

A CEREBELLAR DISORDER IN CHICKS, APPARENTLY OF NUTRITIONAL ORIGIN*

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PLATES 2 TO 4

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In the course of certain experiments having for their object a study of vitamin E deficiency in fowls, an interesting nervous disorder appeared amongst two small groups of White Leghorn chicks. The disease was manifested only in birds receiving certain simplified diets; it was accompanied by characteristic symptoms and by uniform and well defined lesions of the cerebellum.

I. Diets.—The simplified diets were similar to those used by Evans and Burr (1) in vitamin E studies upon the rat, except that roughage was supplied by the inclusion of 10 per cent of paper pulp (Hart, Halpin and Steenbock (2)). Their composition was as follows:

<i>Group I. Diet 107</i>	<i>per cent</i>	<i>Group II. Diet 108</i>	<i>per cent</i>
Skimmed milk powder, Merrel				
Soule.....	15			15
Casein, Merck's technical.....	15			20.5
Cornstarch.....	45			20
Lard.....	3			21
Cod liver oil, Mead's.....	2			2
Yeast, Fleischmann's bakers'				
dried.....	5			5
Salt mixture, McCollum 185 (3).	5			6.5
Paper pulp, Eastman.....	10			10

The chicks were hatched on June 8, 1930. Before being placed upon these simplified diets, they were started in batteries upon the stock

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Diet 20 consisting of mash, made by a local dealer, and supplemented with skimmed milk powder, cod liver oil and yeast.

		<i>per cent</i>	<i>per cent</i>
Mash	Coarse yellow corn meal.....	27.5	
	Wheat bran.....	20.0	
	Wheat flour middlings.....	15.0	
	Ground oats.....	13.5	
	Alfalfa leaf meal.....	7.5	
	Bone meal.....	5.0	
	Skimmed milk powder.....	3.5	
	Calcium carbonate.....	2.0	
	Sodium chloride.....	1.0	
	"Meato".....	5.0	
		81.0	
	Skimmed milk powder.....		15.0
	Cod liver oil.....		2.0
	Yeast, Fleischmann's bakers' dried.....		2.0

The similarity of these three diets with respect to their calculated nutritive value is shown in Table I.

Diets 107 and 108 are seen to differ only in the ratio of fat to carbohydrate. The protein in the diet is of animal origin, save that supplied by the yeast. As regards vitamin content, all diets include sufficient cod liver oil to satisfy the requirements for vitamin A and D. Vitamin B complex is furnished by 5 per cent Fleischmann's dried yeast and milk powder in the simplified diet, and by the cereals and milk powder in the stock diet. No provision is made for vitamin C (Hart, Halpin and Steenbock (2)); and while Diets 107 and 108 are undoubtedly low in vitamin E, the stock diet contains ample. On June 18, when the chicks were 10 days old, the stock diet was replaced in increasing proportions by Diet 107 or Diet 108. On June 29, the stock diet was entirely withdrawn, and the chicks given only Diet 107 or 108. Twenty-seven other chicks of the same hatch were continued upon the stock diet, and served as controls. Up to the present time, they have shown excellent growth and normal appearance and behavior. The peculiar disorder which forms the subject of this paper, was observed only in chicks maintained partly or wholly during their growing period upon the simplified diets.

Reference to the accompanying growth chart will show that the general growth of the chicks on Diets 107 and 108, while not so good

as the controls, compares favorably with that given as "normal" by Card and Kirkpatrick (4), and exceeds that given as "nearly normal"

TABLE I
Nutritive Value of Diets

Diet	Per cent indigestible	Per cent protein	Per cent COH	Per cent fat	Per cent ash	No. of calories per 100 gm.	Nutritive ratio	Ca/P ratio
107	10.0	18.2	54.6	5.2	6.7	338	1:3.6	1.5
108	10.0	22.8	26.6	23.2	8.4	407	1:3.4	1.5
Stock 20	13.5	14.9	42.6	5.1	10.6	285	1:3.8	1.8

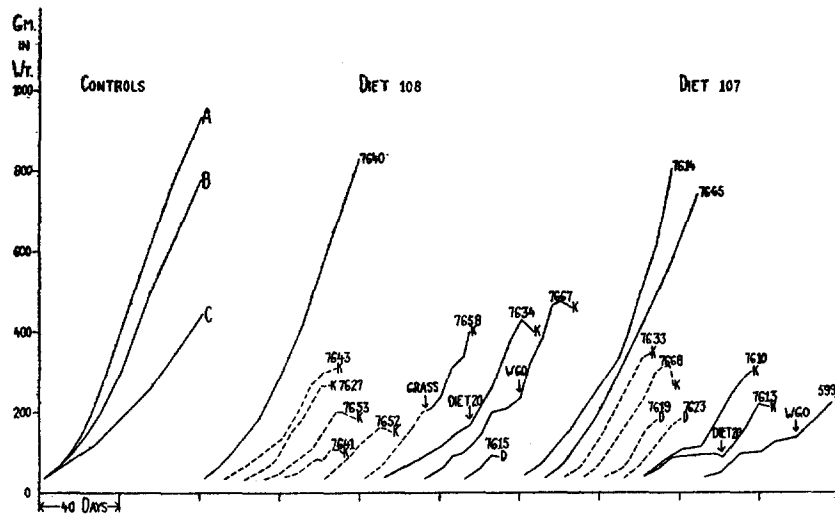


CHART 1. Showing rate of growth of chicks on Diets 107 and 108. Chicks showing cerebellar disorder are charted in dotted line.

A—Composite growth curve of 27 control chicks on Diet 20.

B—Composite curve given by Card and Kirkpatrick (4) as "normal" for chicks.

C—Composite curve given by Hart, Halpin and Steenbock (2) as "nearly normal" for chicks.

by Hart, Halpin and Steenbock (2). With the appearance of the characteristic symptoms, the weight curve in most cases remained

stationary, or declined, as one might expect from the inability of the birds to secure food.

II. Clinical Behavior.—Symptoms pointing to a derangement of function in the central nervous system appeared in many of the chicks with great suddenness after they had been on the diet 3 or 4 weeks. They suddenly became prostrated, lying with legs outstretched and spastic, claws flexed, and head retracted and often twisted, sometimes through a complete semi-circle (Fig. 1). Clonic spasms of the legs were often observed; sometimes coarse tremors. The eyelids drooped, and in the late stages, there was somnolence or stupor. Some of the chicks showed rotary movements to right or left, and before they became completely prostrated, many of them became incoordinate and ataxic, making rocking movements on their legs on rising, or tilting the body to one side or other. It is worth noting that even in the terminal stages of the disease, a true paralysis of the wings or legs never occurred.

The course, duration and intensity of the symptoms varied greatly in different individuals. Death occurred in one chick (7623) on the same day that the symptoms first appeared, and several others were killed on the first day when apparently moribund; others survived for varying intervals up to 16 days. In these, there were transient periods of improvement. As is shown in Table II a few birds showed very trivial incoordination which disappeared as they grew older.

The time elapsing from the institution of the diet to the first appearance of symptoms ranged from 9 to 38 days, the greatest number coming down with symptoms after they had been from 18 to 25 days on the diet. The youngest chick was 19 days old, the oldest 48. In one chick (7641) the symptoms first appeared while it was still receiving small amounts of the stock diet.

III. Pathological Changes.—The lesions found at autopsy were in general limited to the cerebellum, although in two instances, observed in a later experiment, they occurred only in the cerebrum. In most of the birds the alterations were readily detected with the naked eye, the cerebellum appearing swollen, softened, the convolutions flattened or malformed, and discolored reddish or brownish in the affected areas (Fig. 2).

The microscopic lesions, though varying greatly in their extent from small focal areas to large confluent patches involving the greater portion of the cerebellum (Fig. 3), were nevertheless strikingly uniform in character. The essential changes, which will be illustrated in greater detail by the presentation of individual protocols, were: (1) edema, with separation and disruption of the cellular and fibrillar elements; (2) degeneration and necrosis of the Purkinje cells, and of the small cells constituting the granular layer; (3) small hemorrhages scattered through the central white matter, or within the cortical zones; (4) hyaline capillary thrombi in and about the necrotic areas (Figs. 4, 5, 6).

The lesions in the early cases were thus typically degenerative or regressive in character. An inflammatory reaction was noted in but one case, a chick which survived for 12 days after the onset of the symptoms. In this instance, the cellular reaction was exclusively mononuclear, and of slight intensity.

Interesting reparative changes were observed in Chick 7658, which recovered after being given grass, and in which the previously degenerated tissue showed gliosis and calcification.

All other regions of the brain and cord appeared to be free from obvious lesions, although it is probable that the application of more refined neurological technique may disclose minor changes secondary to the cerebellar injury. With the routine methods thus far employed, they have not been noted.

The following protocols represent typical examples of the disease.

Chick 7652.—On June 28, was found reeling and badly picked by other birds in cage. Isolated. July 1, improved, and returned to cage. July 12, again incoordinate and weak. July 14, dying; spastic contractions of legs, with claws flexed. Head retracted. Stuporous. Crop empty. Killed.

Autopsy: No significant gross changes were found in the abdominal or thoracic viscera. The brain showed *softening* and *brownish discoloration of the cerebellum*.

Microscopic: The brain was fixed in 95 per cent alcohol. Sections through cerebellum disclosed marked lesions. There were numerous hemorrhages, chiefly in the white matter. Many of the Purkinje cells are degenerated, or actually necrotic, having lost their nuclear staining and being reduced to formless eosin staining masses. Hyaline thrombi are found in the capillaries in the affected areas, although the alcohol fixation makes one cautious as to this. The nuclei of the granular layer are more widely spaced than in the normal areas, and are

definitely shrunken and pycnotic. In places, they are undergoing fragmentation with the formation of small globular chromatin particles. The fibers of the central white matter are disrupted by edema.

In addition to these purely regressive changes, there is a mild inflammatory reaction. In the meninges, and about many of the small vessels, there is an increased number of mobile mononuclear cells, both lymphoid elements and large macrophages with vacuolated cytoplasm. One of the latter is found in mitosis. No bacteria are seen, and no definite cell inclusions can be recognized.

The *medulla* contains a small recent area of hemorrhage situated in the floor of the 4th ventricle near the lateral recess. There are no other pathologic changes. Sections through cerebrum, optic lobes, spinal cord and dorsal ganglia show nothing abnormal. The other viscera, and skeletal muscles show nothing abnormal.

Chick 7653.—On July 18, suddenly became prostrate and helpless. Lay with legs outstretched, head twisted (Fig. 1). When disturbed, made forced circular movements to left. Killed by decapitation.

Autopsy: On removing brain, a small hemorrhage was noted in cerebellum, the rest of the brain appearing grossly normal. There were small blotchy hemorrhages into the leg muscles, and into serosa over duodenum. The viscera were otherwise normal in appearance.

Microscopic: Cerebellum. The most striking feature is a zone of edema and rarefaction between the granular and molecular layers. The cells in this region are widely separated and the tissue is spongy—in places completely disrupted. Purkinje cells show marked degeneration. Many are densely stained with pycnotic shrunken nuclei and condensation of the Nissl substance. Others show vacuolar or hydropic swelling of the nucleus. Still others are completely necrotic, all nuclear structure being lost, and the entire cell reduced to an amorphous purplish or eosin staining mass. There are small hemorrhages, but a mononuclear reaction, such as was present in Chick 7652, is not found. Hyaline thrombi are seen within the capillaries in the more severely degenerated areas.

Sections through remainder of central nervous system—cerebrum, optic lobes, basal ganglia, medulla, spinal cord and dorsal root ganglia were carefully studied, but no lesions were found in H-E preparations. There were no visceral lesions.

Chick 7627.—Definite symptoms were first noted on June 18, when it was found helpless and stuporous, with head retracted. Unable to stand, but there were spastic movements of the legs, and no paralysis. The chick was killed by decapitation the same day, and the cerebellum removed. A part was used for the inoculation of other chicks, the remaining fragment fixed in 95 per cent alcohol. Grossly no lesions were noted at the time, but the fragment when sectioned after hardening, showed definite softening and brownish discoloration over a large area. Under the dissecting microscope, the tissue presented worm-eaten or a eroded surface in contrast to the surrounding smooth normal tissue.

Microscopically, sections through the fragment showed large areas of edema,

rarefaction and necrosis, without hemorrhage or inflammatory reaction. The remainder of the brain and the thoracic and abdominal viscera were normal.

Chick 7633.—Apparently well until June 22 when it was found lying on its side, with legs extended and spastic, head retracted and eyes closed. Stuporous, but struggled when aroused. Killed.

Autopsy: On exposing the brain, the caudal portion of the cerebellum was covered by edematous pia, and had a striking bluish discoloration, evidently from recent hemorrhage. This extended to the lateral aspects. The remainder of the brain, spinal cord and sciatic nerves were grossly normal. The thoracic and abdominal viscera were also normal.

Microscopic: Cerebellum. Whereas a portion of the cerebellar cortex represents a normal structure, the greater part has undergone extensive softening, and can be distinguished from the healthy tissue in the stained section by its lighter color. The lesions are identical with those seen in previous cases. Edema and disruption of fibers, degeneration or complete necrosis of Purkinje cells, pyknosis of the nuclei of the cells of the granular layer, scattered hemorrhages, and very extensive capillary thrombosis throughout the softened areas. The thrombi are particularly striking in sections stained with Mallory's phosphotungstic acid hematoxylin. There are no perivascular cell accumulations or other evidence of inflammatory reaction (Fig. 3).

In sections taken through the caudal portion of the cerebellum, the degenerative lesions are less pronounced, but there is massive hemorrhage into the central white matter of the lobules.

The remainder of the brain, spinal cord, sciatic nerve and other viscera exhibit nothing of interest. In the skeletal muscle of the leg, there are areas of edema and acute inflammatory lesions of mild intensity, probably due to the trauma inflicted by other birds in cage.

Chick 7668.—On June 20, found prostrate, legs extended, head twisted and retracted. Forced circular movements. Killed on June 22.

Autopsy: On exposure of brain, a faint yellowish discoloration and swelling of the cerebellum was noted, the rest of the brain and cord appearing normal. Upon transection after hardening in alcohol, a very distinct area of encephalomalacia characterized by softening and brownish color, was found to occupy the greater portion of the cerebellum. The best preserved tissue was on the surface, but in places the softening extended to the meninges. There were no gross visceral lesions.

Microscopically, the cerebellum showed massive necrosis, in places proceeding to actual liquefaction. This involved the entire central white matter, but in many places also the granular and molecular layers, reaching the surface of the lobules. The fibers were disrupted, the nuclei of the cells of the granular layer were shrunken, densely stained, often fragmented into small spherules diminishing in size to that of a large coccus. The Purkinje cells have lost their nuclei, and stain diffusely with eosin. The small capillaries are filled with pink staining hyaline masses.

The demarcation of the necrotic areas from the adjacent healthy tissue is in some places abrupt, in others gradual, but there is no limiting reaction. Fatty granule cells are not observed. The meninges are not noticeably altered, although there are one or two small extravasations of red cells, and about one of the cerebellar arterial branches, there is a heavy collar of lymphoid cells.

The medulla and optic lobes, which are included in the section, show excellent fixation and complete absence of lesions. Sections through cerebrum, corpus striatum, brachial enlargement of spinal cord show no lesions. The visceral sections are without interest.

These five cases, strikingly similar in their clinical behavior and in the character of the cerebellar lesions discovered at autopsy, are illustrative of those birds which die or are killed during the active period of the disease. But recovery may take place after a transient exhibition of symptoms, and the following protocol shows well the nature of the residual lesions in such a recovered case.

Chick 7658.—On July 3, 15 days after beginning the administration of Diet 107, the bird showed incoordination and weakness, and was badly picked by other birds in cage. It was isolated and given fresh greens. On July 18, it was still ataxic, swaying and stumbling when it walked. It continued to gain in weight, and remained in fair condition until August 1, when it was killed for examination.

Autopsy: One of the cerebellar lobules was sunken below the level of the adjacent lobules, and had an intense brownish color. The adjacent lobules had a pitted surface, and were also slightly brownish. Upon transection, after fixation in 10 per cent formalin, this brownish area was seen to extend 3 to 4 mm. into the substance of the cerebellum. Nothing otherwise abnormal was found.

Microscopic: An entire cerebellar lobule is found to have shrunken and degenerated, the normal zones and elements being replaced by an indifferent spongy glial tissue in which the cellular elements have no orderly distribution. In the better preserved areas, scattered Purkinje cells are recognizable, but many of them are impregnated with calcium. Many small irregular masses of calcium are scattered through the degenerated area. There are also numerous spongy phagocytes containing clumps of nonrefractile greenish brown pigment, which does not resemble hemosiderin (Fig. 7).

The atrophy and shrinkage of the cerebellar lobules is associated with marked edema of the overlying pia arachnoid.

Other blocks taken through the anterior portion show large areas of gliosis with pigmentation, loss of zonal architecture, disappearance of Purkinje cells. The region of the nucleus lateralis is severely affected.

Amongst the birds which we have studied, there have been two in which the lesions were localized in the cerebrum, and not in the

cerebellum. One chick was found to have a small area of necrosis in the cerebrum, in addition to the characteristic cerebellar lesions. The following protocol illustrates the cerebral localization of the lesions:

Chick 589.—Inoculated subdurally on July 18 with suspension of cerebellum from Chick 7653. Placed on Diet 108. Has remained stunted. On August 11, it was noted that there was marked tremor of legs, a tendency to turn to the right and to keep right eye closed. There was, however, no marked ataxia, and the symptoms seemed atypical. Killed August 12.

Autopsy: Brain showed opaque whitish areas over convexity of cerebral hemispheres on both sides. The cerebellum appeared externally normal.

Microscopic: There are marked lesions throughout both hemispheres of the cerebrum. These consist in large patches of necrosis, in which nuclear staining is lost. The capillaries are distended with hyaline plugs. Some of the areas are superficial, others are in the substance of the hemispheres, some abut upon the third and lateral ventricles (Fig. 8).

Most of the lesions show more or less replacement of the necrotic tissue at the margins by a loose, spongy and in places very cellular neuroglial tissue, in the meshes of which is much purple granular detritus, probably derived from disintegrated nuclear material. Often this is enclosed within mononuclear phagocytes (Fig. 9). The larger vessels are free from thrombi. The fresh necrotic areas are sharply demarcated from the adjacent healthy tissue, and bordered by fresh hemorrhage. *Cerebellum.* There are no lesions of the usual character found on Diet 108. Some of the Purkinje cells are sclerotic and stain densely, but none show complete necrosis. Optic lobes and medulla likewise normal.

Finally, we may present the case of a chick which remained practically free from symptoms, and in which the anatomical findings in the cerebellum were trifling and equivocal.

Chick 7643.—Received Diet 107 from June 18 to August 4. On July 30, it was noted that the chicken was a bit unsteady on its legs. Nothing else abnormal could be observed. The weight increased normally, and when killed on August 4, the bird seemed in excellent condition.

Autopsy: No gross lesions of brain or viscera.

Microscopic: The cerebellum with medulla and optic lobes attached was cut in complete series of 6 micra, every 10th section being mounted. No lesions were found in any portion of the cerebellum, except in Sections 47 and 48. Here there was an area of rarefaction in the central white matter of the convolutions. The nuclei of the glia cells in this area were slightly shrunken and denser than elsewhere. There were no hemorrhages, thrombi or necroses.

The study of this bird shows that slight transitory symptoms may occur with recovery, and without permanent anatomical lesions.

As regards the pathogenesis of the cerebellar lesions, we are inclined to regard the capillary thrombosis as the primary cause of the degenerative changes. When India ink was injected into the carotid arteries, the smallest and earliest lesions remained uninjected, so that occlusion of the capillary stream bed in the affected areas appeared to be a constant accompaniment of the lesions, and in all probability, their primary cause.

A general oversight of our material is given in Table II. Of the 19 chicks on Diets 107 and 108 (7614, 7640, 7665), 3 are still alive, having shown no clear cut symptoms of nervous disorder. They have gained normally and aside from lack of pigment in legs and iris, look and behave like healthy birds. Six chicks (7652, 7653, 7658, 7633, 7668) have shown severe and typical symptoms, and have had characteristic changes in the cerebellum. One of these, (7658) however, recovered partially from the symptoms coincidentally with the administration of green grass, and the cerebellar lesions at the time of death, though extensive, were completely healed. One chick (7643) had slight transitory symptoms, and minimal lesions, discovered only on serial section of the entire cerebellum. Four chicks showed characteristic symptoms, but the cerebellum was not studied. Of the five completely negative cases, 2 (7634, 7613) were transferred to the stock diet 20 on July 18, until they were killed after 25 and 32 days respectively; 2 (7667, 599) received wheat germ oil (550 mg. once weekly) by pipette during the period from July 18 until they were sacrificed on the 25th and 30th days. One (7610) received the unmodified Diet 107 throughout.

The fact that the symptoms and lesions developed only in chicks receiving Diet 107 or 108, and that the 27 other chicks of the same hatch maintained on the stock diet showed normal growth and behavior, seemed to incriminate the diet as in some way related to the disorder. However, it seemed desirable to ascertain whether we were dealing with an infectious disease, and the following experiment was planned to test its transmissibility.

On July 18, twelve 11-day old chicks were inoculated subdurally with a thin suspension in saline of the finely divided cerebellum of

Chick 7653—a case showing typical acute symptoms and lesions. All survived the injection, although three showed transient shock.

On July 22, 5 additional chicks of the same hatch, now 15 days old,

TABLE II
Showing Incidence of Cerebellar Disease in Chicks on Simplified Diets

Chick No.	Diet	Days on diet until		Clinical symptoms	Lesions	
		Appearance of symptoms	Death		Gross	Microscopic
7634	108 ⁽¹⁾	—	K 62	—	—	—
7640	108	35	Alive	±		
7641	108	9	K 21	+++	Brain not examined	
7643	108	17	K 17	±	—	± Serial sections
7652	108	12	K 27	+++	++	+++
7653	108	30	K 30	+++	+	++
7658	108 ⁽²⁾	15	K 43	++ Recovery	++	+++ Healed lesion
7667	108 ⁽²⁾	—	K 58	—	—	—
7615	108	—	D 10		Brain not examined	
7627	108	34	K 34	+++	—	++
7633	107	38	K 38	+++	++	+++
7665	107	—	Alive			
7668	107	32	K 34	+++	++	+++
599	107 ⁽³⁾	—	K 55	+ Atypical	—	—
7613	107 ⁽¹⁾	—	K 54	—	—	—
7614	107	—	Alive			
7619	107	18	D 18	+++	Brain not examined	
7623	107	20	D 20	+++	Brain not examined	
7610	107	—	K 49	—	—	—

⁽¹⁾ Diet 20 substituted on July 23.

⁽²⁾ Grass was given on July 10 and 11.

⁽³⁾ 550 mg. of wheat germ oil given on July 23, 30, and Aug. 7.

were injected subdurally with 0.1 cc. of a saline suspension of the cerebellum of Chick 7627, the tissue being ground with sand in a sterile mortar. All showed severe immediate symptoms following the

injection—convulsive movements, retraction of the head, followed by stupor lasting for an hour or more. Two of the inoculated chicks died a few minutes after the injection, and one after several hours. The three survivors recovered slowly. A control chick inoculated

TABLE III
Showing Incidence of Cerebellar Disease in Inoculated Chicks on Simplified and Natural Food Diets

Chick No.	Diet	Source of injected material	Days on diet until		Clinical symptoms	Lesions	
			Appearance of symptoms	Death		Gross	Microscopic
574	20	Chick 7653	—	D 6	—	—	—
575	20	" 7653	—	K 46	—	—	—
581	20	" 7653	—	K 27	—	—	—
582	20	" 7653	—	D 10	—	—	—
595	20	" 7653	—	D 6	—	—	—
598	20	" 7653	—	K 39	—	—	—
971	20	" 7627	—	K 23	—	—	—
959	20	" 7627	—	K 23	—	—	—
583	108	" 7653	26	K 26	++	+++	+++
584	108	" 7653	13	K 13	++	+	++
588	108	" 7653	15	K 15	++	++	++ (Cerebrum also)
590	108	" 7653	21	K 21	++	+	++
592	108	" 7653	18	K 19	+	—	+
589	108	" 7653	24	K 25	+	++	++ (Cerebrum only)
987	108	" 7627		D 3	—	—	—
992	108	" 7627		D 3 hrs.		Not examined	
972	108	Saline	23	K 23	++	+++	+++

with 0.1 cc. of sterile saline showed no symptoms following the injection.*

* One of the writers has had occasion in the course of work on Fowl Paralysis, to inoculate large numbers of chicks subdurally with suspensions of brain and spinal cord. A severe immediate reaction to the injection of 0.1 cc. of suspension has never been observed. It would seem that the cerebellar suspension used in this experiment was highly toxic—possibly because of the products of autolysis present in the necrotic and softened tissue.

Of the 16 chicks which survived the inoculation, 8 were placed upon the stock Diet 20, and 8 upon Diet 108. The results are summarized in Table III, from which it will be seen that amongst the former group, none of the birds displayed symptoms, or showed pathological changes upon gross and microscopic examination. In striking contrast to this, is the result amongst the group receiving Diet 108. Excluding one chick which died from unknown cause on the third day after injection, every animal of this group manifested

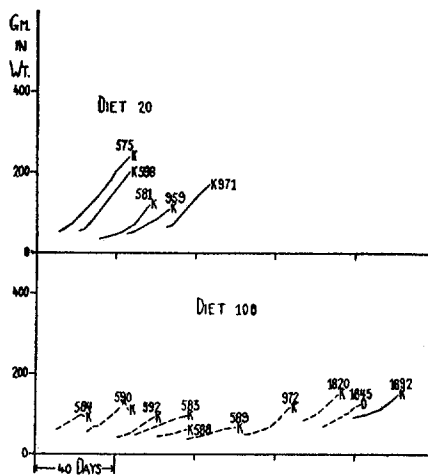


CHART 2. Showing rate of growth of chicks on Diets 20 and 108 which had been inoculated with brain from affected birds. Also 3 uninjected chicks of the same hatch.

more or less characteristic nervous symptoms, appearing from the 13th to the 26th day, and all showed definite and pronounced lesions of the brain.

The growth of the chicks in this experiment (Chart 2) was not satisfactory. They were obtained from a different source and many of them, including controls, were infected with coccidia.

One may deduce from this experiment that the disease is not transmissible by subdural inoculation to chicks maintained on the stock diet.

Three additional chicks of the same hatch were placed upon Diet 108 at the age of 23 days. One (1845) was found dead after 18 days with extensive softening of the cerebellum; a second (1820) showed slight ataxia and tremors on the 23rd day and was killed. In the cerebellum were found multiple discrete early lesions. The third chick (1892) showed no symptoms but was also sacrificed on the 23rd day. No lesions were found in the brain.

DISCUSSION

That the development of this cerebellar disease is related in some way to the diet is sufficiently obvious. Only chicks which received Diets 108 and 107 acquired the disease; controls of the same hatch kept in intimate contact under identical environmental conditions, but receiving only natural food diets, remained free. The nature of the relationship is, however, by no means clear. Among the possibilities which have presented themselves, and which may be briefly considered at this time, are the following:

1. That the disease is due to an infective agent, to which only the chickens on the simplified diet are susceptible. This assumption seems far fetched. The failure to produce the disease by direct subdural inoculation of cerebellar suspension, unless the animals are kept on the simplified diet, makes it impossible to prove or disprove this theory, but against it is the purely *regressive* or *degenerative* character of the pathological picture, as well as the absence of microorganisms in the lesions, or of cell-inclusions suggestive of a virus infection.

2. That the disease is the result of the absorption of some toxic substance from the intestinal tract. The diet itself may contain some substance which is poisonous for the growing chick; or the bacterial flora may be modified by the character of the diet to the detriment of the animal.

3. That the disease is a true nutritional deficiency disease. The satisfactory growth of the birds would indicate that the caloric, protein, and mineral requirements had been adequately covered. (See Table I.) As regards the known vitamins, a deficiency of A and D can be definitely excluded. Vitamin C has been shown by Hart, Halpin and Steenbock (2) to be unnecessary for the chick.

The diet is low in vitamin E, and indeed was devised for the purpose of studying this deficiency in the fowl. As regards the need for the vitamin B complex, B, G, and "B₃" of Williams (5), this may or may not have been satisfied by the incorporation of 5 per cent of Fleischmann's bakers' dried yeast in the diet. The character of the lesions, however, would seem to differentiate the disease from the polyneuritis due to B deficiency.

In this connection, it is interesting to note that Hogan and Shrewsbury (6), using a similar type of simplified diet, observed spasms and tremors of the legs and head retraction, and even "cartwheel" movements which they interpret as "typical neuritic symptoms." However, even with the inclusion of 40 per cent of dried yeast in their diet, 3 out of 7 chicks in one experiment developed symptoms within 3 weeks. It seems improbable that they were dealing with a vitamin B complex deficiency. Since the central nervous system was not studied one can only surmise that they were concerned with the same disease as that here described.

A condition simulating polyneuritis in chicks has been attributed by Hughes, Lienhardt and Aibel (7) to lack of vitamin A, but their histological studies have not yet been published. In our experiments, a deficiency of vitamin A can be excluded.

CONCLUSIONS

Growing chicks maintained on a diet consisting of milk powder, casein, starch, yeast, cod liver oil, salts and filter paper develop ataxia, tremors, retraction or twisting of the head, clonic spasms of the legs, and stupor. These symptoms may appear suddenly, usually between the 18th and 25th day, and may end in death. If recovery takes place, the chicks may go on to normal development.

Definite lesions are found in the cerebellum of the affected chicks. These consist of edema, necrosis and hemorrhages. Hyaline thrombi are found in the capillaries in and about the degenerated areas.

REFERENCES

1. Evans, H. M., and Burr, G. O., The Antisterility Vitamin Fat Soluble E, *Memoirs of Univ. of Calif., Berkeley*, 1927, **8**, 25.
2. Hart, E. B., Halpin, J. G., and Steenbock, H., *Jour. Biol. Chem.*, 1920, **43**, 421.

3. McCollum, E. V., Simmonds, N., and Becker, J. E., *Jour. Biol. Chem.*, 1922, 53, 53.
4. Card, L. E., and Kirkpatrick, W. F., Rearing Chickens. Normal Rate of Growth in White Leghorns and Rhode Island Reds. *Storrs Agri. Exp. Sta. Bull.* 96, 1918.
5. Eddy, W. H., Gurin, S., and Keresztesy, J., *Jour. Biol. Chem.*, 1930, 87, 729.
6. Hogan, A. G., and Shrewsbury, C. L., *Jour. of Nut.*, 1930, 3, 39.
7. Hughes, J. S., Leinhardt, H. F., and Aubel, C. E., *Jour. of Nut.*, 1929, 2, 183.

EXPLANATION OF PLATES

PLATE 2

FIG. 1. Positions assumed by affected chicks. Note retraction and twisting of head.

FIG. 2. Chick 583. Transection through cerebellum, showing extensive area of softening. (Approximately $\times 7$ diam.)

FIG. 3. Chick 7633. Low power showing extensive softening and rarefaction in central portion of cerebellum.

PLATE 3

FIG. 4. Chick 1820. Section through margin of small degenerating area. To the left, normal nuclei of granular layer; to the right, pycnotic nuclei more widely spaced because of edema. A capillary containing hyaline thrombus and two degenerating Purkinje cells are seen at junction of molecular and granular layers.

FIG. 5. Chick 7652. Section through degenerating area in cerebellum, showing necrosis of ganglion cells, pycnosis and fragmentation of granular layer and hemorrhage.

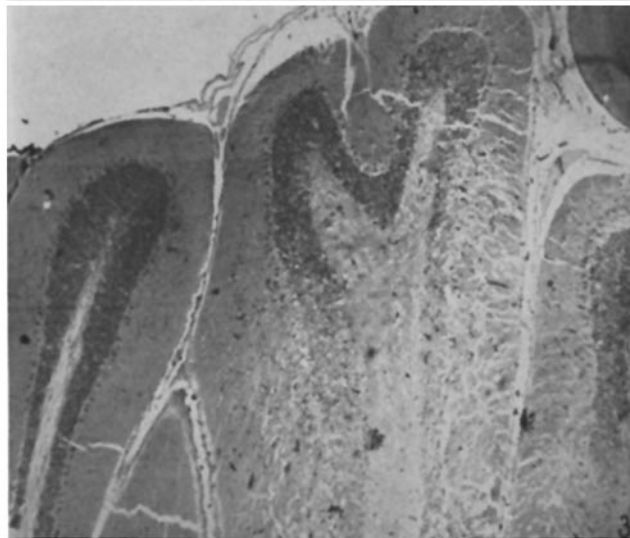
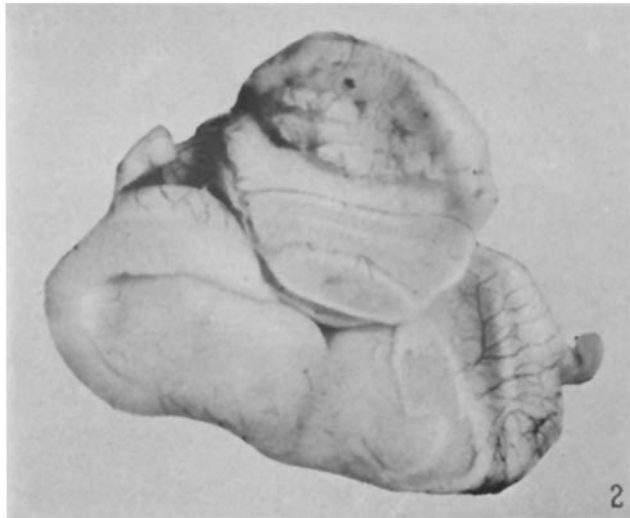
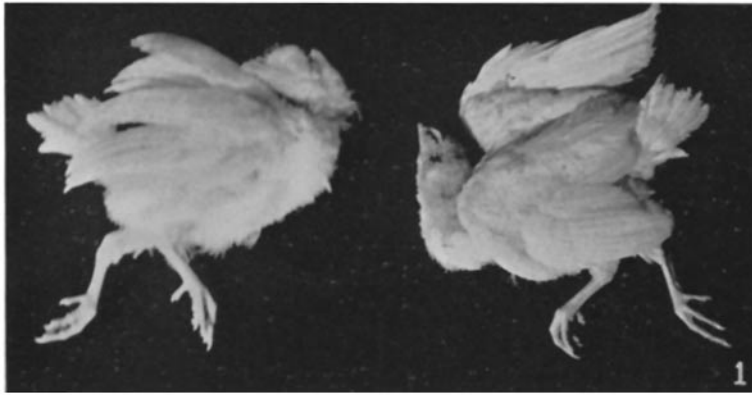
FIG. 6. Chick 1820. Thrombosis of capillary, beginning rarefaction and degeneration of granular layer. (High power.)

PLATE 4

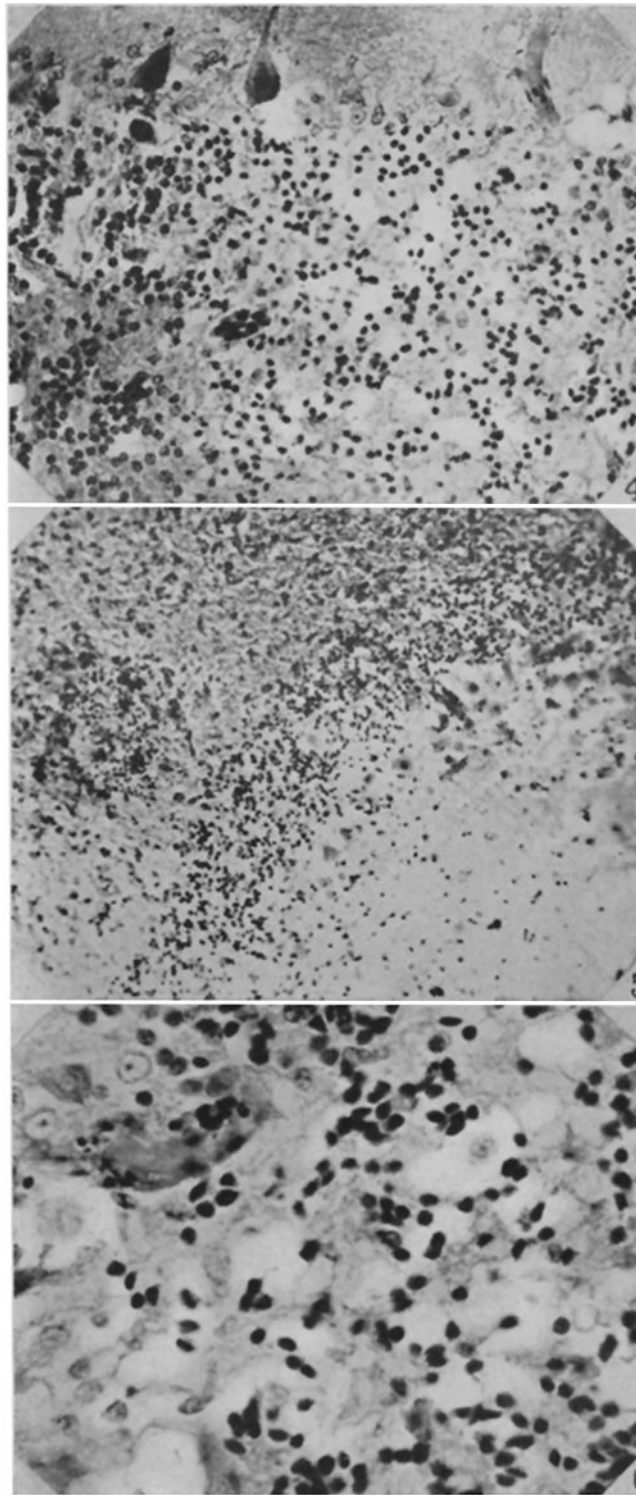
FIG. 7. Chick 7658. Extensive atrophy of entire lobe of cerebellum, with gliosis, calcification, and edema of overlying pia arachnoid. (Low power.)

FIG. 8. Chick 589. Extensive area of recent necrosis in cerebrum.

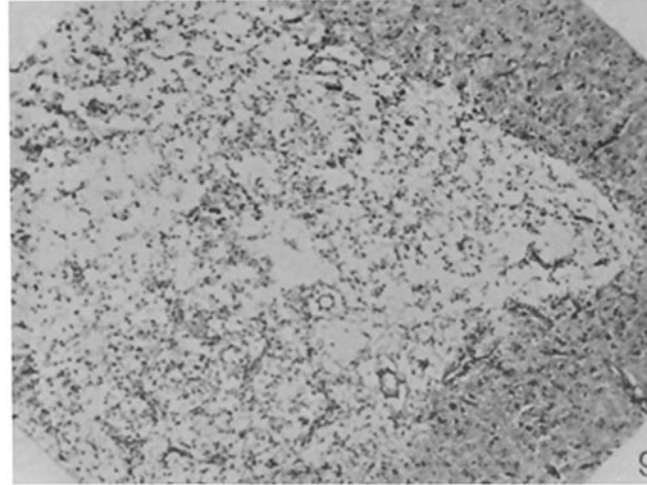
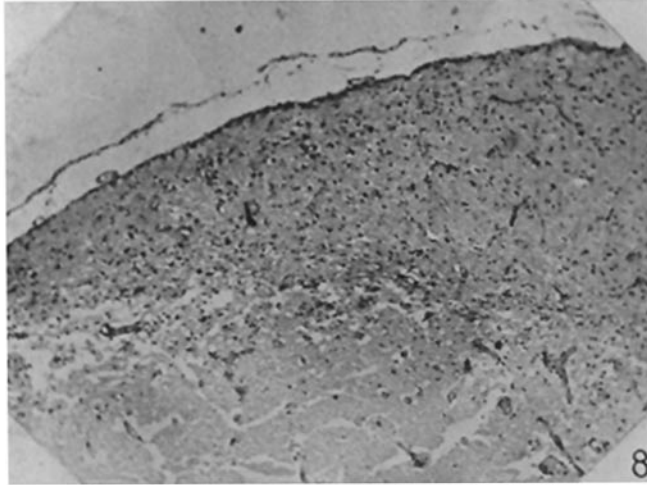
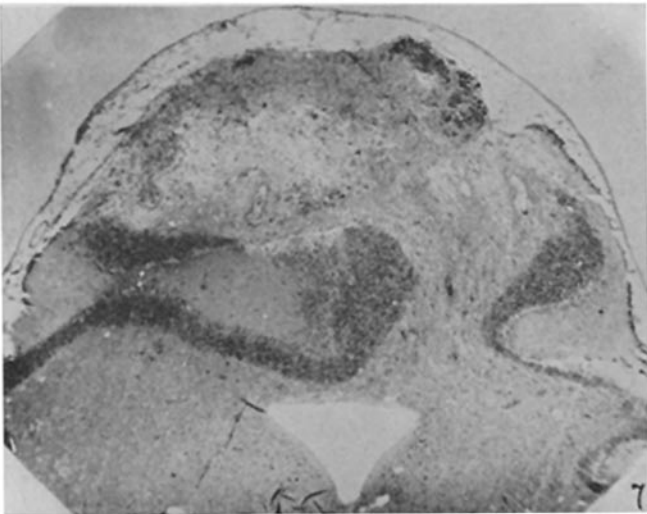
FIG. 9. Chick 589. Replacement of necrotic area in cerebrum by spongy glial tissue.



(Pappenheimer and Goettsch: Cerebellar disorder in chicks)



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