At any time convulsions may occur.

The difficulty of describing in summary the positions of degeneration arises more particularly from the fact that often the amount of degeneration in the same tracts varies to some extent from level to level and degeneration may be evident in a tract at two levels in the

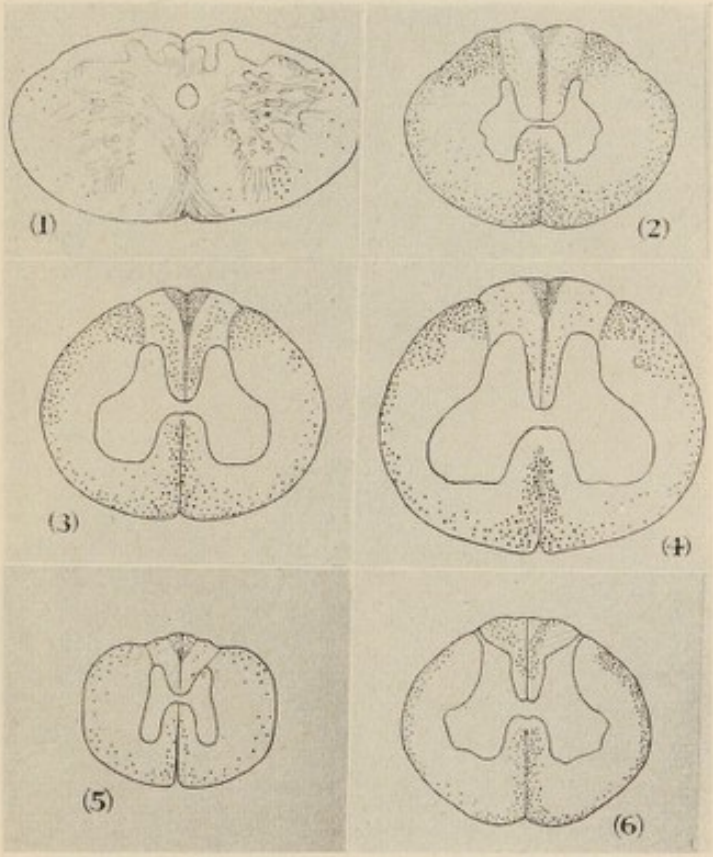

FIG. 10.—Drawings showing degenerating fibres at different levels of the spinal cord as prepared by the Marchi technique. The levels represented are: (1) medulla; (2) 2nd cervical; (3) 4th cervical; (4) 6th cervical; (5) 7th dorsal; (6) 4th lumbar. The column of Goll in this animal shows degeneration—a relatively rare occurrence.

cord whereas at an intermediate level little or no degeneration may be found in the corresponding area.

Thus, of all cords examined up to the present time and showing degeneration in the region of the first cervical, 72 per cent. of cases had degenerated fibres in the column of Burdach, whereas in the same cords in the region of the sixth cervical only 52 per cent. showed degeneration in this tract. Again in the direct cerebellar tract: 44 per

cent. of sections of the first cervical region showed degeneration; 62 per cent. of sections of the third cervical region showed degeneration; 36 per cent. of sections of the sixth cervical region showed degeneration. This irregularity in the appearance of degeneration is apparently a

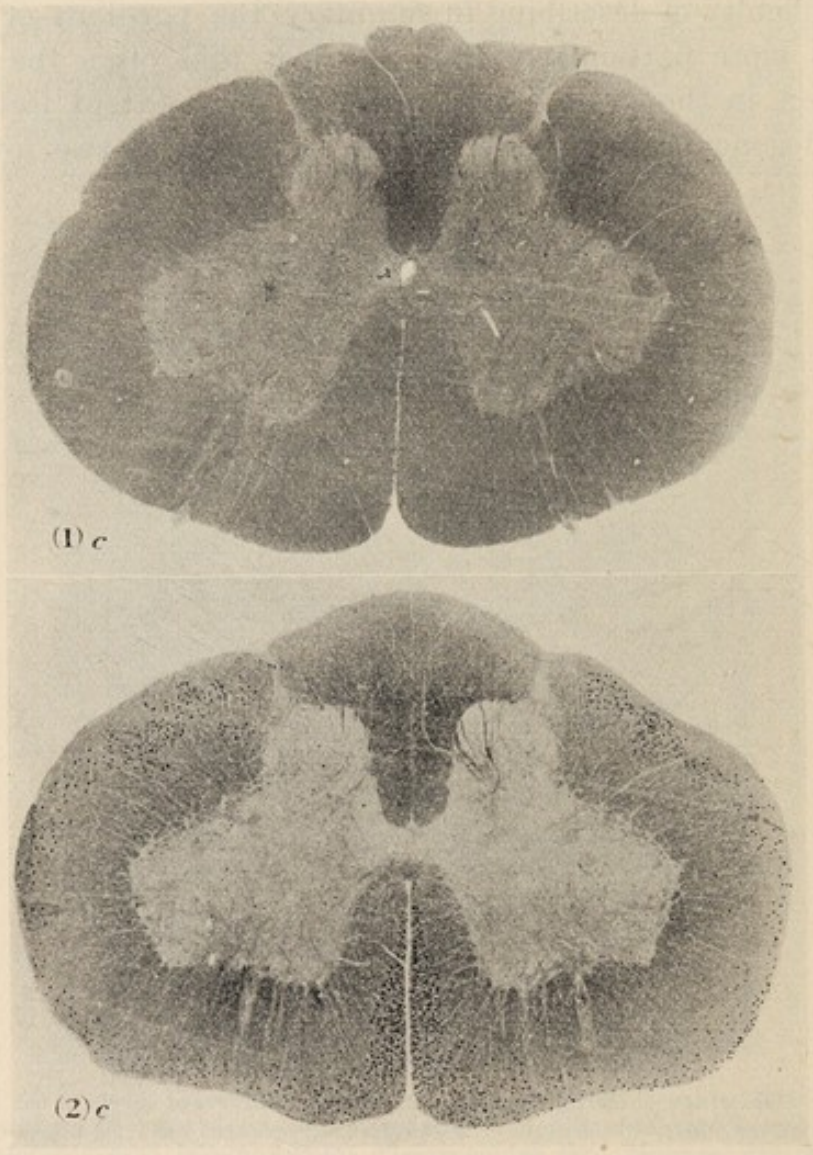

FIG. 11.—Photomicrographs of sections stained by Marchi technique of animals (*c*) and (*e*) of Experiment XII at a level of the 6th cervical. Note the absence of all degeneration from (1) (carotene plus ergot) and the abundant degeneration in (2) (ergot). (Retouched.)

condition also found in the demyelinated fibres of sub-acute combined degeneration associated with pernicious anæmia.

Some other points as regards areas of degeneration demonstrated in these experiments by the use of Marchi and Weigert stains may be mentioned:—

(1) Degenerative changes are more prominent in the cervical than in the lumbar region (fig. 9).

(2) The anterior columns are most affected whichever region of the cord is investigated (fig. 10).

(3) The anterior columns (represented in the human cord by the direct pyramidal tract, a tract said not to be present in the cord of the dog), the antero-lateral tract, especially superficially, the column of

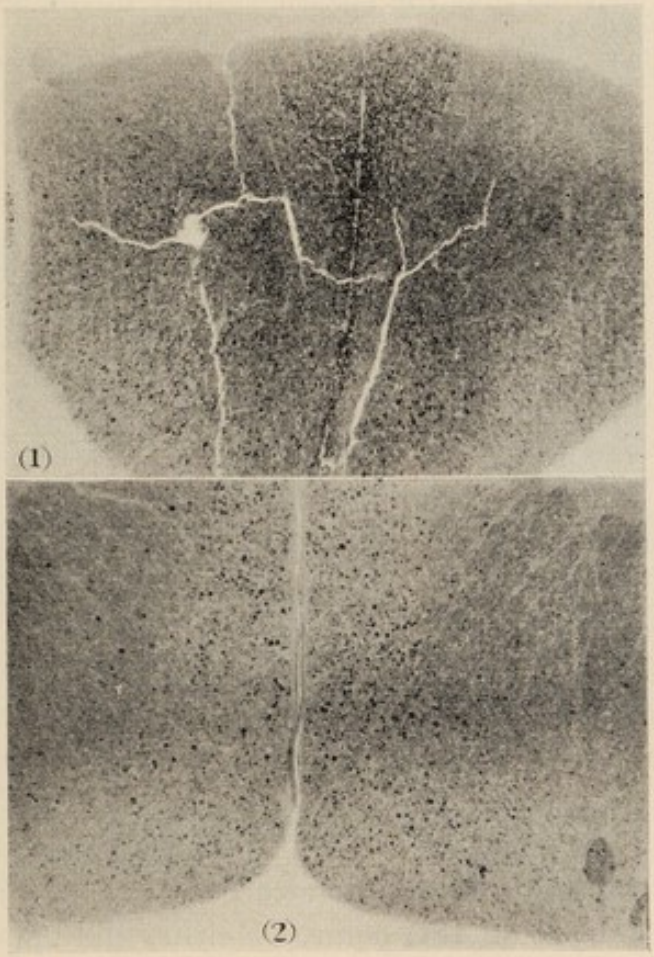

FIG. 12.—Photomicrographs showing cord degeneration by the Marchi technique in (1) posterior columns (note the degeneration in Goll's column) and (2) anterior columns.

Burdach, and the direct cerebellar tracts are the positions most commonly associated with degeneration. In the direct cerebellar tract, the degenerated fibres are often found near the surface, but sometimes they are deeper, away from the surface, whereas sometimes both areas are affected. The fibres of Goll's column are not often affected.

(4) The crossed pyramidal tracts are usually but not always free from degeneration.

(5) The indications are that the ascending fibres of the cord are more particularly picked out, including those passing to the cerebellum.

Areas of degeneration at different levels in the cord can be seen in figs. 9 and 10. The point of interest of fig. 9 is that it shows degeneration in the column of Goll, a rare occurrence in these experiments.

The spasticity of the limbs suggested that the upper motor neurone would show extensive degeneration, but this does not appear to be the case. It seems possible that the spasticity may ultimately be related to

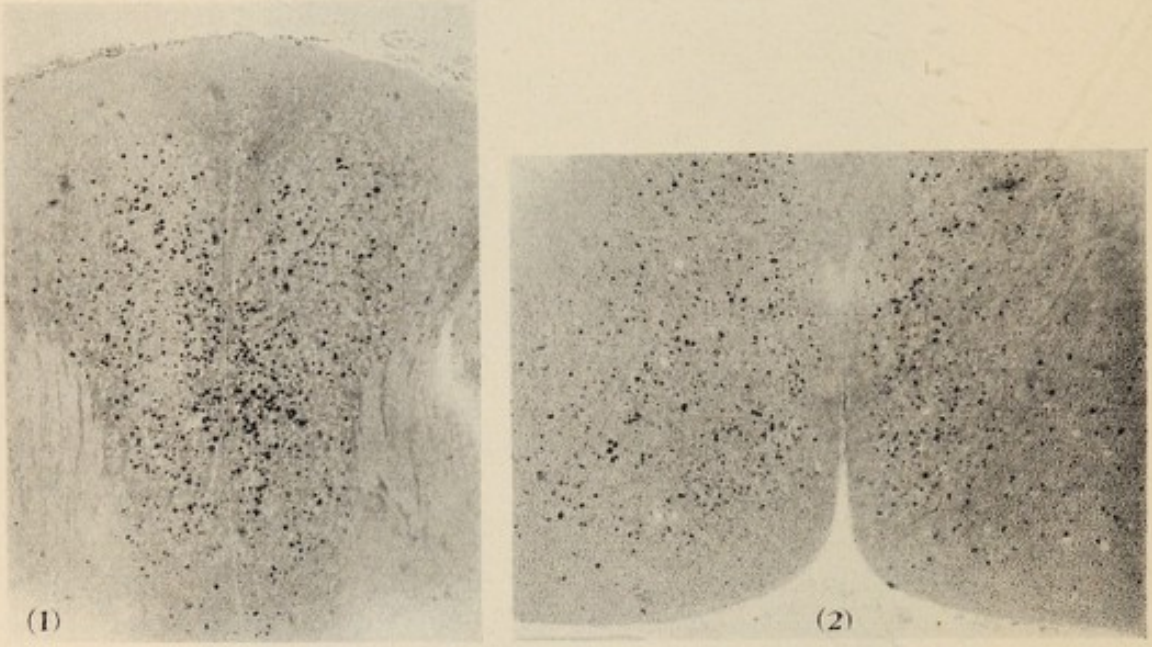

FIG. 13.—Photomicrographs of the spinal cord at the level of the 1st cervical, stained by Marchi technique. (1) Posterior columns (degeneration in Burdach only, the usual occurrence) and (2) anterior columns.

changes in the cord and mid-brain concerned with posture but nothing definite can be said yet on this point. Neither the upper parts of the brain nor the mid-brain has yet been fully examined histologically. The fits, stupid appearance, inco-ordination and loss of balance suggest that further extension of the examination to the upper parts of the central nervous system will show degenerative changes.

The changes in the cord do not seem to be accompanied by any cell invasion or other indication of an inflammatory nature. Neuroglial overgrowth in the positions of degeneration is not great but the blood-supply to these areas appears in Weigert-stained sections to be increased. Probably the relative smallness of sclerotic growth is due to the shortness of the experiments. In longer experiments, e.g., thirty-

one weeks, larger areas of degeneration can be seen when the cord is stained by Weigert's method.

The distribution of cord degeneration suggests strongly that the fibres going to the cerebellum are involved. The clinical condition (inco-ordination, loss of balance) also agrees with this suggestion.

The relation of the clinical condition to the cord changes is not easy to interpret, partly because of the interference of other factors and

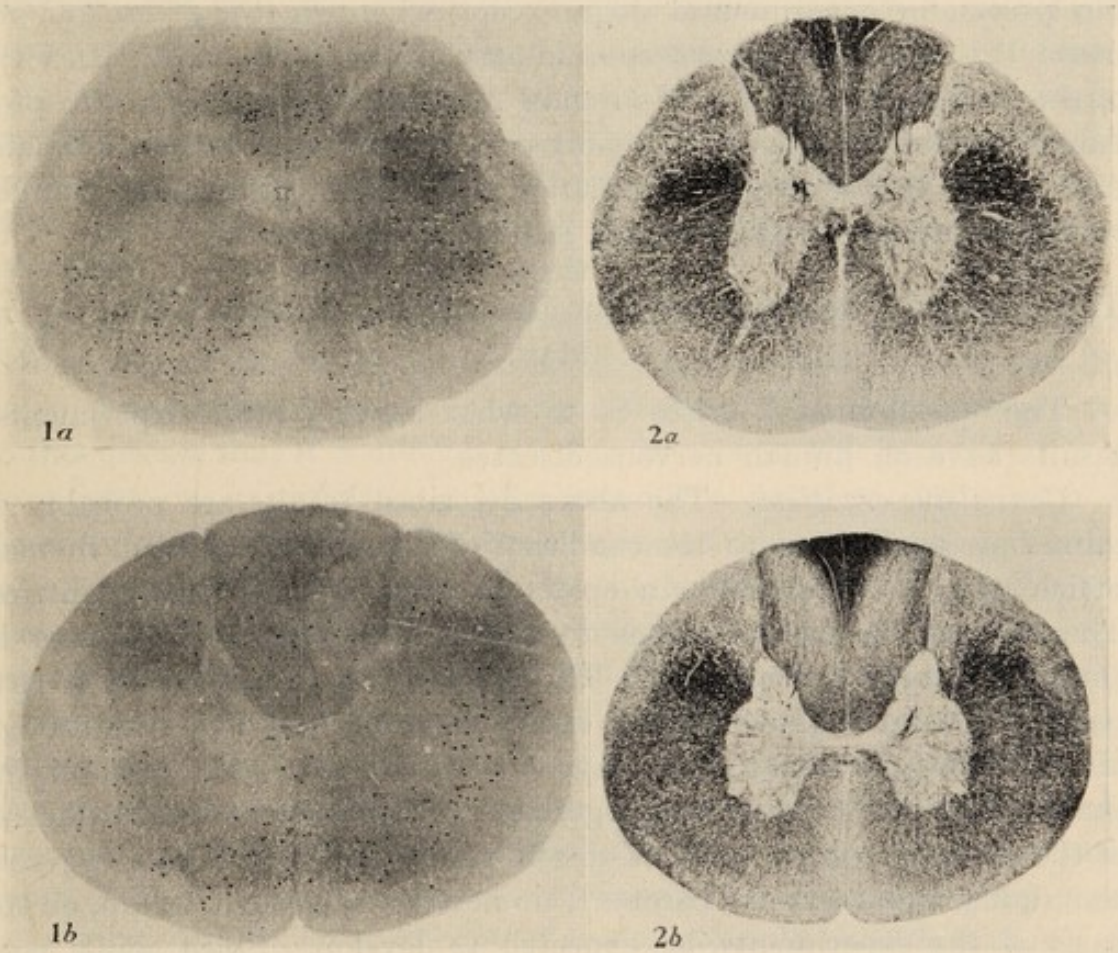

FIG. 14.—Photomicrographs of the 2nd and 4th cervical regions of one cord showing degeneration by (1) Marchi and (2) Weigert technique, respectively, after about five months of ergot feeding (without vitamin A or carotene). 1 (*a*) and 2 (*a*) are photographs of the 2nd cervical level; 1 (*b*) and 2 (*b*) of the 4th cervical level.

partly because of the obvious difficulty of investigating sensory changes in animals. For instance, muscular weakness is undoubtedly to be partly explained by the intrinsic failure of the muscle and may be independent of nervous defect. Again, pathological changes in the eyes are often found which interfere with vision. Sometimes the eyes are affected by xerophthalmia due to a deficiency of vitamin A, but in dogs

receiving a diet deficient in this vitamin and containing ergot another inflammatory condition of the eyes, often affecting the conjunctiva and becoming in some cases suppurative, is sometimes seen. Together with the conjunctivitis, a vascularized keratitis may develop. (I am indebted to my colleague, Mr. Wellwood Ferguson, for examining the eyes of some of these animals.) In one group with vascularized keratitis examined by him, no organisms were grown from the pus exuding from the eyes). This suppurative condition of the conjunctiva and cornea is rarely seen in experimental dogs except when ergot is given, and no doubt this substance plays a specific part in its development. In view of the well-known relation of vitamin A to hemeralopia, it is also probable that the vision is affected more centrally in many of these animals. An attempt to disentangle the relation of all these pathological changes to the behaviour of the animals is now being made.

The Possible Bearing of the Results to Nervous Disease in Man.

The question now arises as to what bearing these experimental results have on human nervous diseases.

Convulsive ergotism.—The above described results are probably of immediate application to the condition of convulsive ergotism in man which appears occasionally in epidemic form in rye-eating countries. They suggest an explanation as to why such epidemics only appear in times of famine and poverty. The explanation usually given is that people eat more ergotized bread during such periods, but this, though true in itself, probably only plays a comparatively small part in the problem. If the people eating ergotized rye bread also received sufficient of the protective foods such as green vegetables, butter and egg-yolk containing vitamin A and carotene, no nervous symptoms would, on the basis of the experiments, be expected to develop. Since spinal cord degeneration has been found to develop in many animals in the absence of vitamin A even when no ergot was included in the diet, it is probable that in human beings also the pathological changes ascribed to ergot may in some cases be due to unergotized rye bread itself when eaten under conditions of restricted protective foods. Another point of interest is that in epidemics of nervous ergotism some members of a family may be attacked and others escape. The reason for this indiscriminate picking out of individuals may be related to the reserves of vitamin A in the livers of the individuals. In my experience with dogs, I have found degenerative changes in the spinal cord only when

the liver was depleted of vitamin A. The periods required for this depletion vary greatly in animals according to the other factors of the diet and to the amount of vitamin A initially stored, and the latter will be a crucial factor in the time of onset of nervous ergotism in individuals.

If the experimental results can be directly extended to human nervous ergotism, as seems likely, they indicate that not only can the condition be prevented by supplying foods containing vitamin A and carotene, but also that when the condition has developed immediate improvement in health will follow the administration of such foods. Complete cure is unlikely since nerves in the central nervous system cannot regenerate, and the extent to which function returns will depend on the extent and intensity of the nerve degeneration at the beginning of treatment, but in animals at least the "curative" effect as indicated by clinical improvement is very great.

Pellagra.—The cord changes produced in some of the experimental animals are so similar to those associated with pellagra in man, and the mode of producing them is so closely related to the conditions conducive to this disease that it is difficult to avoid the assumption that they throw new light on its ætiology. Like convulsive ergotism, pellagra is a disease of poverty and is often but not exclusively found among maize-eating populations, and especially in the southern part of the United States. Two types of pathological change are found in this disease—one affecting the skin, especially the hands and parts of the body exposed to sunlight, and the second affecting the nervous system and producing both cerebral and cord symptoms. It is natural to suppose that the skin changes are secondary to those of the nervous system, and indicate a release of the trophic control of the affected skin areas. On the other hand, the two types of change are sufficiently dissociated in many cases as to suggest that two mechanisms are responsible—the breakdown of one resulting in the skin changes and of the other the nervous changes. Experimental evidence also supports the suggestion of a double mechanism which may, however, in some way at present unknown work in harmony. Since the work of Goldberger and Tanner [24] it has been widely accepted that pellagra is a deficiency disease due to the absence from the diet of sufficient vitamin B_2 (or vitamin G of the U.S.A.). This conclusion depended on the curative effects on the skin lesions of pellagrins produced by a series of substances such as yeast, meat and milk. The group of foods having this curative effect was such as to preclude the action being due to a then known vitamin, and the

hypothetical active substance was called the P-P, or pellagra-preventing, factor. Confirmation of this work from another angle seemed to be forthcoming in animal experimental work. The condition known as "black tongue" in dogs was regarded by Goldberger, Wheeler, Lillie and Rogers [25] as analogous to pellagra in man and reacted to the pellagra-preventing factor as did pellagra itself. Goldberger and Lillie [26], and also Chick and Roscoe [27], extended the investigations to rats and in consequence of the skin lesions produced in rats by diets deficient in vitamin B_2, Chick and Roscoe called this substance the anti-dermatitis factor. Recent work described by Aykroyd [28] has, however, led to some doubt as to the identity of vitamin B_2, in so far as the properties of this substance have been elucidated by rat-feeding experiments, and the P-P factor and the problem must still be regarded as unsettled.

Now the results described above suggest that vitamin A or carotene also plays a part in the prevention of pellagra or at least in its nervous manifestations. There is no reason to believe that the experimental diets of the dogs were deficient in vitamin B_2 for they contained separated milk, yeast, meat and often wheat germ, all sources of this substance. On the other hand, sources of vitamin A or carotene prevented the nervous degenerations completely, so that it seems probable that pellagra is due to a double deficiency in the food—the skin changes resulting from a B_2 deficiency and the nervous changes from an A deficiency. The experimental results described above in which the effect of yellow and white maize were compared, confirm the suggestion that a deficiency of vitamin A and carotene is the cause of the nervous changes in pellagra. Yellow maize containing carotene did not allow the spinal changes to occur, but actually prevented such changes being produced even by ergot. When white maize formed the cereal basis of the diet the nerve degeneration appeared (see p. 26). Again, the geographic distribution of pellagra in man seems to be in accord with these results. In America, and especially in the Southern States of the U.S.A. where pellagra is very prevalent, white maize or corn is generally eaten. In Java and the Dutch East Indies where yellow maize is eaten (although how extensively as compared with white maize I do not know), Jansen and Donath [30] report that pellagra is almost never seen. The above suggestions also receive support from observations published in 1928 by Underhill and Mendel [31] who described the effect of various substances, and especially of carotene (5 mg. daily), in curing black-tongue in dogs, a condition referred to above as being cured by the P-P factor of

Goldberger. Underhill and Mendel [31] confined their attention particularly to the mouth and alimentary canal and general condition of the experimental animals, and did not examine the nervous system. Incidentally, it is clear that the condition of "black tongue" as investigated by these various workers cannot be the same disease.

The final point as regards the ætiology of pellagra requiring consideration is whether maize or other cereals play an active part in the development of the disease owing to the presence in it of a toxic factor. Some of the experimental results above described suggest such to be the case. If this is true, then the embryo of the maize is probably richer in this toxic substance than the endosperm. I attempted to test this point experimentally but the results were too indefinite to allow any conclusions to be drawn.

To sum up, the evidence suggests strongly that a deficiency of vitamin A or carotene is the deciding factor in the development of nervous degenerative changes in pellagra. Deficiency of the pellagra-preventing factor seems to be responsible for the skin changes and there is a possibility that a toxic element in maize and other cereals, which is ineffective in the presence of sufficient vitamins A and B_2, must receive consideration. It seems probable that pellagra can be completely prevented and cured by the inclusion in the diet of green vegetables, carrots, milk, butter, egg yolk and mammalian liver. It is possible that, when the condition is very severe, the alimentary disorder may prevent the absorption of these foodstuffs and, if so, it will be necessary to inject carotene or concentrated vitamin A preparations intravenously.

Lathyrism.—Lathyrism, so extensively seen in India, is a disease of which I have no direct knowledge, but the clinical and pathological descriptions and history suggest that the experimental work on dogs may also throw some light on its ætiology and treatment. Clinical descriptions of lathyrism and the dietetic conditions under which it develops have been published by Stockman [32] and Acton [33] and others. The main facts appear to be that it develops in people : (1) who eat largely of lathyrus sativus peas ; and (2) in times of famine and drought when their diet is largely restricted to these peas.

There is still some doubt as to the causal agent of lathyrism. For instance, Anderson, Howard and Simonsen [34] found that the seeds of khesari (*Lathyrus sativus* L.) were harmless to ducks and monkeys, whereas akta (*Vicia sativa* L. var. *angustifolia*) produced symptoms in pigeons, ducks and monkeys. Attempts have been made by Stockman

[35], Dilling [36] and Acton and Chopra [37] among others to isolate a toxic agent from lathyrus peas. Stockman [35] found an alkaloid in small quantities which he thought might be responsible for the condition; Acton and Chopra [37] found an amine, and Anderson, Howard and Simonsen [34] thought that the base divicine found in akta might be the toxic agent. Dilling [36] isolated two alkaloids in very small quantities which caused increased spinal reflexes followed by paralysis in frogs, and weakness and paresis of the hind limbs of mice. Acton and Chopra [37] state that the toxic factor can be removed from the peas by soaking them in water.

Experimental work on animals with the object of producing lathyrism has never been very satisfactory. In no case, so far as I know, has good evidence been adduced that the weakness and spasticity produced in animals by diets containing lathyrus peas or by injecting chemical preparations made from these peas are associated with the degenerative cord changes seen in human lathyrism. Many of the experimental diets used by workers have been so defective that it would be surprising if the animals had not developed severe illness independently of the specific signs and symptoms of lathyrism. With the knowledge now at hand it ought to be simple to produce in experimental animals the spinal cord degeneration found in human lathyrism if the diets are more complete than those previously used by investigators and yet deficient in vitamin A and carotene. I have not yet been able to investigate this problem owing to the difficulty of getting a supply of the peas, but in a casual experiment I was interested to find characteristic cord changes in one animal whose diet consisted of beans 30 to 150 grm., separated milk powder 20 grm., meat 10 grm., yeast 5 grm., orange juice 3 c.c., sodium chloride 1 grm. (see Exp. XIV). Whether this result was due only to a deficiency of vitamin A, or to this deficiency acting in association with a toxic factor in the beans, cannot be stated. Another dog on a similar diet with the addition of cod-liver oil did not develop any degenerative changes in the spinal cord. The beans used in this experiment were dried haricot beans. Animals receiving dried peas did not, during the short period of the experiment, show any degenerative changes. I hope, however, to be able to investigate this problem in the near future.

It ought also to be an easy matter to see whether vitamin A or carotene has the specific curative and preventive effect in human lathyrism. As the result of a field study of lathyrism in India, Young advanced the suggestion that lathyrism is primarily a deficiency

disease, and that vitamin A deficiency is the factor concerned. While the facts adduced by Young could be equally well used to support the possibility of other deficiencies being responsible for this disease—for instance, a deficiency of vitamin B_2—the experimental results above described are in support of Young's suggestion.

EXPERIMENT XIV.

Spinal Cord Degeneration in a Dog whose Diet included Dried Haricot Beans.

Age beginning: $10\frac{1}{2}$ weeks.
Duration of experiment: About 3 months.
Basal diet: Separated milk powder, 20 grm.; lean meat, 20-10 grm.; peanut oil, 10 c.c.; orange juice, 3 c.c.; dried yeast, 2·5 grm.; salt, 2 grm.: irradiated ergosterol (vitamin D) 0·05-0·1 c.c.

Animal	Diet variable	Cord degeneration as shown by Marchi staining in sections examined	Clinical notes
(*a*)	Dried beans	Degeneration in anterior, antero-lateral columns and direct cerebellar	Very quiet; gait not normal, but no very definite symptoms noticed
(*b*)	Dried beans Cod-liver oil	No degeneration	Normal

CORD CHANGES IN PERNICIOUS ANÆMIA.

Finally, the problem arises as to whether these experimental results have any relationship to the subacute combined degeneration of the cord associated with pernicious anæmia. It must be stated at once that the discussion on this point is largely hypothetical, and my own observations on this disease, although of an elementary nature, make it clear that the problem in this case is not so directly related to the animal results as are convulsive ergotism, lathyrism and pellagra. At the same time the demyelination of the cord fibres often found in pernicious anæmia is so characteristic a change that its production and prevention by the above described methods suggest that there may be a similar specific factor or factors involved in both the clinical and the experimental conditions. Nor is the exceptional nature of this pathological condition the only reason for suspecting some common factors in ætiology. Whatever the primary cause of pernicious anæmia may be, there seems little doubt that the liver is implicated in this disease since the administration of liver (Minot and Murphy [39]), by supplying a hormone (a compound containing β-hydroxyglutamic acid (West and Howe [40]) and ι-γ-hydroxyproline (Dakin, West and Howe [41]), brings about great improvement in the blood condition. There is,

however, general agreement that watery extracts of liver, while they cure the blood condition over the period, do not stop the degeneration of the cord. Indeed, it is said that the nervous symptoms are often more severe to-day, since the liver treatment was introduced, than they were previously; for, whereas the blood condition is generally brought back to normal by liver extracts, and the life of the patient lengthened, the cord changes become progressively worse. Ungley and Suzman [42] have recently found that whereas the nervous symptoms often become worse during treatment with liver extracts, curative changes and general improvement both in the blood-picture and in the nervous condition result when whole liver is administered. If this is the case, it seems probable that the substance present in the whole liver, and absent from the extracts of the liver, acting in this way on the nervous system, is vitamin A. My own experience in connection with the cord changes of pernicious anæmia is very small. The nervous symptoms in one patient under my charge suffering from pernicious anæmia became definitely worse when ordered whole liver, and later, liver extract and cod-liver oil. Unfortunately, she was an out-patient during treatment, so that it is very difficult to make any deductions from this case. She was admitted to hospital later in a comatose condition, with a bacterial infection of the kidneys, and post mortem her liver contained only a small amount of vitamin A (79 blue units per gramme), although much more would have been expected in view of her suggested treatment.

It appeared to me at one time possible that the liver in pernicious anæmia could not store vitamin A in a normal way, but this cannot be always the case because in another patient suffering from pernicious anæmia who had died of a pyelo-nephritis the liver was found to be very rich in vitamin A (10,560 blue units per gramme of liver). This liver was kindly sent to me by Dr. Perdrau. It is possible, of course, that although the pernicious anæmia patient may be able to store vitamin A, there is sometimes a failure of an unknown nature which prevents this substance exerting its normal function on the central nervous system. An analogous instance suggesting the possibility of such an explanation is seen in diabetes mellitus, since, according to Pollak [43], the pancreas of patients deceased of this condition usually contains insulin in fair quantities, i.e., roughly half that of normal people.

The two main pathological conditions associated with pernicious anæmia, i.e., the blood changes and the cord changes, so often develop independently and react so differently to treatment that the idea that each is controlled by a separate chemical mechanism seems likely. It

would certainly be interesting if both of these chemical failures could be associated with failure of one organ, viz., the liver. It is obvious, however, that the known facts of this problem are too few at present to allow anything more than surmise, but the hypothesis has sufficient support to warrant further investigation along these lines.

It is also clear that there is a possibility of a relationship between this experimental work and other clinical conditions associated with degenerative changes in the spinal cord. Subacute systemic lesions of the cord, regarded by Orr and Rows [5] as falling in the hæmatogenous category, are also found in Addison's disease and cancer cachexia, and it would be of interest if it were found that carotene and vitamin A prevented or influenced these changes. On the other hand, there are the degenerative cord changes found in tabes dorsalis and general paralysis and said by these same workers to arise from toxins ascending the peripheral nerves in the lymph stream, which must receive consideration from this new angle. Again, the well-known remissions seen in disseminated sclerosis suggest that there may be some protective mechanism of the nervous system such as is exerted by carotene and vitamin A, which at times comes into action and not only prevents extension but even, in the early stages at least, has a curative influence. It is evident that in this clinical condition also the protective effect of these substances is worth testing. It may be, of course, that the protective influence of carotene and vitamin A can only be exerted in the case of specific nerve toxins obtaining access to the nervous system in a particular way, for instance, via the blood-stream and not via the lymph. Or, it may be that they readily antagonize and prevent the changes associated with a high cereal diet and ergot but give no protection against the toxin of treponema pallidum or that producing disseminated sclerosis. At all events these points can be easily tested clinically.

It is true that so far as this experimental work is concerned pathological changes in the spinal cord have only arisen as the result of a gross deficiency of vitamin A or carotene in the diet. It is doubtful, however, whether under ordinary dietetic conditions a deficiency so intense is of common occurrence in this country. On the other hand, an insufficiency of vitamin A and carotene in the general diet of highly organized communities is undoubtedly very widespread. The question arises as to whether the partial deficiency of these protective substances is responsible for slighter, but possibly more frequently seen, abnormalities of the nervous system. For instance, the effect of deficiency in

vitamin A intake in producing epithelial hyperplasia often associated with bacterial invasion suggests that this substance may have a similar influence on the development of nervous tissue. Women in pregnancy not only have to be provided with sufficient vitamin A to keep their own tissues working but must themselves pass on large quantities to the developing fœtus. Does the nervous system of the infant suffer when this supply is deficient? How such a partial deficiency of vitamin A or carotene might influence the human nervous system at different periods of life is not known. Anybody with experience of feeding young animals on experimental diets must be impressed by the difference in energy and activity among puppies brought up respectively on diets rich and poor in vitamin A. Similar differences in human dietary in early life may be of equal significance.

As regards the effect of gross deficiency of vitamin A described above, although all attention has been given to the spinal cord it must be added that the mental changes evidence by the general behaviour of the animals are also great and demand investigation. It is indeed clear that the functioning of the more highly developed parts of the brain is greatly influenced by dietetic constituents and the possibility that cerebral development and activity are to some extent retarded by dietetic defects short of those tested in this experimental work must be borne in mind.

One final point must be referred to. The anti-infective action of vitamin A and carotene is on a firm experimental basis. Clinically the uses and limitations of this action have not been worked out, but even so experience indicates that the phenomenon will undoubtedly prove to be of significance in the case of some human infections. The question presents itself, whether the protective effect of vitamin A and carotene against demyelination of nerve fibres and their anti-infective action against some kinds of bacterial infection may not have special significance in the case of the nervous system. Is it not possible, for instance, that the beneficial effect of the malarial treatment of general paralysis of the insane is due to the liberation of large quantities of vitamin A and other protective factors from the livers of patients infected with the malarial parasite? It might be worth while before giving malarial treatment in such patients to fill their liver up with this vitamin by giving diets rich in this substance and cod-liver oil. It is possible that the uncertainty of this strange form of treatment would disappear if this factor were taken into consideration.

The foregoing experimental work demonstrates that nutritional influences on the central nervous system, both in the sense of protec-

tive and harmful action, are sufficiently important to warrant their consideration in the clinical investigation of many pathological changes of the nervous system.

Summary.

(1) When diets containing a large amount of cereal (other than yellow maize) and deficient in vitamin A or carotene are eaten by young puppies, degenerative changes in the spinal cord in the form of demyelination of the nerve fibres, which can be readily observed by Marchi's method of staining, can generally be observed.

(2) The addition to the diet of 2 to 5 grm. of ergot daily under these conditions hastens and intensifies these degenerative changes. Rye germ also hastens the changes and in some experiments wheat germ has been found to have a similar but less pronounced effect.

(3) The presence in the diet of any rich source of vitamin A such as liver oil (mammalian or fish), whole milk, butter or egg yolk or some source of carotene such as green vegetables or carrots, or carotene itself, prevents or diminishes these degenerative changes even when ergot is eaten.

(4) Spinal cord degeneration of this type does not seem to develop until the reserves of vitamin A in the liver are dispersed. Since these stores may be very large in well-fed animals, the time of onset of the symptoms of spasticity, inco-ordination and weakness varies greatly in different litters of animals. In the case of ergot, the changes are seen usually after two to four months on the diet.

(5) Not only do vitamin A and carotene prevent the degenerative changes but even in affected animals, the improvement in the clinical condition on the addition of either of these substances to the diet is very great.

(6) The possible bearing of these results on convulsive ergotism, pellagra, lathyrism and the subacute combined degeneration in pernicious anæmia, tabes dorsalis, disseminateds clerosis and even on the malarial treatment of nervous syphilis is discussed.

The expenses of this investigation were covered by a grant from the Medical Research Council, to whom my thanks are due.

I wish to acknowledge with gratitude, the assistance I have received in the course of this work from Mrs. Mellanby, Miss Ella Surie and Miss Joan Hopgood. I have to thank also the British Drug Houses, Ltd., for supplying the vitamin D in the form of irradiated ergosterol (radiostol) and vitamin A (mammalian liver oil) used in this experimental inquiry.

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