A STUDY OF THE "VIBRATOR" TURKEY

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ABSTRACT

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by Ronald Arthur Peterson

A study of the vibrator turkey was undertaken to determine a possible histological cause of the condition. Other factors investigated were rate of tremor, livability, length of incubation period, growth rate and feed conversion. All birds were Michigan State University, commercial or vibrator strain Broad Breasted Bronze turkeys.

A histological examination of the cerebellum showed no difference in the number of Purkinje cells between vibrators and non-vibrators.

The tremor rate of the vibrators, measured by the use of high speed motion pictures, was about 30 cycles per second.

Brain weights were taken to determine whether any atrophy had occurred in the brain. Comparisons between non-vibrators and vibrators, based on percent of body weight in day-old poults, showed only non-significant differences. In four-day old poults, the mean body weight of non-vibrators was significantly greater than in vibrators. When brain weights were calculated as percent of body weight, significant differences were noted in total brain weight, cerebrum and medulla oblongata weights between controls and vibrators; however, no significant difference was found in the relative cerebellar weights.

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The greatest poult mortality occurred during the first two weeks after hatching (about 40 percent mortality for the vibrator, about 10 percent mortality for the non-vibrators). Death rate from two weeks to five months of age was approximately the same for both vibrator and non-vibrator turkeys.

In feed conversion, the vibrator turkeys were about as efficient as the commercial non-vibrator turkeys. Growth rate was measured from intermingled and non-intermingled groups. In addition to the three strains of turkeys mentioned above, non-vibrating half-brothers and half-sisters of the vibrators were used in one trial. Sexes were not separated in this study. Growth rate of the vibrator strain turkeys was somewhat slower at the start of the growth period, but by four or five months of age there was no statistical difference in body weight between the vibrators, Michigan State University and commercial strain of turkeys.

There was no difference in the length of incubation period in vibrator and non-vibrator turkeys.

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By

Ronald Arthur Peterson

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TABLE OF CONTENTS

LIST OF TABLES

Table | Page

INTRODUCTION

One of the causes of poor hatchability may be the presence of unfavorable genes in the parent stock. Lethal genes generally cause the death of the embryo at some particular stage of development, or disable the offspring so that it is unable to hatch. Semi-lethal genes usually allow the hatching of some but not all of the offspring.

There are also a number of genetically determined variations which cause death shortly after hatching or inhibit the offspring in its ability to survive in competition with non-affected individuals. In turkeys, the vibrator is the only nervous condition which has been reported to be caused by a simple sex-linked recessive gene.

This study of the "vibrator" turkey was undertaken to find a possible histological cause of the condition. Other factors studied were the rate of tremor, livability, length of incubation period, growth rate and feed conversion.

REVIEW OF LITERATURE

At the Michigan Agricultural Experiment Station during the hatching season of 1956, an abnormal condition, in newly hatched Broad Breasted Bronze poults, was observed. The poults exhibited a very rapid shaking or vibration of the head and neck. A study of the hatching records in 1958 (Coleman et al., 1950) indicated that the vibrating condition was due to a single sex-linked recessive gene. The gene character symbol was given as "vi" and the poults were called "vibrators".

Riddle (1917) was the first, to the author's knowledge, to report an inherited nervous disorder in birds. He designated this condition, occurring in pigeons, as hereditary ataxia with the cause appearing to be a simple recessive gene. When walking, the birds were very unsteady resulting in somersaulting forward or backward.

In 1924-25, Knowlton (1929) observed a condition in Barred Plymouth Rock chickens which he described as "congenital loco". The symptoms indicated an apparent lack of control of the neck muscles. The neck was usually bent upward and backward over the body with the head being held in a slightly twisted position and the beak pointing upward. The chicks were unable to stand, although, if placed on their feet and supported by their abdomen, some could retain a standing position for a short period of time. When unaided, chicks would attempt to move or to right themselves, they were only able to push themselves along on their backs or sides. They were never able to gain enough control over their bodies to find and eat food. In all other respects, the chicks appeared to be the same as the controls. Autopsy showed no definite abnormality which could be said to cause the symptoms. The author suggested that there may be some deficiency in the structure controlling equilibrium, since this condition had symptoms somewhat like those exhibited by pigeons, whose sense of equilibrium had been artificially destroyed. Knowlton, in attempting to prove that congenital loco is associated with an impairment of equilibrium, severed the acoustic nerve, destroyed the semi-circular canals, or damaged the brain root controlling equilibrium of non-affected birds. Symptoms resulting from these operations indicated that congenital loco is associated with an impairment of equilibrium. The genetic principles involved in this condition proved that it was a simple recessive gene.

Congenital tremor was described by Hutt and Child (1934) in Single Comb White Leghorns. The symptoms were described as a shaking motion, from a barely perceptible amount, to such an extreme that the bird could not stand. The rate of tremor ranged from 6.5 to 16 complete vibrations per second in one- and two-day-old chicks. Any type of excitement did not noticeably increase the rate of tremor, which was continuous while the chick was standing. When the chick would squat and rest, the tremor would disappear. The tremor appeared to be most severe at hatching time, but as the survivors grew older, the symptoms gradually disappeared. Death losses in the affected chicks reached 85 percent within one month of age and only one out of 35 reached sexual maturity. The genetic basis was interpreted as a simple autosomal recessive character.

Cole (1957) reported on an inherited lethal condition. This condition, that occurred in a strain of Broad Breasted White turkeys, was identical in all known respects to the congenital loco described by Knowlton. The poults appeared in excellent condition when hatched; however, they did not live for more than a week even with hand feeding. Cole, therefore, considered the character as an obligate lethal.

Scott et al. (1950) reported that a sex-linked semi-lethal nervous disorder was found in Rhode Island Red chickens. This disorder was not discernible in newly-hatched chicks. A mild but rapid movement of the head and neck was observed as early as 18 days of age, but in most of the chicks, the movement showed up by the end of 4 weeks. With advancing age, the shaking became more severe. The shaking movements had become so violent by 10 weeks of age that relatively few "shaker" individuals could walk without stumbling. The condition seemed to be aggravated by excitement. By the time the chicks were 14 weeks of age, the majority of them were unable to stand and death resulted from starvation. A histological examination was made of the tissue in the central nervous system. The tissues were fixed in Zenker's fluid and in 10 percent formalin. The Zenker-fixed tissues were stained with hematoxylin and eosin. The formalin-fixed tissues were prepared according to Mahon's technique for the demonstration of myelin. The affected birds showed one consistent lesion, cerebellar Purkinje cell degeneration and loss. In the histological sections, the degenerated cells appeared to stain darker. The severity of the symptoms increased directly with an increase in Purkinje cell destruction. These researchers were unable to demonstrate any significant demyelination of nerve fibers, and stated that their findings were in accordance with the accepted view relating Purkinje cell function to muscular coordination.

Godfrey et al. (1953) reported on a condition in day-old chicks which they referred to as "jittery". In this condition, the head shook rapidly and was retracted over the back. Death loss was very high with only one to two percent of the affected birds reaching maturity. The adults showed the same symptoms as the chicks; in addition, when frightened they tended to circle rapidly. Breeding tests showed that the gene involved was a sex-linked recessive. Upon histological examination there was a marked deterioration of the Purkinje cells of the cerebellum. The Purkinje cells appeared to be fewer in number, with 10 to 50 percent of the exising cells staining more intensely, than in the controls. There was no relationship between Purkinje cell number decrease and severity of muscle incoordination as found by Scott et al. (1950); however, the condition seemed to be physiologically similar to the shaker condition.

Dynendahl (1958) reported a hereditary tremor in ducks which he determined was caused by a genetic recessive gene or genes. The tremor was quite pronounced and involved most of the skeletal muscles. When the bird was standing there were approximately 10 skeletal muscle contractions per second which increased upon excitement. The condition was observed immediately after hatching and the tremor apparently ceased during rest. Balance was poor, with the ducklings usually lying on their backs unable to right themselves, and so most of the ducklings died of starvation. Histological examination of the nervous system at various levels showed no definite abnormalities.

Markson et al. (1959) reported on a sex-linked recessive nervous disease affecting light Sussex pullets. Symptoms were first observed at about two to three months of age. When the pullets were at rest, there were slight rippling tremors of the head and neck. When excited, they became somewhat ataxic with the tremor becoming more pronounced. The condition did not seem to interfere with growth and rearing. The abnormality was confined to atrophy of the cerebellum, which ranged from about one-half the size of the cerebellum of the control birds down to a shrunken nodule. The cerebellum retained its distinctive shape and gross structure even in the shrunken nodule state. Microscopic changes were also confined to the cerebellum. The major abnormality was located in the Purkinje cells which were in various stages of degeneration. There was consequent swelling and disintegration of the Purkinje dendrons, which led to the collapse of the molecular layer and disappearance of their axons which, in turn, resulted in a decrease of white matter. These authors concluded that this degeneration led to the consequent atrophy of the cerebellum.

Winterfield (1953) described a condition in New Hampshire chickens in which the birds exhibited a weaving and bobbing movement of the head. When excited they became very ataxic and the condition varied in severity. For example, one individual had fine head tremors while another lacked coordination in locomotion. Histological examination of the cerebrum and cerebellum was made using sections stained with hematoxylin and eosin. Changes were found in the molecular and granular layers of the cerebellum which were in a degenerative process which was demonstrated by the loss of Nissl substance. There was a decreased number and degeneration of Purkinje cells. The condition was caused by a sex-linked recessive factor with the livability being about 95 percent.

Kawahara (1955) noticed, in a strain of Barred Plymouth Rocks, a slight tremor of the head and neck. The birds showed a 50 percent mortality by 20 weeks of age. Histological examination of the brain indicated a degeneration of the Purkinje cells in the cerebellum. The condition was the result of a recessive sex-linked gene.

Similar conditions have been reported in other animals; for example, Anderson and Davis (1950) reported a condition in a 3-month old calf where there was gross disorganization of normal cortical structure with degenerated Purkinje cells and a rudimentary cerebellum.

Jennings and Sumner (1951), during six weeks of observation, described a condition in an Ayrshire calf with symptoms of posterior incoordination. Histologically, the abnormality occurred in the cerebellum in which the molecular layer was smaller, few microglia or astrocytes present, and the Purkinje cells were reduced in number along with apparent degeneration as compared to the normal calf.

In young mice, Braverman (1953) observed spastic paralysis, tremor and convulsions which he determined to be caused by a mutant gene. Histological examination of the brain and spinal cord revealed no lesions. The author offered a cerebellar hypothesis as an explanation.

Gregory *et al*. (1944) reported a type of congenital hereditary spasm in Jersey cattle. These calves exhibited continual intermittent spasmodic movements of the head and neck. When standing, spasms of both front and hind legs were observed. Death occurred within days to a few weeks of age. The condition was caused by a recessive autosomal lethal gene.

OBJECTIVES

The present investigation of the Michigan State University vibrator turkey was undertaken to determine:

1. A possible anatomical or histological cause of the vibrator condition.
2. Rate of vibrations.
3. Livability of vibrators as compared to non-vibrator turkeys.
4. Length of incubation period of vibrators as compared to non-vibrator turkeys.
5. Growth rate of vibrators as compared to non-vibrator turkeys.
6. Feed conversion of vibrators as compared to non-vibrator turkeys.

GENERAL PROCEDURE

Throughout the experiments, three strains of Broad Breasted Bronze turkeys were used: 1) a commercial strain; 2) Experiment Station strain; and 3) the vibrator strain.

The poults were hatched in Jamesway 252 incubator-hatchers which were operated as recommended by the manufacturer. Pedigree hatching trays were used. All poults that were reared at the Michigan State University Poultry Science Research Center were started in Petersime Model 25D chicken brooder batteries. At four weeks of age, these poults were transferred to Oaks Model 324, 4-deck broiler finisher batteries. At approximately eight weeks of age, all the poults were moved to 5 X 16 foot wire separated sun porches. One week later part of the poults were placed on an alfalfa, brome and ladino clover range. See Tables 1, 2, 3 and 4 for rations fed.

Body weights were taken monthly using a gram scale up through four weeks of age. In the two- to five-month age period, weights were taken to the nearest one-tenth of a pound using a standard weighing scale.

Brain weights were taken on one- and four-day old poults. Brains were removed by clipping the soft cranial bones away with a small (over-all length about 105 mm) curved sharp pointed dissecting scissors. The whole brain was then lifted out and cut into the major areas (cerebrum, cerebellum and medulla oblongata). Brain weights were taken to the nearest one-hundredth of a gram on a Mettler (Type B5) analytical balance. Histological sections were fixed in an autotechnicon Model 2A.

Statistical analysis was made using the student's t test and analysis of variance (Dixon, 1957).

Table 1. Turkey pre-starter fed 0 through 2 weeks of age

Ingredient	1/2 ton	
Ground yellow corn	239.1	
Pulverized heavy oats	40	
Wheat standard middlings	40	
Alfalfa meal, 17% prot.	40	
Soybean oilmeal, solv. 44%	350	
Fishmeal, red	100	
Meat & bone scraps, 50%	70	
Dried whey	20	
Ground limestone	10	
Salt, iodized	5	
$MnSO_4$	0.5	
Vitamin A palmitate 5000/gm.	3.7	
Vitamin D 1500/gm.	3	
Vitamin B_{12} 6 mg/lb.	1	
Terramycin TM-5	10	
Choline Chloride, 25%	1	
Methionine	0.7	
Vitamin E 20,000/lb.	0.5	
Niacin		20 grams
Riboflavin		2.5 "
Calcium pantothenate		6 "
Pro-Gen (Arsanilic)	0.5	
Fat	40	

Table 2. Turkey starter fed 3 through 8 weeks of age

Ingredient	1/2 ton	
Ground yellow corn	300	
Pulverized heavy oats	50	
Wheat standard middlings	50	
Alfalfa meal, 17% prot.	50	
Soybean oilmeal, solv. 44%	350	
Fishmeal, red	60	
Meat and bone scraps, 50%	60	
Dried whey	20	
Ground limestone	20	
Steamed bone meal	5	
Salt, iodized	5	
$MnSO_4$	0.5	
Vit. A palmitate 5000/gm.	1	
Vit. D 1500/gm.	1	
Vit. B_{12} 6 mg/lb.	0.25	
Terramycin TM-5	1	
Choline chloride, 25%	0.4	
Methionine	0.25	
Vit. E 20,000/lb.	0.2	
Niacin		20 grams
Riboflavin		0.5 "

Table 3. Turkey grower fed 9 through 16 weeks of age

Ingredient	1/2 ton	
Ground yellow corn	465.55	
Pulverized heavy oats	60	
Wheat standard middlings	75	
Alfalfa meal, 17% prot.	40	
Soybean oilmeal, solv. 44%	220	
Fishmeal, red	30	
Meat and bone scraps, 50%	30	
Dried whey	10	
Ground limestone	20	
Steamed bone meal	30	
Salt, iodized	5	
$MnSO_4$	0.5	
Vit. A palmitate 5000/gm.	1	
Vitamin D 1500/gm.	1	
Vitamin B_{12} 6 mg/lb.	0.25	
Terramycin TM-5	1	
Choline chloride, 25%	0.4	
Methionine	0.1	
Vitamin E 20,000/lb.	0.1	
Niacin		10 grams
Riboflavin		0.5 "

Table 4. Turkey finisher fed 17 through 24 weeks of age

Ingredient	1/2 ton
Ground yellow corn	497.8
Pulverized heavy oats	70
Wheat standard middlings	100
Alfalfa meal, 17% prot.	30
Soybean oilmeal, solv. 44%	200
Meat and bone scraps, 50%	20
Dried whey	5
Ground limestone	18
Steamed bone meal	45
Salt, iodized	5
$MnSO_4$	0.5
Vitamin A palmitate 5000/gm.	1
Vitamin D 1500/gm.	1
Vitamin B_{12} 6 mg/lb.	0.25
Terramycin TM-5	1
Choline chloride, 25%	0.4
Methionine	0.25
Vitamin E 20,000/lb.	0.25
Niacin	5 grams

EXPERIMENT I:

The purpose of this experiment was to determine the number of vibrations, per second, of the vibrator poults. The poults used in this experiment varied from one- to three-days of age. In order to accurately count the number of vibrations per second, it became necessary to record the vibrations graphically since the rate was too rapid to count visually. Several methods were tried and that of photographic recording proved most satisfactory. High speed 16 mm. motion pictures were taken at the Michigan State University Mechanical Engineering Laboratories, at an average speed of about 1,200 frames per second. Time was recorded every 1/120th of a second on the side of the film by a light in the camera. Results were obtained with the use of a frame counting projector designed so it could be stopped without scorching the film. The number of vibrations was determined by:

$$\frac{\text{Number of vibrations counted}}{\text{Number of frames}} = \frac{\text{vibration per sec.}}{\text{frames per sec.}}$$

The filming procedure was as follows:

1. Two films were made of individual vibrators.
2. Two films were made of one vibrator and one non-vibrator side by side.
3. Six films were made of two vibrators per film.

The results are shown in Table 5. The average vibrations were calculated as about 30 times per second, with a range from 20 to 46 per second. When the beak was viewed photographically, each individual tremor appeared to form an eliptical circle. One problem encountered in the photographing of the birds was that of keeping the poults awake. Under the heat from the flood lamps required for photographing, the poults became drowsy and tended to sleep.

Table 5. Observations of vibrations per second of individual vibrator Broad Breasted Bronze turkeys less than one-week old

Bird No.	Average vibrations per second	Range of vibrations per second
1	30.4	28.2 - 32.1
2	30.9	26.4 - 34.8
3	39.8	32.1 - 46.8
4	23.9	20.5 - 28.6
5	28.1	25.6 - 32.3
6	36.5	36.5 1/
7	33.5	28.3 - 41.7

1/ Unable to obtain more than one observation

When the poults were sleeping or under the influence of ether, there were no visible vibrations. Observations made of embryos at various stages of development revealed that only at 26-28 days of incubation, when some of the vibrator poults could support their heads, were vibrations observed. The muscles of the entire length of the neck appeared to be involved in the tremor. At no time were ataxic symptoms observed in the vibrator poults. Hutt and Child (1934) measured the rate of tremor in one- and two-day old chicks with congenital tremor and found the tremors to range from 6.5 to 16 complete vibrations per second. The tremors stopped when the birds were at rest. Similarly, Dynendahl (1958) reported that ducks with hereditary tremor stopped trembling when resting. When active, these ducks had exhibited tremors of about 10 muscular contractions per second.

EXPERIMENT II:

The purpose of Experiment II was to determine the livability, growth rate and feed conversion of the vibrator turkey as compared to other strains.

Trial A:

Fifty-four (54) Michigan State University strain, 54 commercial strain and 54 vibrator poults were started April 6, 1961, in commercial chick brooder batteries at the Michigan State University Poultry Science Research Center. At four weeks of age they were moved into broiler finisher batteries. When 7½ weeks old the poults were moved into 5 X 16 foot wire separated sun porches with wire floors. Two replications of each strain with 18 birds per replicate were reared separately, while the remainder were reared intermingled. The birds were weighed monthly, with feed weigh-back on the same day. The body weight and date were recorded on the date of death for birds that died.

Trial B:

Only growth rate and mortality were measured in this trial period. Twenty-nine (29) Michigan State University strain, 48 vibrator strain and 19 half-brothers and half-sisters of the vibrator poults, hatched on April 19, 1961, were divided into six replications and started in commercial brooder batteries. Each replication contained about 8 vibrator, 5 Michigan State University strain and 3 non-vibrating half-brothers and half-sisters of the vibrators. At four weeks of age, they were moved into finishing batteries. At eight weeks of age, they were moved into 5 X 16 foot wire separated sun porches and the replications were combined. When nine weeks old, the poults were moved to an alfalfa, brome and ladino turkey range. All birds were weighed monthly and death losses were recorded on date of death. The feeding program for all birds is listed in Tables 1, 2, 3, and 4.

Mortality was measured from 0 to 5 months of age (Tables 6, 7, 8 and 9). Death losses were higher in the vibrator poults (Trial A, 44.4 percent and Trial B, 37.5) during the period of 0 to 2 weeks of age as compared to the non-vibrating poults (Trial A, 5.5 and 13.0 percent and Trial B, 10.3 and 15.7 percent mortality). In all strains tested, death rate from 2 weeks to 5 months of age was comparable (Trial A, commercial strain 19.6 percent; vibrator strain 20.0 percent; Michigan State University strain 27.6 percent; Trial B, vibrator strain 33.3 percent; non-vibrating half-brothers and half-sisters of vibrators, 37.5 percent; Michigan State University strain, 42.3 percent total mortality). Apparently, if the vibrator survives the first two weeks, its livability is as good as the non-vibrator strains. It was evident that one reason for the high early mortality of the vibrator poults was starvation. The vibrators appeared to have greater difficulty in finding food and water. Another possible factor was that the vibrator poults had been inbred to a greater extent than had the non-vibrators. This could account for the higher early death loss in the vibrator poults. Wilson (1948) found as inbreeding increased, chick mortality to 8 weeks of age also increased. Waters and Lambert (1936) found that mortality increased very rapidly as the degree of inbreeding increased. The poults were started in brooder batteries where the heat source was located back under a darkened area. Here the poults tended to group together. This might have accounted for some of the early death loss. Although feed was placed on an egg flat and water was placed part way into this heated area, some of the weaker individuals apparently did not find the food.

Table 6. Trial A - Mortality of three strains of Broad Breasted Bronze turkeys to two-weeks and five-months of age when reared separately (18 birds per replicate)

Strain	Reps.	Percent mortality: 0 to 2 weeks		Percent mortality: 2 weeks to 5 mos.		Total percent mortality to 5 months of age	
Commercial	1	16.6	(3) 1/	20.0	(3)	33.2	(6)
	2	0		27.7	(5)	27.7	(5)
M.S.U.	1	5.5	(1)	23.5	(4)	27.7	(5)
	2	16.6	(3)	26.6	(4)	38.8	(7)
Vibrators	1	44.4	(8)	20.0	(2)	55.5	(10)
	2	44.4	(8)	20.0	(2)	55.5	(10)

1/ Number of birds that died in parenthesis

Table 7. Trial A - Mortality of three strains of Broad Breasted Bronze turkeys to two-weeks and five-months of age when reared intermingled (6 birds per replicate)

Strain	Reps.	Percent mortality 0 to 2 weeks		Percent mortality 2 weeks to 5 mos.		Total percent mortality to 5 months of age	
Commercial	1	0		0		0	
	2	0		16.6	(1)	16.6	(1)
	3	0		16.6	(1)	16.6	(1)
M.S.U.	1	16.6	(1) 1/	20.0	(1)	33.2	(2)
	2	0		66.6	(4)	66.6	(4)
	3	33.3	(2)	0		33.3	(2)
Vibrators	1	50.0	(3)	0		50.0	(3)
	2	33.3	(2)	25.0	(1)	50.0	(3)
	3	50.0	(3)	33.3	(1)	66.6	(4)

1/ Number of birds that died in parenthesis

Table 8. Trial A - Summary of total mortality of three strains of Broad Breasted Bronze turkeys at two-weeks and five-months of age

Strain	Total number of birds in test	Percent mortality				Total percent mortality to 5 months of age	
		0-2 wks.		2 wks.-5 mos.			
Commercial	54	5.5	(3) 1/	19.6	(10)	24.0	(13)
M.S.U.	54	13.0	(7)	27.6	(13)	37.0	(20)
Vibrators	54	44.4	(24)	20.0	(6)	55.5	(30)

1/ Number of birds that died in Parenthesis

Table 9. Trial B - Summary of total mortality of two strains of Broad Breasted Bronze turkeys at two-weeks and five-months of age when reared intermingled

Strain	Total no. of birds in test	Percent Mortality 0 to 2 weeks		Percent Mortality 2 wks. to 5 mos.		Total percent mortality to 5 months of age	
M.S.U.	29	10.3	(3) 1/	42.3	(11)	48.2	(14)
Vibrators	48	37.5	(18)	33.3	(10)	58.2	(28)
Pheontypic 2/ non-vibrators	19	15.7	(3)	37.5	(6)	47.2	(9)

1/ Number of birds that died shown in parenthesis

2/ Half-brothers and half-sisters of vibrators

In the comparison of feed conversion to 5 months of age, the Michigan State University strain turkeys required less feed per pound of gain (4.15 and 4.13 lbs.) as compared to the commercial kinds (4.33 and 4.60 lbs. of feed) and the vibrator turkeys (4.63 and 5.05 lbs. of feed) strains (Table 10). One possible reason for the Michigan State University strain poults requiring less feed per pound of gain was that at the 3-5 month age period, the females were significantly lighter than the other strains tested (Table 11). With the commercial and vibrator strain turkeys being heavier, they probably required more feed for body maintenance than the Michigan State University strain. The variation that occurred between replications might be accounted for by the fact that there was an unequal number of each sex per replicate. There probably is a difference in feed conversion between sexes. No statistical analysis was made because only group data were obtained. The vibrator turkeys strain was just about as efficient as the commercial strain turkeys.

An analysis of the monthly body weights of the three strains of male turkeys from hatching time to five months of age was made using the analysis of variance (Table 12). When hatched, the vibrator poults weighed significantly more at the 1 percent level than the other two strains. One reason for this could be that some of the vibrator hens were older than the hens used as a source of hatching eggs for the commercial and Michigan State University strains. The older turkey hens produced larger eggs. Scott and Phillips (1936) found a high positive correlation between Narragansett turkey-egg size and day-old poult size. The vibrator poults at one month of age weighed significantly less (at the 5 percent level) than the commercial strain. This might be due to the vibrator condition causing greater difficulty for the poults in getting

Table 10. Feed conversion in three strains of Broad Breasted Bronze turkeys to five-months of age (male and female)

Strain	Reps.	Pounds feed/lb. gain
Commercial	1	4.60
	2	4.33
M.S.U.	1	4.15
	2	4.31
Vibrator	1	4.63
	2	5.05

Table 11. Trial A. Average monthly body weights of three strains of Broad Breasted Bronze female turkeys reared separate and intermingled1/

Strain		No. birds tested	April	May	June	July	Aug.	Sept.
			Body weight (lbs.)					
(a)	Commercial	16	45.1 2/	1.1	4.3	8.6	11.5	13.9
(b)	M.S.U.	9	42.9	1.0	3.7	7.4	9.7	11.5
(c)	Vibrator	9	54.4	0.9	3.6	8.4	12.0	13.4
(d)	Intermingled	25	49.8	1.3	4.2	8.7	11.4	13.4
	F Value		12.19**	2.6	2.33	5.47**	8.80**	11.72**
Strains Significantly different		c-ab d-a,b c-d				b-a,c,d	b-a,c,d	b-a,c,d

** Significnat at $P < 0.01$ level

1/ Both males and females raised together

2/ Hatching weight in grams

Table 12. Trial A. Average monthly body weights of three strains of Broad Breasted Bronze male turkeys reared separate and intermingled 1/

Strain	No. Birds tested	Body weight (lbs.) April	May	June	July	Aug.	Sept.
(a) Commercial	9	43.7 2/	1.3	4.9	11.0	15.8	19.5
(b) M.S.U.	15	45.3	1.2	4.6	10.5	15.3	18.3
(c) Vibrator	7	55.9	1.0	4.2	10.2	15.2	18.9
(d) Intermingled	9	50.6	1.2	4.5	10.5	15.4	19.2
F Value		40.23**	3.4*	2.47	2.76	0.61	1.66
Strains significantly different		c–a,b d–a,b c–d	a–c				

* Significant at $P < 0.05$ level

** Significant at $P < 0.01$ level

1/ Both males and females raised together

2/ Hatching weight in grams

started eating. There was no significant difference found in body weight from two to five months of age.

An analysis of variance was made of the body weights of female poults raised intermingled with males mentioned above (Table 8). At hatching time the female vibrator poults were significantly heavier (at the 1 percent level) than those of the Michigan State University and commercial strains. The Michigan State University females were significantly lighter in weight (at the 1 percent level) than the commercial and vibrator strains during the three to five months period. One possible reason for the lower weight of the Michigan State University strain females was that the parents of these birds had been selected primarily for higher hatchability, with little attention given to body weight.

In another trial comparing the Michigan State University and Vibrator strains of males, the vibrators were significantly lighter (at the 1 percent level) during the first two months of life (Table 13). At three months of age, the male vibrators were significantly lighter at the 5 percent level than the Michigan State University Strain. There was no significant difference from four to five months of age between Michigan State University strain, non-vibrating half-brothers of vibrators, and the vibrator strain males. The female turkeys were significantly different only at hatching (Table 14). Phenotypic non-vibrating half-sisters of vibrators weighed less than the Michigan State University strain and vibrators (52.5 vs 62.6 vs 61.1 gms. respectively) at hatching time. At three months of age, the phenotypic non-vibrating half-sisters of vibrators weighed significantly less (at the 5 percent level) than the Michigan State University strain (6.8 lbs. vs 7.8 lbs.). Although

Table 13. Trial B. Average monthly body weight of two strains of Broad Breasted Bronze male turkeys reared intermingled 1/

Strain	No. birds tested	Body weight (lbs.) April	May	June	July	Aug.	Sept.
(a) M.S.U.	8	62.4 2/	1.3	4.2	9.6	14.1	19.4
(b) Vibrators	13	61.9	1.0	3.4	8.4	13.5	18.5
(c) Phenotypic 3/ Non-vibrators	4	58.0	1.2	4.3	9.1	14.1	17.5
F Value		1.17	6.11**	7.73**	3.51*	2.02	1.79
Strains significantly different			a,b	b-a,c	a-b		

* Significant at $P > 0.05$ level

** Significant at $P > 0.01$ level

1/ Both males and females raised together

2/ Hatching weight in grams

3/ Half-brothers of vibrators

Table 14. Trial B. Average monthly body weight of two strains of Broad Breasted Bronze female turkeys reared intermingled[1/]

Strain	No. birds tested	Body weight (lbs.) April	May	June	July	Aug.	Sept.
(a) M.S.U.	7	62.6[2/]	1.2	3.9	7.8	11.3	13.4
(b) Vibrators	7	61.1	0.9	3.3	6.9	10.8	13.1
(c) Phenotypic [3/] Non-vibrators	6	52.5	1.0	3.2	6.8	10.1	12.4
F Value		10.9**	3.42	2.6	3.59*	0.23	2.98
Strain significantly different		c-a,b			c-a		

* Significant at $P < 0.05$ level

** Significant at $P < 0.01$ level

1/ Both males and females raised together

2/ Hatching weight in grams

3/ Half-sisters of vibrators

somewhat lighter in body weight during the first two months, during the third, fourth and fifth months of life the vibrator is non-significantly different in weight from the Michigan State University strain, commercial strain and non-vibrating half-brothers and half-sisters of the vibrators.

<u>EXPERIMENT III:</u>

In some nervous disorders in birds reported by other workers, the atrophy of the cerebellum has been a prominent lesion in birds exhibiting head and neck tremors along with ataxic symptoms. An example of this in chickens was reported by Markson <u>et al</u>. (1959).

This present experiment was run to determine if any atrophy had occurred in the brain of vibrator poults. Brains were taken from both one-day old and four-day old poults. The brains were lifted out of the lower cranial case after the top cranial bones had been cut away. The brains were then cut into three parts--the cerebrum, cerebellum and medulla oblongata--then while the parts were still wet, they were weighed on an analytical balance.

There was no significant difference between day-old poult brain weights as percent of body weights of controls and vibrators in total, cerebellum, cerebrum and medulla oblongata brain weights (Tables 15, 16, 17, 18, 19, 20). There was a significant difference between control and vibrators in total brain weight, cerebrum and medulla oblongata weight of four-day old poults. The cerebellum weights were non-significantly different (Tables 15, 16, 17, 18, 19, 20). There was a high rate of starve-outs in the vibrators; thus, the four-day old non-vibrators were significantly heavier in body weight than the vibrators ($P < 0.02$ level). Assuming that the brain was not affected by the starving, this would account for the higher percentages of brain weight per unit of body weight in the vibrator than in the non-vibrators. A "t" test run on the medulla oblongata weights, not adjusted for body weights, showed no significant difference. It could be concluded that there was no significant difference between the cerebellum, cerebrum, and medulla oblongata weights of vibrator and non-vibrator poults.

Table 15. A comparison of cerebellum weights as percent of body weight in control and vibrator Broad Breasted Bronze turkeys at one-day and four-days of age

	Control		Vibrators
Age in days	1		1
Number of birds	8		8
Percent cerebellum weight	0.31		0.33
t value		0.80	
Age in days	4		4
Number of birds	13		13
Percent cerebellum weight	0.40		0.36
t value		1.186	

Table 16 A comparison of medulla oblongata weights as percent of body weight in control and vibrator Broad Breasted Bronze turkeys at one-day and four-days of age

	Control		Vibrator
Age in days	1		1
Number of birds	8		8
Percent medulla oblongata weight	0.87		0.86
t value		0.43	
Age in days	4		4
Number of birds	13		13
Percent medulla oblongata weight	0.76		0.93
t value		2.90*	

* Significant at $P < 0.05$ level

Table 17. A comparison of brain weights as percent of body weight in control and vibrator Broad Breasted Bronze turkeys at one-day and four-days of age

	Control		Vibrators
Age in days	1		1
Number of birds	8		8
Percent brain weight	2.38		2.44
t value		0.153	
Age in days	4		4
Number of birds	13		13
Percent brain weight	2.21		2.61
t value		2.70*	

* Significant at $P < 0.05$ level

Table 18. A comparison of cerebrum weights as percent of body weight in control and vibrator Broad Breasted Bronze turkeys at one-day and four-days of age

	Control		Vibrators
Age in days	1		1
Number of birds	8		8
Percent cerebrum weight	1.24		1.19
t value		1.72	
Age in days	4		4
Number of birds	13		13
Percent cerebrum weight	1.06		1.32
t value		2.783*	

* Significant at $P < 0.05$ level

Table 19. A comparison of total brain weight as percent of body weight in male and female Broad Breasted Bronze turkeys at one-day and four-days of age

	Male		Female
Control:			
Age in days	1		1
Number of birds	5		3
Percent brain weight	2.44		2.38
t value		0.153	
Age in days	4		4
Number of birds	8		5
Percent brain weight	2.07		2.44
t value		1.93	
Vibrator:			
Age in days	1		1
Number of birds	5		3
Percent brain weight	2.39		2.34
t value		0.44	
Age in days	4		4
Number of birds	9		4
Percent brain weight	2.61		2.60
t value		0.03	

Table 20. A comparison of body weights of four-day old vibrator and control Broad Breasted Bronze turkeys used for brain weight purposes.

	Control		Vibrator
Number of birds	13		13
Body weight	73.3		61.2
t value		2.53*	

* Significant at $P < 0.02$ level

EXPERIMENT IV:

The purpose of this experiment was to determine if there was any difference in length of the incubation period between vibrators and non-vibrators. Hatching eggs were placed in pedigreed hatching baskets stacked vertically to observe the time and number of individual non-vibrator and vibrator poults hatching without removing them from the incubator. Three different hatches were used, with pooling of the information. The data was based on a mathematical hatching time value which was obtained by the observation sequence (0_1, 0_2, 0_3, etc), times the number of poults hatched at that particular observation. There was no definite period of time between observations. Statistically, there was no difference found between the vibrators and controls in the length of incubation periods (Table 21).

Table 21. A comparison of length of incubation period of control and vibrator Broad Breasted Bronze turkeys

	Control	Vibrator
Number of birds	77	40
Median mathematical hatching time value*	5.98	6.93
t value	0.39	

* Based on observation sequence (O_1, O_2, O_3, etc.) times the number of poults hatched at that particular observation. A lower value represents earlier hatching.

EXPERIMENT V:

In most nervous disorders reported by other workers in birds, it was observed that there was a degeneration of the Purkinje cells in the cerebellum. The purpose of this experiment was to determine histologically, if degeneration and loss had occurred in the Purkinje cells of the vibrator poults. Whole brains of day-old poults were lifted out of the lower cranial case after the top cranial bones had been cut away. The brains were then cut into three parts: cerebrum, cerebellum and medulla oblongata. The spinal cord was then removed from some of the individuals by cutting the dorsal surface of the vertebral column away. The brain and spinal cords were fixed, embedded in paraffin and stained. See appendix Pages 50, 51, 52, 53, 54, 55 and 56. The sections were cut at 7-10 microns in thickness in series at intervals of approximately 60 microns.

A histological examination of the Purkinje cells, located between the molecular and granular layers of the cerebellum was made. An area count was made of the Purkinje cells which were easily discernible in each of the above stains. No difference in the number or structure of the Purkinje cells between the controls and vibrators was discernible. No abnormalities were found upon histological examination of the entire central nervous system of 15 vibrator and 15 non-vibrator poults. No abnormal Purkinje cells were found when a histological examination was made of the cerebellum of two vibrator and one non-vibrator turkeys which were six-months of age.

Of the nervous disorders reported in fowl in which histological examinations were made, Winterfield (1953) observed in chickens a decrease and degeneration of Purkinje cells. Kawahara (1955) reported degeneration of the Purkinje cells in chickens. Markson _et al._ (1959) described the major abnormality as being located in the Purkinje cells which were in various stages of degeneration. Godfrey _et al._ (1953) observed upon histological examination that there was a marked deterioration of the Purkinje cells. Scott _et al._ (1950) reported that their birds had a consistent lesion in which there was a loss and destruction of Purkinje cells.

In comparison with the reports from other workers, the vibrator turkey is dissimilar in that there is no obvious degeneration or loss of Purkinje cells of the cerebellum, although Dynendahl (1958) did not find any evident lesions in the central nervous system of ducks with hereditary tremor.

GENERAL DISCUSSION

In this study of the vibrator condition in Broad Breasted Bronze turkeys, the exact cause of the tremor-type symptoms was not determined. Using the beak as a point of reference, it was determined that the rate of tremor was about 30 elliptical cycles per second. Hutt and Child (1934) observed 6.5 to 16 complete vibrations per second in congenital tremor chicks, while Dynendahl (1958) reported 10 muscle contractions per second in hereditary tremor ducks along with poor balance. Scott _et al_. (1950), Godfrey _et al_. (1953), Markson _et al_. (1959), Winterfield (1953), and Kawahara (1955) all reported tremor in chickens with nervous conditions but did not indicate the rate. The rate of tremor in the vibrator was much faster than has previously been reported in other conditions.

The tremor in the vibrator poult appears to occur only when the bird is supporting its head and neck against gravity. The rate of tremor is irregular, in that when the bird moves its head quickly from one position to another, the tremor seems to stop. There is no visible tremor when the vibrator is asleep or under the influence of ether. Little is known as to exactly how sleep and ether function in bringing about depression in the central nervous system. According to Guyton (1956), sleep appears to occur due to the inactivity of the neurons in the higher brain centers, such as in the cerebrum. Ether's main effect is as a depressor in the central nervous system.

According to MacIntosh and Barnister (1952), ether affects the most recent phyogenetically developed area in the central nervous system, which would be the cerebrum, next would be the depression of the cerebellum and so on down through the more primitive centers in the medulla oblongata area. Taking into consideration the fact that the tremor does not occur in the vibrator during sleep and under the influence of ether, it might be concluded that the cause of the disorder if located in the central nervous system is somewhere other than in the medulla oblongata.

Scott _et al_. (1950), Godfrey _et al_. (1953), Markson _et al_. (1959), Winterfield (1953), Kawahara (1955), and Dynendahl (1958) all made histological examinations of the central nervous systems of birds showing various degrees of tremor and ataxic symptoms. These investigators, with the exception of Dynendahl, found practically the same lesion in the cerebellum in varying degrees; that is, degeneration and loss of the Purkinje cells. Dynendahl (1958) found no definite lesions. Upon histological examination of the cerebellum in the vibrators, no difference was found in Purkinje cell number nor was degeneration evident.

Ruch and Fulton (1960) in discussing Parkinson's disease indicated that the tremor (regular and rapid) associated with lesions in the basal ganglia was involuntary, that is, occurring only during rest and sleep.

In discussing cerebellar tremor, Ruch and Fulton (1960) described the condition as intentional as the tremor occurs during intentional movements instead of at rest. A better description of the cerebellar tremor might be an ataxic tremor. One might compare the tremor to certain actions taken when first learning to drive an automobile, when you tend to drift off your intended course, then in an attempt to return to the course one applies the controls too vigorously, thus over-corrects and then overshoots the intended course again. As the above process is repeated the result will be an oscillation similar to an irregular tremor which increases in severity as the movement progresses. The cerebellum probably exerts both facilitory and inhibitory influences on movements initiated by the cerebral motor cortex. Ruch and Fulton (1960) also compared the function of the cerebellum in relation to the cerebral cortex to that of a feed back system. For example, when a stimulus input command enters into a control unit to take a new position, this starts a power source which then moves an object toward the new position. The degree of movement is dependent upon the difference between the starting position and the desired position. As movement is continued toward the desired point, information is fed back to the control unit where again the current position is related to the intended position and new orders are fed into the power source. Thus the difference between the present and intended position is progressively reduced. If underdamped, systems like the above are subject to overshoot and oscillate in making rapid transients, or to be sluggish if overdamped. The cerebellum might be considered similar to a feed back stabilizing or controlling network in determining if a system is too slow in its control or tends to oscillate.

Based on the above information the tremor in the vibrator is probably voluntary and the cause is probably located somewhere in the cerebellum or in the nerve trunks that bring information to the cerebellum. Histologically, no gross lesions were found in the vibrator cerebellum. The vibrator condition appears to differ from other nervous conditions reported in birds, since most of the other authors reported histological degeneration and loss of the Purkinje cells in the cerebellum. They also reported various ataxic symptoms along with tremor. In the vibrator turkey, there were no ataxic symptoms observed; therefore, it might be concluded from the lesser symptoms of the vibrators that the lesion, if occurring in the cerebellum, would not be as pronounced as in the more severe cases reported.

The tremor in the vibrator poults occurs at hatching time, or at pipping time, if the individuals who are strong enough to hold their heads up will vibrate. The vibrator condition is similar to the condition reported by Dynendahl (1958) in ducks, Godfrey et al. (1953), Knowlton (1929), Cole (1957), Hutt and Child (1934) in chickens, in that all are apparent at hatching time. The vibrator condition is dissimilar from those reported by Scott et al. (1950) and Markson et al. (1959) in chickens in which the condition appeared somemonths after the chicks had hatched.

In comparing the vibrator poults in the age period of 0 to 2 weeks, 37 to 44 percent died as compared to a death loss of 5 to 13 percent for the controls. Death rate in turkeys from 2 weeks to 5 months of age was 10 to 27 percent in Trial A and 33 to 42 percent in Trial B with not too much difference in percentage between the vibrator and control strains within each trial. In most nervous conditions reported in birds, /Kawahara (1955), Dynendahl (1958), Godfrey et al. (1953), Scott et al. (1950), Cole (1957), Hutt and Child (1934)/ mortality of the affected birds greatly exceeded that of the control birds. This was not true of the conditions reported by Winterfield (1953) and Markson et al. (1959). These authors found livability of the affected birds to be almost as good as that of controls.

The vibrator poult appears to survive equally as well as the controls if they reach two weeks of age. The early vibrator poult mortality appeared to be due to apparent starving out. A possible reason could be that the vibrator symptoms made it more difficult to find food. Another factor could be that the vibrator poults started out in a weaker condition than the controls due to inbreeding, which was used to maintain the trait. Also, these weaker individuals tended to hang under the heat source which was in a darkened area in the chicken brooder batteries. They may not have found the feed during the first few days of life, even though they were dunked in feed and water when started. A trial should be run on a well-lighted floor with feed and water placed near and under heat lamps. Under these conditions, the vibrator poults may have a greater opportunity for coming in contact with the food, and the more aggressive individuals might induce some of the weaker ones to start picking at the food. Forced feeding should also be attempted to determine if some factor other than inability to find food is causing death. The vibrator condition seems to differ from most of the other conditions reported in that it apparently is not lethal.

Although the phenotypic half-brothers and half-sisters survived just about as well as controls, the vibrators should be out-bred to determine if inbreeding did influence the early death rate. Waters and Lambert (1935) and Wilson (1948) indicated that inbreeding increases mortality.

The vibrator condition doesn't affect body weight during the four and five month period of life but the vibrator poults are somewhat lower in body weight during the first three months of life than the controls. Markson _et al_. (1959) were the only investigators to report on growth rates of chickens having a nervous condition. They indicated that there was no difference between control and affected birds.

There is apparently no effect from the vibrator condition in length of the incubation period between controls and vibrators.

There is a possible commercial use for this condition in that it is caused by a single sex-linked recessive gene (Coleman _et al_., (1960). Therefore, if the proper matings are made, the birds will be self-sexing. By mating a homozygous recessive vibrator make to a non-vibrator female, the F_1 generation offspring will be self-sexed, with all the females being vibrators and all the males heterozygous non-vibrators. This marker gene will be useful commercially only if further tests show that it has no deleterious effects.

CONCLUSIONS

1. The tremor of the vibrator poults was determined to be approximately 30 complete vibrations per second.
2. In a comparison of livability between vibrator and non-vibrator poults, the greatest mortality (about 40 percent) occurred during the first two weeks of life in the vibrators. Cause of death was apparent starvation. Death rate from two-weeks to five-months of age was comparatively the same for both vibrator and non-vibrator poults.
3. The vibrator poults were just about as efficient in feed conversion as the commercial non-vibrator strain of turkeys.
4. Growth rate of the vibrator strain turkey was somewhat slower at the start of the growth period, but by four or five months of age there was no statistical difference in body weight between the vibrator, Michigan State University and commercial strain of turkeys.
5. Brain weight comparisons between non-vibrator and vibrator poults, when based on percent of body weight of day-old poults, was found to be non-significantly different. In four-day old poults, the non-vibrators were significantly larger in body weight than vibrators. When brain weights as percent of body weight of the control was taken, there was a significant difference in total brain weights, cerebrum and medulla oblongata as compared to vibrator turkeys. There was no significant difference found in the cerebellum weight.
6. There was no significant difference in length of incubation period between non-vibrator and vibrator poults.
7. There was no difference observed between the vibrator and non-vibrator turkeys in the number of Purkinje cells of the cerebellum.

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APPENDIX I

FIXATION PROCEDURE*

1.	10 percent Formalin		6 hr.
2.	Alcohol . . .	70 percent	1 hr.
		80 percent	1 hr.
		95 percent	1 hr.
		Absolute	1 hr.
		Absolute	2 hrs.
3.	Equal parts absolute alcohol and xylol		1 hr.
4.	Oil of cedarwood		1 hr.
	Xylol		1 hr.
5.	Paraffin I (60°C.)		1 hr.
6.	Paraffin II (tissuemate) (60°C.)		2 hrs.
7.	Imbed and section		

* Procedures from Histopathologic technic and practical histochemistry by R. D. Lillie, 1957

Some tissues were fixed with Zinker's Fluid

1. Fix for 24 hours.
2. Wash in running water 12-24 hours.
3. Run through alcohols, etc. as usual procedure stated above.
4. Imbed and section.
5.* De-wax as usual - xylene, absolute alcohol, 95 percent alcohol and distilled water.
6. Alcoholic iodine solution for 5-10 minutes.
7. Wash in tap water.
8. Sodium thiosulfate solution 5 minutes.
9. Running tap water 10-20 minutes.

*5-9: To remove mercuric chloride which interferes with staining.

From: Lillie, 1957

APPENDIX II

TISSUE STAINS

ROUTINE HEMATOXYLIN AND EOSIN STAINING PROCEDURE:

After sections have stood in hot air oven (60°C.) for 1 hour:

1.	Xylol	3 minutes
2.	Xylol	3 minutes
3.	95 percent alcohol	3 minutes
4.	Tap water	3 minutes
5.	Hematoxylin stain	8-10 minutes
6.	Tap water	5 minutes
7.	Acid-alcohol	up to 1 minute
8.	Running tap water	5 minutes
9.	Ammonia water	1 to 2 minutes
10.	Distilled water	3 minutes
11.	Eosin stain	1 to 2 minutes
12.	95 percent alcohol	1½ minutes
13.	Absolute alcohol	1½ minutes
14.	Carbol-xylol	3 minutes
15.	Xylol	3 minutes
16.	Xylol	3 minutes

Mount in Permount

If Zinker's fixative is used, insert the following steps between steps 3 and 4:

a.	Iodized alcohol	4-5 minutes
b.	95 percent alcohol	3-4 minutes
c.	Sodium thiosulfate	10-20 seconds

From: Lillie, 1957

LUXOL FAST BLUE PERIODIC ACID SCHIFF HEMATOXYLIN STAIN:

1.	Xylene. 2 changes	3 minutes
2.	Absolute alcohol	3 minutes
3.	95 percent alcohol	3 minutes
4.	0.1 percent Luxol Fast Blue in the oven at 60° overnight	
5.	Rinse in 95 percent alcohol	
6.	Rinse in distilled water	
7.	0.05 percent lithium carbonate	few seconds
8.	Differentiate in 70 percent alcohol	20 to 30 seconds
9.	0.05 percent lithium carbonate	few seconds
10.	Differentiate in 70 percent alcohol	
11.	Rinse in distilled water	
12.	0.5 percent periodic acid solution	5 minutes
13.	Rinse in distilled water	2 changes
14.	Schiff's solution	15 to 30 minutes
15.	Sulfurous acid solution	3 changes, 2 minutes each
16.	Tap water	5 minutes
17.	Harris's hematoxylin	1 minute
18.	Tap water	5 minutes
19.	Acid alcohol	briefly
20.	Tap water to blue	
21.	95 percent alcohol	2 changes, 2 minutes each
22.	Absolute alcohol	2 changes, 2 minutes each
23.	Xylene	2-3 changes, 3 minutes each
24.	Mount in Permount	

RESULTS: Myelin . . blue, green
Nuclei . . dark blue
Capillaries . . red
Fungi, and P.A.S. pos. elements . . rose to red
Cytoplasmic nucleoproteins . . bluish purple

Procedure from Manual of Histologic and Special Staining technics,

Armed Forces Institute of Pathology, 1957

Lillie's variant of the Weil-Weigart Method: *

Material should be fixed two days in 10 percent formalin, then transferred to 2.5 percent potassium bichromate to complete a total of four days chromation. Then dehydrate with graded alcohols, clear in benzene or gasoline, and imbed in paraffin. Section, bring paraffin sections to 80 percent alcohol.

1. Transfer to a mixture of equal volumes 4 percent iron alum and 1 percent alcoholic hematoxylin solution (1-5 days old only) and stain in paraffin oven at 55-60°C. for 40 minutes.
2. Wash in water and decolorize one hour in 0.5 percent iron alum.
3. After the iron alum, wash in water and blue in 1 percent borax, 2.5 percent potassium ferricyanide solution for 10 minutes.
4. Wash in water, and counterstain 5 minutes in a 1:1,000 solution in 1 percent acetic acid of safranin O.
5. Dehydrate, clear and mount through an acetone, acetone and xylene, xylene sequence in synthetic resin.

RESULTS: Blue-black myelin,

Yellow to brown to black RBC, red nuclei and tigroid

Pink background

From: Lillie, 1957

Mallory's Phosphotungstic Acid Hematoxylin Stain:

The solution is prepared by dissolving 1 gm. hematoxylin and 20 gm. phosphotungstic acid in a liter of distilled water. The addition of 177 mg. of potassium permanganate will ripen it at once.

Zinker fixation was prescribed by Mallory. After sections are de-paraffinized, etc.

1. Iodine in 95 percent alcohol (0.5 percent) five minutes.
2. 0.5 percent sodium thiosulfate five minutes (or 5 percent for 1 minute)
3. Wash in tap water.
4. 0.25 percent potassium permanganate five minutes.
5. Wash in water.
6. 5 percent oxalic acid five minutes (Mallory 10-20 minutes).
7. Wash in running water 1-2 minutes.
8. Stain in phosphotungstic acid hematoxylin overnight (12-24 hours).
9. Dehydrate rapidly in 95 percent and absolute alcohol or in acetone, clear with a 50 percent mixture of the dehydrating agent and xylene, then two changes of xylene.

   Mount in permount.

RESULTS: (for astrocytes and glia fibrils)

From: Lillie, 1957