A STUDY OF THE ORIGIN AND DIFFERENTIATION OF PIGMENT IN THE HAIR OF THE HOUSE MOUSE (MUS MUSCULUS)

THESIS FOR THE DEGREE OF M. OF S.

C. A. RICHARD JOHNSON

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by

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This study was conducted under the direction of

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DEDICATION

This thesis is respectfully dedicated to the memory of my father and mother, who themselves denied the privilege of higher learning inspired their son to seek the best of educational opportunities.

OUTLINE

A STUDY OF THE ORIGIN AND DIFFERENTIATION OF PIGMENT IN THE HAIR OF THE HOUSE MOUSE (Mus musculus)

PART I

INTRODUCTION

1. STATEMENT OF THE PROBLEM AND THE OBJECTIVES OF THE INVESTIGATION.
2. SCOPE OF PROBLEM
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INTRODUCTION

1. STATEMENT OF THE PROBLEMS AND THE OBJECTIVES OF THE INVESTIGATION.

The study of hair color and the investigation of the principles underlying hair coloration have commanded the attention of scientists for many years. The causes of hair type structure and color intensities have long been comparatively well understood. The relation of hair structure and color types in the human race have been accurately determined. The actual granular characteristics which are responsible for the production of color have been defined, and within limitations have served as the fundamental basis for generalization and classification.

Investigations dealing with the actual physical process of the production of color granules have been, to a certain extent, neglected. This investigation attempts the study of the hair follicles in the skins of seven colored strains of mice to determine and to locate the actual cells that produce the colored granules, and the structural elements within these cells which concern themselves with the development of the granules.

The purposes of the investigation were as follows: (a) to locate the point of origin of the granules within the cell and to determine the cell structure which produces these granules, (b) to determine the part of the hair follicle in which granules first appear. (c) the determination of the development of the follicles through the different ages, (d) the observation of changes in the hair itself until it emerges on the surface of the skin, (e) the determination of the exact age of the individual at which hair erupts through the skin, (f)

the determination of the exact age of the individual at which color granules appeared in the follicles of the hair. (g) to photograph under the microscope the process of color formation in a static form for the particular ages, (h) the drawing of the actual cell structures which were revealed under high power oil immersion lens to be in the actual process of color granule production, the interruption of which was accomplished by obtaining the tissue at that stage from the living mouse specimen, (from the combination of the pictures and the accurately enlarged drawings of the individual granule producing cells conclusions can be suggested as to the origin of the granules), (i) the devising of a classification system into which the successive developmental changes of the hair follicle could be placed. (j) the study of the structural changes in the developing hair of the mouse and a comparison of these changes with those in the human hair. It was necessary to prepare stained slides for studying with the oil immersion lens of the microscope.

2. SCOPE OF THE PROBLEM.

Representative specimens from the following seven color strains of mice were used in the investigation:

Black and Tan

Chocolate (intense brown)

Light-bellied black agouti

Pink-eyed black agouti

Chinchilla

Dark-eyed black agouti

Dilute brown

The albino stock of mice, apparently devoid of color granules, was used for structural comparisons with the colored strains. Accordingly mice from the albino stock were subjected to the same techniques as were employed in the colored strains.

The number of individuals used in each strain varied. The black and tan stock had 58 specimens and represented the largest number in the experimental groups. Next in order of number came the chocolate stock with 46 representatives. The dilute brown stock had 34 individuals, and the dark-eyed black agouti had 32. The light-bellied black agouti stock was represented by 15 mice, the pink-eyed black agouti by 12, and the chinchilla strain by 8 individuals. The control stock, the albino, had 13 specimens. Thus there were 205 colored individuals, 13 albinos and 10 mice whose material was spoiled, making a grand total of 228 animals used. See Table I.

The main part of the investigation was concerned with animals at birth, one-day old, and-two days old. The total number of individuals in these age groups comprised 105; 19 in the birth, 47 in the 1-day and 39 in the 2-day stock.

These very young mice (see Table II) were distributed as follows: Black and tan - 5 in birth age, 9 in the 1-day age, and 9 in the 2-day age group. Chocolate - 4 at birth age, 9 in the 1-day age, and 7 in the 2-day age group. Light-bellied agouti - 3 in birth age, 5 in the 1-day age, and 2 in the 2-day age group. Pink-eyed black agouti - 4 in the 1-day age, and 4 in the 2-day age group. Chinchilla - 2 in the 1-day age and 2 in the 2-day age group. Dark-eyed black agouti - 4 in the birth age. 5 in the 1-day age, and 5 in the 2-day age group. Dilute brown - 3 at birth age, 13 in the 1-day age, and 10 in the 2-day age group.

Table I

TOTAL OF STRAINS OF MICE

Color strain of Mice	Number of Individuals
Black and Tan	58
Chocolate	46
Dilute Brown	34
Dark-eyed Black Agouti	32
Light-bellied Agouti	15
Pink-eyed Black Agouti	12
Chinchilla	8
Total of colored strains	205
Albino stock	13
	218
Faulty technique	10
Grand total of specimens	228

Table II

Total Of The Seven Strains Of Mice

Color strain	Three initial age groups age. birth	1 day	2 day
Black and Tan	5	9	9
Chocolate	4	9	7
Light-bellied agouti	3	5	2
Pink-eyed black agouti	0	4	4
Chinchilla	0	2	2
Dark-eyed black agouti	4	5	5
Dilute brown	3	13	10
Total	19	47	39

3. REVIEW OF LITERATURE.

The subjects with which this paper deals have apparently not been previously investigated.

The following is a review of the articles having a bearing on the subject at hand.

Davenport and Davenport (1, 1910) in working on human hair and skin color conclude that the grades of color may be due to the fluctuations in the number of pigment granules present.

Dyson (2, 1911) in the main confirms the work of Merowsky regarding the nuclear origin of melanin granules. He regards these as the mother substance of pigment.

Little (3, 1913) cites Riddle's view that melanin pigment is the result of an oxidation process. Tyrosin and other compounds are acted on by an oxidizing agent (enzyme) tyrosinase, producing melanin compounds. Riddle thought that yellow (lowest), brown, and black (highest) are progressive stages in oxidation.

Hausman (4, 1920; 5, 1924; 6, 1928) has prepared several interesting papers on the pigment granules in the human head hair. He conducted a comparative racial study and developed a classification system. He notes the size and shape of the color granules and also shows a relationship between the type of the cuticle and the diameter of the hair shaft.

Harris (7, 1925) concludes that pigment tends to afford a protection against the lethal action of certain photodynamic substances in light.

Day (8, 1925) has studied and classified hair and observed the development and succession of hairs. Retention of the hair has been ob-

served and a system of regeneration has been described. He has noted the succession of type of hairs in a single follicle.

Hance and Murphy (9, 1926) show that hard X-rays prevent the formation of pigment in the hair follicles of mice, with the result that hair previously black comes in white.

Hance (10, 1927) alone in this experiment shows that X-rays caused the colored hair to fall out and to be replaced by white hair.

Just (11, 1927) lists a bibliography of 69 titles, in French, relating to the coloration of hair.

Pawlas (12, 1927) intimates that black pigment is formed by the oxidation of mother substances which circulate in the body fluids and is deposited in special cells where it is transformed into pigment under the influence of certain oxidizing catalysts. In the higher animals the mother substance is probably dioxyphenylalanine, in lower animals tyrosine.

Van Bemmeler (13, 1928) suggested the possibility of making genetic studies from photographs made of the human hair.

Maximow and Bloom (14, 1928) discuss the normal histology of the skin and show the developmental changes and structural attributes of a normal hair.

Luhring (15, 1928) concludes that pigment occurs in granules or in diffuse and colloid form, and that there is no transition between yellow and black pigment.

Peck (16, 1929) showed that there was a relationship between the amount of color granules produced and the amount of light. The greatest amount was produced by those individuals treated with ultra-

violet light, the least produced by the light coming in the room through the windows.

Koller (17, 1930) demonstrates that pigment is due to the action of the oxidase tyrosinase.

Dawson (18, 1930) studied the surface hair development of guinea pigs and made notations on the developmental features of surface hair. Five hair types were described. Age and growth features were also described.

Hentschel (19, 1930) found pigment forming cells present as brownish toned structures at the earliest stage of hair germ formation. When the bung is formed granules are found in the lowest cell strata of the outer root sheath. Pigment starts in the upper part of the papilla and lays hat-like around through the matrix stratae. (Trans. from German).

Needham (20, 1931) discusses the enzyme tyrosinase. He suggests the time of appearance of human hair in the embryo. He shows that pigment of ectodermal origin comes before pigment of mesodermal origin.

Boyd (21, 1932) showed that the coat color pattern is laid down in the earliest stage of the individual. This was done by a series of transplants of skin which developed hair color characteristic of the source from which it originated. He puts forth the idea that color pattern is initiated and determined prior to birth.

Laidlaw (22, 1932) cites Block who contends that "dopa" is an indicator of the presence or absence of the natural ferment which is responsible for melanin production.

Jacobsen and Klinck (23, 1934) discuss the formation of melanin in the human body and point out the difficulty of its isolation due to its specific chemical properties.

Jacobsen (24, 1934) reviews the chemical aspect of the melanin problem in his article on Melanin II. He lists a bibliography of 58 sources relating to the chemical aspects of melanin.

Jankowsky (25, 1935) proposes two theories on the relationship between hair color and pigment. One theory is based on the differing qualities and quantities of pigment, and the second recognizes degrees of presence or absence of an essential chemical element. He concludes that hair color depends on the free natural blend of a single pigment substance.

Einsele (26, 1937) developed methods for measuring the amounts of color pigments in the hairs of mice of varying genetic constitution to show the gene effect on the quantities and kinds of pigment present. He concludes (1) differences exist among the hair melanins of mice such that a unit quantity of melanin from one genotype absorbs less light than the same quantity from another genotype. (2) Different genotypes show different granule size and distribution. (3) There is a proportionate weight of granules in the hair depending on its source.

Reed and Sanders' (27, 1937) observations conflict with those of the author of this thesis. They report that there is no pigment in the developing hair follicles of newly born mice. They found that pigment pattern is already determined at birth in mutations of white-bellied agouti (A^w), dominant spotting (W) and black and tan (a^t).

Daniel (28, 1938) determined the absorption spectra of melanins from the hair of mice of various colors, of known genotypes. They do not differ significantly. Hence the changes in coat color seem to be due to quantitative variations in pigment rather than qualitative. This seems to be the same pigment that is present in at least two breeds of horses. A table is given showing the absorption curves of the genotypes studied.

Dunn and Einsele (29, 1938) studied the members of the albino series and found that in combination with black, the reduction in intensity of color by graded steps from full color (black) to white is accomplished by a parallel graded reduction in the quantity of melanin as measured by weight. The chief reduction is the decrease in the size of the granules. It is probable that the type of melanin molecule is the same in all genotypes.

Strickler (30, 1940) finds that hair does not erupt on the dorsum until the third day of life of a mouse with a majority of specimens exhibiting hair eruption for the first time on the fourth day. Skin pigmentation is found at 3 days of age on the dorsum of the mouse.

Wright (31, 1917) in his study has indicated that enzymes are factors in granule production and suggests the following hypothesis:

(1) Melanin is produced by oxidation of protein through action of enzymes.

(2) This reaction occurs in the cytoplasm, probably by enzymes secreted from the nucleus.

(3) The chromogens used, particularly the ones oxidized, depend on the character of the enzyme present.

(4) Hereditary differences are due to the enzyme element in the reaction.

Trotter (32, 1932) in Dr. Cowdry's book has a complete discussion of the development of hair. A bibliography of subjects relating to the topic is given.

Cowdry (33, 1932) in his book refers to Fürth who suggests that melanin formation depends on the action of tyrosinase or allied enzymes. The latter split the chromogen groups from the protein

molecule, these groups, oxidized by the tyrosinase, undergo condensations and take up sulphur and iron holding groups and other compounds. The entire complex forms melanin.

PART II

GENERAL TECHNIQUE

1. SECURING THE MATERIAL
2. FIXING THE SKIN SECTION
3. EMBEDDING
4. MOUNTING
5. SECTIONING
6. STAINING
7. DISCUSSION OF THE STAINING TECHNIQUE

GENERAL TECHNIQUE

1. SECURING THE MATERIAL.

In the present study individuals from the following strains of mice were used:

	Stock	Genetic Formula
(1)	Albino	aabbccdd
(2)	Black and Tan	A^tA^tBB CC DD
(3)	Pink-Eyed Black Agouti	AABBCCDDpp
(4)	Chinchilla	AABB $C^{ch}C^{ch}$DD
(5)	Dark-Eyed Black Agouti	AABBCCDD
(6)	Dilute Brown	aabbCCDDii
(7)	Light-Bellied Black Agouti	A^WA^WBBCC DD
(8)	Chocolate	aabbCC DD

Originally the foundation stock animals were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Descendants of the animals, the genetic formula of which are given above, served as the stock material from which animals were taken for this investigation. All of the individuals used in this experiment were raised in the rodent colony at Michigan State College in the summer terms of the years 1936, 1937, and 1939.

When a stock female showed signs of pregnancy she was removed from the stock breeding cage and placed in a freshly cleaned and littered individual cage, supplied with food and water. A small tuft of shredded paper was placed in the cage for the mouse to use as nest material.

The mice in the stock breeding cages were fed Fox Chow* and hard dog biscuit. The composition of the Fox Chow was as follows.

Purina Fox Chow Analysis:

Crude Protein	not less than	20%
Crude Fat	not less than	3%
Crude Fiber	not more than	6%

Ingredients: Wheat germ, dried skim milk, liver meal, barley malt, fish meal, dried meat 3% alfalfa meal, corn grits, soy-bean oil meal, cereal feed (from commercial wheat and rye), molasses, dried beet pulp, 0.75% cod liver oil (containing 1000 U. S. P. units of vitamin A and 150 A.O.A.C. check units of Vitamin D per gram), 1% iodized salt, 300,000 U. S. P. units of Vitamin A per 100 lbs of feed derived from 2% Pur-A-Tee-Carotene (containing 1,500,000 U. S. P. units of Vitamin A per pound).

When the pregnant females were isolated some were placed on a balanced grain diet and some were kept on the regular Fox Chow - dog biscuit diet. Fresh water in glass bottles with a dropper tube attached was kept before the animals at all times.

Each pregnant female was carefully identified by means of a label fastened to the base of the cage which bore her complete identification as to breed, genetic formula, date of isolation, and the experimenter's name.

At approximately the same time each day the litter cages were inspected to see if any young had been born. The regular inspections

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*Purina Fox Chow, Purina Mills, Ralston Purina Company, Davenport, Iowa.

of the cages were made in an effort to obtain the exact time of birth. As a result, the age of the mice in this experiment is accurate to within a fraction of a day. In a number of individual cases the exact hour of birth was observed. All observations were carefully recorded. The isolated animals were examined, and as soon as young were born the birth was recorded on the back of the label on the cage. On each succeeding day one mouse from the litter was removed and killed for study. In the latter part of the experiment two individuals were removed from the litter in the cage and killed each day.

The fundamental data were recorded in a large cloth-bound book and kept as a permanent record of the experiment.

In addition to the above facts other information was entered in the record relative to the method of treatment of the individual sample of skin.

2. FIXING THE SKIN SAMPLE.

The stock specimens (ages, birth to 9 days) were periodically taken from the littering cages and killed with ether. A longitudinal incision was made on the ventral side of the specimen, extending from the posterior portion of the abdomen anteriorly to the apex of the lower jaw. This made the skin tissues of the specimen very accessible to the fixing reagent. Such animals were then placed in Bouin's Fluid* for three hours.

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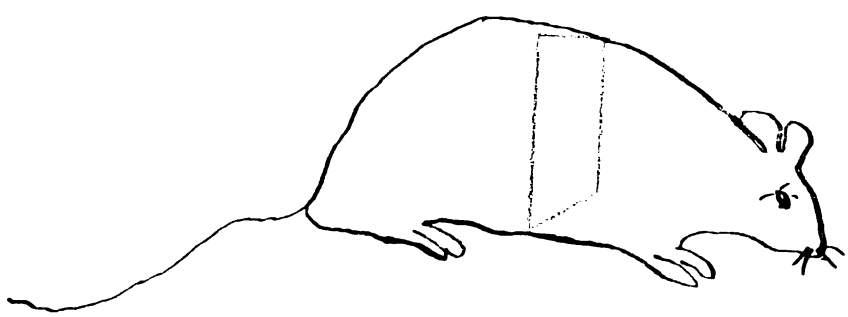
* Picric Acid, Saturated aqueous solution - 75 parts
Formol - 25 parts
Acetic Acid - 5 parts

At the expiration of three hours the mouse was removed from the fixing fluid and washed in 70% alcohol to remove the excess amount of fixative. The specimens were then put into individual bottles containing fresh 70% alcohol, in which they were permanently kept.

In securing a representative section of skin from the body of the mouse it was deemed wise to select the tissue from the same region on all the mice studied. The sample chosen was a strip including portions of the dorsal, lateral and ventral surfaces. A region was selected about half way between the anterior and posterior legs, running from the mid-ventral region up to the mid-dorsal surface. All of the skin samples were removed from the right side of the specimen.

The object in selecting such a specific region was not alone to obtain a characteristically pigmented area but also to secure one in which the general contour and direction of the growing hair would be uniform from sample to sample, affording the maximum opportunities for study. T he region of the animal roughly between the mid-dorsal and mid-ventral line extending over the side was thought to possess all of the desired qualities of hair position and pigment gradations. The hair in this area grows uniformly, the individual follicles descend into the tissue at about a 30 degree angle, and the tip of the hair extends toward the caudal part of the animal.

The incision to remove the skin was made in the manner indicated by the accompanying diagram -



The asymmetry of the skin sample was to enable the writer to section the specimen lengthwise of the hair follicles. In sectioning the tissue one therefore would always be able to distinguish the region of the animal from which the skin was secured, and to feel reasonably sure of the direction of slant of the hair follicles in the section. Such careful marking of the skin permitted sections to be made in a manner so as to strike the major per cent of hair follicles in a longitudinal plane.

3. EMBEDDING.

Two methods of embedding the tissue in paraffin were used. Specimens #1 - 120 were embedded in paraffin employing the alcohol-xylol method. Specimens #121 - 228 were embedded by the dioxane method. A brief discussion of the methods employed in both systems is in order.

(a) Alcohol-xylol Method.

In the alcohol-xylol method the tissue was taken from the 70% alcohol container and treated with pure alcohol to insure the removal of excess water. Impregnation with paraffin was accomplished by permitting the tissue to remain in a paraffin bath overnight. The impregnated skin was embedded in labeled paper forms as indicated by the diagram. It was oriented to facilitate the procedures of sectioning.

View showing orientation of skin in paper form.

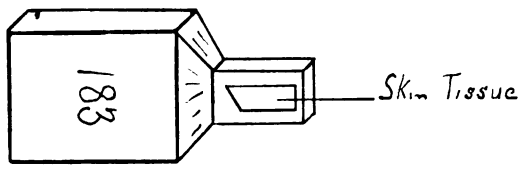
(b) Dioxane Method.

Specimens #121 - 228 were embedded by the dioxane method. Covered glass vials filled with dioxane prepared the tissue for impregnation

with paraffin. A mixture consisting of 1/3 dioxane and 2/3 melted paraffin further insured the removal of water from the tissues. Pure paraffin was employed for final impregnation. The tissues then were ready for mounting.

4. MOUNTING.

The embedded tissues were mounted on wood blocks. The accompanying diagram illustrates the method of affixing the specimen on the wood block.



The above procedure rendered the tissues ready to enter the sectioning technique.

5. SECTIONING.

The skin tissues were embedded in hard (56°-58°) paraffin. These mounts were sectioned on the microtome. The physical conditions being adjusted, serial sectioning was accomplished from 5u to 10u in thickness depending on the nature of the individual tissue. These sections were placed on an albumen fixed slide and allowed to dry. Three slides were made from the material obtained from each specimen. This afforded an opportunity to employ various staining techniques on the same specimen and make critical comparisons of the material. The sections now were ready to enter the process of staining technique.

6. STAINING.

At first in the staining technique is was necessary to use a trial and error method in order to find out which procedure would clearly reveal cell structures and outlines and to display the pigment granules to advantage.

Essentially ten methods of staining were employed. A comparison of the stained specimen of one technique could be made with a similar section stained with another technique. This would assure one of the relative value of the stain.

The fact should be cited that each of the following techniques was used in outline form, however, as an individual modification proved its value various alterations were incorporated into the specific staining technique. These modifications employed rendered the tissues properly stained and suited to the study purpose. Comparisons of the final stained specimens proved to be grounds for acceptability of evidence.

An outline of the staining techniques used is given in the following section.

(1) Hematoxylin Staining

1. Xylol - 10-15 minutes
2. Absolute alcohol - 1 minute
3. Pass through 95%, 70%, 50%, 35% alcohol - leaving ½ minute in each
4. Delafields Hematoxylin, until properly stained
5. Wash in water 5 minutes
6. Pass up through 70% alcohol ½ minute each
7. Acid alcohol (70%) - 30 seconds

8. Dip in 70% alkaline (.01% bicarbonate of soda)
9. 95% alcohol - 1 minute
10. 100% alcohol - 3 minutes
11. Carbol xylol - 5 minutes (until clear)
12. Mount

(2) Eosin and Hematoxylin Staining

1. Two changes of xylol - 3-4 minutes
2. 95% alcohol - 2-3 minutes
3. Iodized alcohol - 4-5 minutes
4. 95% alcohol - 3-4 minutes
5. 5% solution of aqueous sodium thiosulphate - 10-20 seconds
6. Wash in tap water, 2 changes - 1-2 minutes each
7. Overstain in Harris Hematoxylin - 3-4 minutes
8. Wash in water, 2 changes - 1-2 minutes each
9. Differentiate in acid alcohol (1% HCL in 70% alcohol)
10. Dip quickly in tap water, neutralize in (1% of NH OH)
11. Wash in 2 changes of tap water - 1-2 minutes each
12. Stain in 1% aqueous solution of eosin - 30-60 seconds
13. Wash in two changes of tap water
14. 95% alcohol - 2-3 minutes
15. Absolute alcohol - 2-3 minutes
16. Carbol xylol, 2 changes - 3-4 minutes
17. Neutral xylol, 2 changes - 3-4 minutes
18. Mount in balsam

(3) Heidenheins Iron - Hematoxylin

1. Pass the slides through xylol - 10 minutes

   Absolute alcohol - 2 minutes

   95% alcohol - 1 minute

   Water - 1 minute

2. Put into iron alum - until properly mordanted
3. Wash in water
4. Stain 0.5% Hematoxylin - 1 hour
5. Rinse 5 minutes in water
6. Place into iron alum (extract excess stain)
7. Wash in several changes of water

   (Insure removal of iron alum) causes fading

8. 95% alcohol

   100% alcohol

   xylol

9. Mount

(4) Methyl Green

1. 2 changes of xylol (remove paraffin)
2. 95% alcohol - 2-3 minutes
3. 95% alcohol (fresh) - 2 minutes
4. Pass through 95% 70%, 50%, 35% alcohol - 1 minute each
5. Methyl green stain (to correct intensity)
6. Wash in water - 5 minutes
7. Pass up 50%, 70% alcohol

   Acid 70% alcohol (1 drop HCL)

8. Destain to correct intensity
9. Dip in 70% alkaline alcohol (.01% bicarbonate soda)
10. 95% alcohol - 1 minute
11. 100% alcohol - 3 minutes
12. Carbol - xylol until clear
13. Mount in Canada balsam

(5) Methyl Green and Eosin

1. 2 changes of xylol (to remove paraffin)
2. 95% alcohol - 2-3 minutes
3. 95% alcohol, fresh - 2 minutes
4. Pass through 70%, 50%, 35% alcohol - 1 minute each
5. Methyl green stain to correct intensity
6. Wash in water - 1 minute
7. Dip in eosin to correct intensity
8. Wash in water
9. Pass up 35%, 50%. 70% alcohol
10. Destain in acid alcohol (1 drop HCL)
11. Dip in alkaline alcohol (.01% bicarbonate of soda)
12. 95% alcohol - 1 minute
13. 100% alcohol - 3 minutes
14. Carbol xylol until clear
15. Mount in Canada balsam

(6) Methyl Green - Acid Fuchsin

1. 2 changes of xylol (to remove paraffin)
2. 95% alcohol - 2-3 minutes
3. 95% alcohol, fresh - 2 minutes
4. Pass through 70%, 50%, 35% alcohol - 1 minute each
5. Methyl green stain to correct intensity
6. Wash in water - 1 minute
7. Acid fuchsin to correct intensity
8. Wash in water
9. Pass up through 50%, 70% alcohol
10. Destain in acid alcohol (1 drop HCL)
11. Dip in fresh 70% alcohol
12. 95% alcohol - 3 minutes
13. 100% alcohol - 3 minutes
14. Carbol xylol until clear
15. Mount in Canada balsam

(7) Acid Fuchsin Stain

1. 2 changes of xylol to remove paraffin
2. 95% alcohol - 2-3 minutes
3. 95% alcohol, fresh - 2 minutes
4. Pass through 70%, 50%, 35% alcohol - 1 minute each
5. Acid fuchsin stain to correct intensity
6. Wash excess stain in several changes of water
7. Destain in 95% alcohol (carefully)
8. Dip in fresh 70% alcohol

9. 95% alcohol - 1 minute
10. 100% alcohol - 3 minutes
11. Carbol xylol until clear
12. Mount in Canada balsam

(8) Methyl Green, Eosin, Acid Fuchsin Staining

1. 2 changes of xylol
2. 95% alcohol - 2-3 minutes
3. 95% alcohol, fresh - 2 minutes
4. Pass through 70% 50%, 35% alcohol - 1 minute each
5. Acid fuchsin to correct intensity
6. Wash excess in water
7. Eosin stain until correct intensity
8. Wash off excess stain in water
9. Methyl green stain to correct intensity
10. Wash off excess stain in water
11. Pass up through 50% and 70% alcohol
12. Destain very carefully in acid alcohol
13. Dip in fresh 70% alcohol
14. 95% alcohol - 1 minute
15. 100% alcohol - 3 minutes
16. Carbol xylol until clear
17. Mount in Canada balsam

(8) 1% Safranin Staining

1. 2 changes of xylol to remove paraffin

2. 95% alcohol - 2-3 minutes
3. Fresh 95% alcohol - 2 minutes
4. Pass through 70%, 50%, 35% alcohol - 1 minute each
5. 1% Safranin stain (rapid acting) to desired intensity
6. Wash off excess stain in water
7. Pass up through 50%, 70% alcohols - 2 minutes each
8. Destain in acid alcohol to desired intensity
9. Dip in fresh 70% alcohol
10. 95% alcohol - 1 minute
11. 100% alcohol - 3 minutes
12. Carbol xylol until clear
13. Mount in Canada balsam

(10) Hematoxylin. Ferric Alum Staining
(Formula from Dr. Carl Gower)

1. Mordant in 2.5% ferric alum - 2 hours
2. Dip several times in water
3. Place in Hematoxylin - 2 hours
4. Wash in running water - 5-10 minutes
5. Destain in .2% ferric alum solution until gray - 15-20 minutes
6. Place in running water for 10-15 minutes
7. Dip several times in distilled water
8. Dehydrate 70% 95%, 100% alcohol
9. Carbol xylol until clear
10. Mount in Canada balsam

7. DISCUSSION OF THE STAINING TECHNIQUES

No exact scale or comparative standard of the staining techniques used is possible. Two slides apparently given the same attention in the technique would exhibit varied end results, one being highly acceptable and the other only moderately serviceable. Extra factors made it necessary for one to attempt following the most successful method of procedure, duplicate the good features, and incorporate those processes into the procedure for the future slides.

The following then is a personal evaluation of the staining systems and their adaptability to the type of tissue used in this study and the individual modifications employed by the author.

Most of the stains used have more or less specific purposes as they display certain parts of the tissue. Some stains show affinity for elements of the nucleus or of the cytoplasm. Each stain thus used was purposely employed.

(1) Regular hematoxylin staining.

Hematoxylin follows well after any type of fixing agent and is known as a general all purpose stain. It showed general staining qualities rather than selectivity.

(2) Eosin and Hematoxylin staining.

Hematoxylin, a general stain, was used to stain for general features. Eosin, an anilin dye was used as a contrast stain to display cellular structures not fully exploited by the general stain.

(3) Heidenhain's Iron Hematoxylin.

This type of staining was for general purpose observation. The results did not display to good advantage the particular features desired in the study.

(4) Methyl Green.

Methyl Green is one of the best nuclear anilin stains. It stains the chromatin of the nucleus causing it to become well differentiated. It was found serviceable as it contrasted in color with the brown color granules.

(5) Methyl Green and Eosin.

Methyl green, a good nuclear stain, was tried in combination with eosin as a contrast stain. Each affected its own characteristic cellular structure.

(6) Methyl Green and Acid Fuchsin.

Methyl green, a good nuclear anilin stain, was added to acid fuchsin, an excellent stain for cytoplasmic structures. This combina - tion was employed on a representative number of slides and produced good results.

(7) Acid Fuchsin.

Acid fuchsin alone is a good cytoplasmic stain. Slides with this stain alone accentuated the cytoplasmic structures to the exclusion of similar features of the nucleus.

(8) Methyl Green, Eosin, and Acid Fuchsin.

A combination of the three stains mentioned in the previous paragraph was employed on a specific group of slides. It was found that the addition of any third stain did not accomplish a worthy purpose.

(9) 1% Safranin.

Safranin is an important basic dye. Its staining reaction is rather rapid and correct differentiation is accomplished in the destaining process.

(10) Hematoxylin Ferric Alum Stain. (Dr. Carl Gower)

The major portion of the tissues in the later part of the experiment were stained using the Hematoxylin ferric alum staining procedure outlined by Dr. Gower. The stain is specific for nuclear cell structure and performs equally well in general staining. The iron alum acts as a mordant in preparing the tissue for the reaction of the stain. Strong ferric alum solution is allowed to mordant the tissue for a two hour period before it is stained for a similar length of time. Destaining, accomplished in a weak solution of ferric alum, causes the sections to assume their characteristic silver gray color. When this color is obtained the destaining process is complete. Destaining is usually accomplished in twenty minutes. After washing, dehydration and clearing, the slides are ready for mounting.

Material obtained by the use of this stain was always well prepared. The cell structures were clearly outlined, and the internal structure clear and definite. The quality of color was of the desired intensity to afford maximum cell structure clearification. However the quantity of color present was not too great to cause the clouding or the excluding of any of the delicate nuclear or cytoplasmic portions. The color itself, gray, contrasted with the brown color of the minute granules, rendering them easily detectable.

This last method of staining contained all of the qualities desired of a particular stain. This fact was sufficient reason for employing it in a major per cent of cases in the later part of the experiment.

Representative types were stained with each technique to convince the author of its relative value or of its relative inadvisability.

In the staining processes regulation Coplin staining jars were used. The jars were cleaned and fresh supplies of reagents were placed

in them whenever it became evident in the slightest degree that a change would be beneficial. This careful observation and management of the reagents afforded the maximum possibility for proper staining performance.

Care was used in the staining technique. No slide was ever allowed to become exposed to the air for periods of time as this would render the tissue less impregnable to the stains. Precaution was taken also in the management of the slides to insure their being kept from dust or foreign materials of any type which would cause the stains to be ineffective.

During the actual processes of staining, a microscope was placed near the jars. This afforded opportunity for superficial examination of the slides under low power objective to get a better estimate as to the degree of penetration of the stain. In the destaining processes, both in the method employing 95% alcohol and in the 70% acid alcohol method, the low power microscope examination was again used to make certain of the exact stage to which the sections had been destained. When the intensit y of staining had been reduced to the proper degree, the slides were run up through the alcohols and made ready for permanent mounting under cover glass.

In general the specimens were classified into the specific color strains, and into age groups within each strain. Critical examinations and comparisons were made among sections from a particular strain and age group in regard to staining techniques. Comparisons were also afforded between the various age groups. Perhaps the most important comparisons afforded was that of the individuals of one strain contrasted with a comparable age group of another stock. Gross and specific comparisons

were made possible by an examination of the colored strains of animals with the albino stock specimens in both stained and unstained material. Sections of albino skin had no pigment granules to cover up cell structures. Comparisons between albino and colored skin sections often clarified obscure structural situations in the colored skins.

PART III

STUDY OF THE PREPARED SLIDES

1. GENERAL STUDY
2. ERUPTION OF THE HAIR
3. STUDY OF THE FOLLICLES
   - a- Description of the follicles
   - b- Classification of the colored strains
4. MICROPHOTOGRAPHS OF THE OBSERVED AREAS
   - a- General mechanics
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STUDY OF THE PREPARED SLIDES

1. GENERAL STUDY.

The processes of technique previously described had as their purpose the production of material best suited for the study of color origin in the hair. The requirements of clearness and well defined material, properly stained and mounted, were at all times kept foremost in mind in the processes of preparation. The entire stock of material that was prepared had been classified into the color strains of animals and into definite age groupings. All of the individuals that were killed on the day of birth were put into one division and those killed on the first day were arranged in chronological order in the next section. Similarly the remaining specimens of the particular color stock were systematically arranged in age groups.

The boxes were labeled bearing the name of the color strain of the animal which they contained. The animals from the following strains of mice were arranged into age groupings from birth as far as the 9 day period.

Black and Tan

Chocolate

Light-bellied black agouti

Pink-eyed black agouti

Chinchilla

Dark-eyed black agouti

Dilute brown

Preliminary examinations of all of the slides immediately afforded one a reliable indication of their relative values. Written records were kept of each individual slide and notations made concerning the observed features and any outstanding character displayed by the particular slide. In this manner a system of information was compiled which dealt with each slide revealing its relative merits for future study based on the evaluation of the sectioning, staining, orientation, and preparation of the section. In later and more careful observations desirable slides were selected and exposed to even more careful investigation. By such a process of elimination and selection a desirable set of slides was procured for each of the strains and age groups of mice under investigation.

The piece of imbedded tissue was oriented in such a way as to secure longitudinal sections of the hair follicles. Failure to cut the follicles in this plane, and imperfections in staining reduced the number of slides serviceable for study. These possibilities were recognized early in the investigation. Hence, three slides were made from each specimen, thus considerably increasing the probability of securing at least one with properly sectioned and stained material.

In a continuation of the selective process a more or less arbitrary system was used which designated individual slides as being characteristic of their particular age group. These slides which by examination proved to be correctly stained, sectioned and in addition were characteristic of their age group, formed the basis of study for their specific stock. The slides thus selected possessed to a high degree the combined features sought and exhibited to the greatest advantage those particular objects which were to be carefully noted.

As the examination of the slides progressed a system of marking and location of individual hair follicles and even cell structures became necessary. It became evident that if these individual cells were to be compared with other cells a system of location was mandatory. As a basis for these frequent locations the micrometer readings on the movable stage were recorded in a log record of the slide in a manner like a fraction $\frac{15}{53}$, the numerator indicating the horizontal reading and the denominator the scale reading in the direction at right angles to the first reading.

2. ERUPTION OF THE HAIR.

An interesting sideline of the study developed when the exact time the hair erupted through the skin was investigated. The slides which were prepared for the main body of the investigation provided the material on which the study was conducted. Two hundred five slides were inspected. The various color strains of mice were examined. The data thus compiled appear as table 3 and graphs 1 to 8.

No attempt was made to calculate the actual number of follicles that had produced hair to such a degree of advancement that it extended through the surface of the skin. In this means of classification the individual sections were examined under a low power objective and observations made to determine whether the developing hair had progressed to such a stage that some of the shaft extended free from the surface of the skin. As soon as the section exhibited this evidence that individual animal was classified as falling into the hair erupted class.

In the specimens of 2-3-day age groupings it became increasingly difficult to locate hair extending free from the surface. In each case

the entire number of sections on the slide was examined in order to determine if there was hair erupted. In the two youngest groups--birth, 1-day--every section was examined to observe the hair growth and it was found that none of them had produced hair of sufficient development to be extended free above the skin.

There were variations within the individual strains. However, the number of specimens examined, 205, is too small to justify conclusions. The results are only suggestive.

The black and tan stock, graph #1, did not exhibit any eruption of hair in the birth or 1-day old age groups. There were 9 specimens in the two-day age group, 3 of which (33%) showed hair erupted. Of the ten specimens in the 3-day age group, 8 (or 80%) showed hair at the surface. The group of 25 specimens whose age ranged from 4 to 8 days all (100%) were clearly seen to have matured hair above the skin.

The chocolate stock, graph #2, had no specimens exhibit hair prior to the second day. There were 13 specimens of this age. In the 2-day old group there were 7 individuals, 3 of which (42.8%) evidenced eruption of hair. The three-day old group had 7 representatives, 5 of which (72%) showed free surface hair. There were 19 individuals in the 4-9-day age group. All of these (100%) exhibited eruption of hair through the surface.

The light-bellied agouti stock, graph #3, had 10 specimens in the birth, 1-day and 2-day age grouping. None of these individuals exhibited hair above the surface of the skin. Two specimens were in the 3-day old group and both (100%) exhibited erupted hair. There were 3 members in the 4-5-day age grouping, all of which (100%) show erupted hair.

In the dark-eyed black agouti stock, graph #4, 9 specimens were in the birth and one-day age; none of these evidenced free surface hair. In the 2-day age group 5 specimens were examined and 1 (20%) showed hair extending free from the skin. The members of the 3-day age group, 5, all (100%) showed hair erupted. Likewise the 13 members of the 4-9 day age group showed 100% eruption.

The pink-eyed black agouti group, graph #5, had 4 members in the birth and 1-day age groups none of which show surface hair. The 2-day age group contained 5 individuals; 1 (20%) had erupted hair. The 3-day age group had three members, all of which (100%) had free surface hair. There was 1 individual in the 4-day age group. It had erupted hair.

The chinchilla stock, graph #6, had 4 individuals in the birth, 1-day and 2-day age groupings, none of which exhibited eruption of hair. The 4 individuals in the 3- and 4-day age classification all (100%) showed erupted hair.

In the dilute brown stock, graph #7, neither the 3 members of the birth age grouping nor the 13 members of the 1-day old age exhibited surface hair. Of the 10 individuals 2 days of age 2 (20%) exhibited hair eruption. The 4 members of the three-day age group all showed hair extending above the surface. The 4-day age group with 4 individuals in like manner all (100%) showed the eruption of hair.

A summary, graph #8, of the 7 colored strains of mice shows a most interesting comparison of the strains. There are 19 individuals in the birth age grouping, none of which exhibit surface hair. The 47 specimens in the 1-day age group did not show hair eruption. Thirty nine specimens represented the 2-day age group; of these 10 (or 25.6%) exhib-

ited eruption of hair. The 3-day age group comprised 33 individuals, 29 of which (87.8%) had free hair extending above the skin surface. The age grouping from 4–9 days contained 67 specimens all of which (100%) showed erupted hair.

Due to the comparatively small numbers in this experiment, these data are not conclusive. They merely serve as an indication of the hair eruption trend.

An examination of the graphs made for the individual strains afford a quick means of comparison of the specific strains. As indicated by graph #8, eruption of hair takes place beginning with the second day of the individual's life. At this time roughly one-fourth show developed hair beyond the skin. The third day witnesses a vast majority erupting hair. By the fourth day all have completed the act.

Reference should be made to the fact that the tissue from the specimens used in this experiment was obtained from the mid-region of the animal, running from a mid-dorsal line over the lateral portions to the mid-ventral region, embracing a major portion of the skin tissue between the front legs and the flank of the mouse. The facts presented are known to be true only for the region of the body studied.

Strickler (30, 1940) finds, from a gross examination, that hair does not first appear on the dorsum until the third day of life of the mouse. The majority exhibit dorsal hair on the fourth day. All show hair erupted by the fifth day.

This evidence differs from that obtained by the author. In this thesis it is observed that hair eruption begins on the second day, a major per cent (87%) show free surface hair on the dorsum on the third day, and all have dorsal hair by the fourth day.

The deviation in the data obtained is explained by the fact that the author employed the use of a microscope in observing the sections. This afforded a more accurate means of examination than that of the previous investigator who used a hand lens.

3. STUDY OF THE FOLLICLES

a- Description and classification of the follicles.

Attention was turned to a study of the individual hair follicles, for they were the chief objects of observation as they contained the secret of the origin of the color granules.

It soon became evident that one would be able to classify the follicles into several more or less distinct types, basing the classification on the progressive changes exhibited in the normal growth procedure of the hairs. Several provisional systems of classifications were devised, such as the counting the numbers of pigment granules and letting them serve as a basis for classification. This system was abandoned because even at the earliest stage of development the color granules are comparatively numerous and defy counting. No set standards based on counting could be devised.

Instead of counting the color granules adjacent to the papilla, a system of classification was devised which noted the area near which the granules were located. In the earliest stages of the growth of a hair, diagram 1, it was observed that the granules seemed always to be congregated in an area near or around the tip of the papilla. This initial stage of classification was designated as follicle type #1. It

represented the area in which apparently pigment first made its appearance. Progressively the other stages or types in our arbitrary system were defined.

Follicle type #2, diagram 2, was designated as the group in which the granules were arranged over the apex of the papilla and extended proximally to a point approximately half the distance from tip to base of the papilla along the sides.

Follicle type #3, diagram 3, was designated as the group which had developed pigment along a major portion of the sides of the papilla, but the advance of the granules had not progressed to any considerable extent into the developing hair shaft.

Type #4, diagram 4, comprised the group of follicles in which the granular area surrounded the sides and tip of the papilla, and granules were found throughout the length of the immature hair shaft.

Further classification of the hair follicles beyond this stage was not necessary as this investigation concerns itself with the origin of color granules. Further differentiation of the hair produced progressively developmental stages which lend themselves to classification in like manner. This again is another problem which is most interesting and could serve as a topic of some future study.

At the outset of the study of the follicles these types were rather readily observed. Accordingly a sketch was made of each stage and these sketches were used as standards in the further classification of follicles.

Twenty-five longitudinally sectioned follicles were deemed sufficient for determining the class to which any skin specimen belonged.

The system here employed is of scientific value in that it can serve as a basis for determination of the progressive stage that the individual hair follicle has attained in its development. It indicates whether the follicle is in the initial stages of pigment formation or whether it has passed into the stage where the process is but a continuation of growth and enlargement.

The system here devised can be used by other investigators and will afford them the means of identifying the developing hair follicles to determine which individual follicle is in the actual process of initiation of the color granules. Appropriate material can be selected from which they can carry out study to determine the structural elements which concern themselves with color origin.

Reference has previously been made to the fact that observation of specimens beyond the two-day stage proved of little import in that the purpose of this study concerned itself with the origin of color granules. This is definitely a problem of the first stages in the development of the follicles.

The entire group of graphs and data sheets dealing with the classification of the hair follicles proport to the evidence as exhibited by the first stages, namely birth, 1-day and 2-days.

The classification of hair follicles enabled us to determine whether the various colored strains showed differences in follicular characteristics.

The animals of the more intensely colored varieties were examined and classified first. Progressively the mice of the other strains which possessed less color intensity were examined later. This procedure

tended to permit one to be relatively sure of the classification of the intense strains and in a similar manner afford the same comparative assurance for the less intensely colored varieties.

Classification of the follicles of the stock progressed through the entire series of the seven color strains. Graphs #9-24 were made to present the data obtained for these stocks. A summation of these results was graphed (graphs #23 and #24) to indicate the relative value or significance of the system employed.

Black and Tan. The black and tan strain, graph #9 exhibited the following classification. There were 5 animals who were killed and examined at birth; 147 follicles were classified. Of these 88 fell in follicle type #1, 51 were type #2, 7 were type #3 and only one was seen to be of type #4.

Nine animals were of the 1-day age in this strain. A total of 225 follicles were examined and classified in these 9. These fell into the following follicle types, 12 were type #1, 37 were in type #2, 82 in type #3, and 94 were seen to be of type #4.

There were nine animals in the 2-day age specimen group. A total of 225 follicles were classified; 15 were found to be type #1, 16 seen to be type #2, 37 fell in type #3 and 157 were classed as type #4.

Transformation of this data into percentage calculations reveal the following information (graph 10):

Of the 147 follicles in the initial stage, 59% were type 1; 34.7% were type 2; 4.8% were type 3; and but .09% were of type 4.

In the 1-day stage of development the 225 follicles classified fell into these percentage relations: 5.3% are seen to be of type 1; 16.4% exhibit type 2 qualifications; 34.4% are found to be type 3; 41.7% are classified as type 4.

The 2-day stage of development assumes these percentage ratings: 6.6% are in type 1; 7.1% are of class 2; 16.4% are of class 3; and 69.7% fall into class 4.

The following classification resulted for chocolate stock of mice (graph 11):

There were 4 animals who were killed and examined at birth. Due to staining difficulties resulting in intense impregnation of stain, the color granules if evident tended to be covered by the stain and unobservable to the eye. It was evident that there was pigment present but due to ineffective staining it was thought best to omit this group as the classification would probably be inaccurate.

Nine animals of this stock were in the 1-day age grouping. Ninety one follicles were classified. Five were found to be in type 1; 25 were of type 2; 45 were found to be type 3; and 16 were seen to fall in type 4 classification.

Seven animals of this color strain were in the 2-day age class. There were 155 follicles classified in this grouping. Eleven were found to be in type 1; 17 were considered type 2; 36 were listed as type 3; and 92 appeared to be type 4 classification.

Transforming the above information into percentages (graph 12) reveals the following information:

The birth stage having been stained improperly was excluded.

In the 1-day old stage, 91 follicles; 5.5% are of type 1; 27.4% are in type 2; 49.7% are seen to be type 3; and 19.7% fall into type 4 follicles.

The 2-day old stage had 155 follicles classified of which 7% were of type 1; 10.9% were listed as type 2; 23.7% were classed as type 3; and the remaining 59.3% were in type 4.

The light-bellied agouti stock was examined (graph 13) and revealed the following distribution. There were 3 animals in the initial stage of development. Seventy five follicles were classified in this group. Thirty six of these follicles were in type 1; 29 were classed as type 2; 10 were listed as type 3 and there were no follicles which came in class 4.

Five animals were in the 1-day stage of development. One hundred twelve follicles were classified. Ten were of type 1; 26 were of type 2; 54 came to be type 3; and 22 were classified as type 4.

Two animals represented the 2-day old age with 41 follicles classified. There were 2 which were in type 1; 4 in type 2; 14 in type 3; and 21 in type 4.

Transposing these data into a percentage scale (graph 14) the record reveals that of those of the birth stage, 48% were of follicle type 1; 38.6% classed as type 2; 13.3% as type 3, and 0 percentage points for type 4.

The 1-day old specimens with 112 follicles give 8.9% listed as type 1; 23.2% as type 2; 48.2% were of type 3; and 19.6% were of the final type 4.

The 2-day old individuals with 41 follicles classified came under the four types as follows: 4.87% were type 1; 9.7% were type 2; 34.1% were listed as type 3; and 51.2% came to be listed as type 4.

The dark-eyed black agouti stock had representatives in all of the three age groups (graph 15). There were 4 individuals in the birth stage with 100 follicles being classified. Sixteen were found to be of type 1; 52 were of type 2; 31 were type 3; and 1 follicle was classified as being type 4.

The 5 one-day-old individuals presented 100 classified follicles. Five follicles were of type 1; 32 were of type 2; 49 were listed as type 3; and 14 were classed as type 4.

The 2-day-old age group had 5 specimens represented and a total of 89 follicles were classified in this group. Three follicles were of type 1; 4 were of type 2 variety; 25 were of type 3; and 57 were of the last type 4.

Reference to the above in percentage scales (graph 16) exhibit the following follicle relationships. The birth stage with 100 follicles show 16% in type 1; 52% in type 2; 31% in the third type and but 1% coming in type 4.

The 1-day-age specimens with 100 follicles classified fall into the categories of 5% in type 1; 32% in type 2; 49% in type 3; and 14% in final type 4.

The last stage, the 2-day-age group, show 3.3% in type 1; 4.5% in type 2; 28.1% in the third type group; and 64.4% in the fourth type.

The pink-eyed black agouti stock (graph 17) had but two groups of ages represented in the stock. No specimens were obtained for the initial or birth age period.

Four specimens came in the 1-day age group and from them 60 follicles were classified into the 4 types. Fourteen were of type 1; 18 appeared as type 2; 20 were of type 3; and 8 were of type 4.

Four individuals came in the 2-day age group having 100 of their follicles classified into types. Four were of type 1; 17 were of type 2; 31 appeared to be type 3; and the remaining 48 were type 4.

Transposing this data onto the percentage scales (graph 18), we find that of the 60 follicles classified from the 1-day old individuals,

23.3% are type 1; 30% are of type 2; 33.3% are of the third type, and 13.3% are of type 4.

The 2-day old grouping whose 100 follicles formed the material for classification revealed 4% in type 1; 17% in type 2; 31% in type 3; and 48% being type 4.

The chinchilla stock (graph 19) had 2 specimens representing the 1-day age class and 2 specimens representing the 2-day age class. Fifty follicles were classified in each of these age groups. In the 1-day old specimens 9 follicles were found to be of type 1; 20 to be type 2; 16 to be type 3; and 5 to be type 4.

The two-day old class showed 1 follicle in type 1; 7 in type 2; 12 in type 3; and 30 in the final type 4.

In percentages (graph 20) these data exhibit the 1-day age group to have 18% in follicle type 1; 40% in type 2; 32% in type 3; with 10% being classed as type 4.

The 2-day age group shows 2% of the follicles as type 1; 14% as type 2; 24% as type 3; and 60% being classed as the last type 4.

The dilute brown stock (graph 21) presented representatives in all of the age groups and offered a larger number of specimens for age group placement.

The initial stage had 3 specimens with 68 follicles. Sixty-five were found to represent type 1; 3 were considered type 2; and none of the specimens were advanced as far as the third and fourth types.

The 1-day age group comprised 13 specimens from which a total of 306 follicles were classified. Twenty eight were of type 1; 84 were of type 2; 146 were type 3; and 48 were seen to be type 4.

In the 2-day age group there were 10 specimens affording a total of 250 follicles for examination. These resolved themselves into the four types. Sixteen follicles were in type 1; 37 in type 2; 75 in type 3; and 122 being listed as type 4.

More significance is attached to these data when they are expressed as percentages (graph 22). The birth age stage whose 68 follicles were classified displays 95.6% in type 1; 4.4% in type 2 and no individuals exhibiting the requirements of types 3 and 4.

The 1-day age which presented 306 follicles for classification revealed 9.1% falling in type 1; 27.45% in type 2; 47.7% in type 3; and 15.68% being in type 4.

The 2-day age group afforded 250 follicles for classification, and showed that 6.4% came in type 1, 14.8% were type 2, 30% were of type 3, and a total of 48.8% were in type 4.

In the 7 strains of mice studied throughout the three age groups, birth, 1-day and 2-day, a total of 2,245 hair follicles were classified into the 4 types (graph 23).

In the first or birth stage there was a total of 19 individuals from which 390 follicles were classified into the four types. Two hundred five were found to be of type 1; 135 came as type 2; 48 were listed as type 3 and only 2 came in the type 4 classification.

The 47 one-day-old individuals possessed 944 classified follicles. Eighty three follicles were in type 1; 242 came in type 2; 412 were found in type 3, and 207 exhibited type 4.

The final group of 39 two-day-old specimens had a total of 911 follicles, classified in the 4 types. Fifty two were placed in type 1; 102 in type 2; 230 in type 3; and the majority, 527 in type 4.

Presenting these data on the percentage scale (graph 24) affords one a most accurate comparison of frequencies of types, and forms a basis for conclusions.

The 390 follicles classified from the 19 individuals of the birth-age show 52.56% to be of type 1; 34.6% are shown to be type 2; 12.3% as type 3; and a mere .51% are seen to be of the final type 4.

The 944 follicles from the 47 one-day-old individuals show the following percentage distribution: 8.7% are found in type 1; 25.6% are in type 2; 43.6% are listed in type 3; and 21.9% are in type 4.

The 911 follicles which are from the 39 two-day-old individuals are classified with these results: 5.7% list in type 1; 11.2% assume type 2; 25.24% are in type 3, and the majority 57.8% are placed in the final type 4.

Comparisons of the follicles in the seven color strains of mice for the three age groups under investigation present rather interesting features. Basing our comparisons of the colored strains on the percentage graphs, we notice a number of features which are deviations from the average of all of the stocks as revealed by the summary graph (graph 24).

In the birth-age-group we find that in general there is a tendency for a major per cent to be in the first follicle type with a lesser representation in the second and third types. The black and tan and light-bellied black agouti stocks most nearly conform to the general average for the entire group. Marked deviation is noticed in the dark-eyed black agoutis in that at the birth stage there are more representatives of follicle type two than there are representatives in follicle type 1. The remaining portion of the placement scale assumes average proportions.

This deficiency in type 1 among the dark-eyed black agoutis might, conceivably, have been due to classifying some young as of the birth-age, while in reality they might have been born soon after the previous inspection of the cage nearly 24 hours previously. The remaining possibility is that this particular strain may develop at a rate different from the others and possess a characteristic development rate of its own. However, only a small number of specimens was observed in the class, which would militate against the second explanation in favor of the first one.

In a like manner a deviation from the average is noted for the members of the initial group in the dilute brown stock. Here it can be noted that the follicles fall almost entirely in type 1, and the remaining few per cent appear to be type 2 with no representatives in types 3 or 4.

The explanation for this marked deviation from the average may be that the individuals obtained for this age grouping were but an hour or so old. Thus the development of the hair would not have been seriously initiated. The individuals here obtained would be most immature. In contrast to this stock the dark-eyed black agoutis seemed to exhibit unusual rapidity of follicle development. The young mice from the two strains may actually have differed in age by a few hours.

Similar to the aforementioned strain, there is the suggested possibility that the dilute brown stock may develop hair follicles and pigmentation more slowly than the average of the other strains of mice. This latter suggestion, which is only an hypothesis, must of necessity be further examined before any type of positive statement can be formulated.

In an examination and comparison of the 1-day-old specimens of the various color strains there are significant variations among the different color types. The average or standard for this age grouping is

revealed to be centered around type 3 follicles and approximately the same number of individuals being classified in type 2 as in 4. A small number of specimens fall in type 1. With this as a standard we examine the various color strains and note their individual variations.

The intense colored black and tan stock shows a deviation from average in the unusual number of type 4 follicles. Instead of the stock reaching its maximum in follicle type 3, the maximum is attained in type 4.

It may be suggested that the abundance of granular material may have made the classification of black-and-tan follicles more accurate. The possibility that intensity of color is a determining factor here is further suggested when a gross examination of the animal shows that the skin of the black and tan stock colors at a more rapid rate than skins of the other color stocks. This factor no doubt is connected with the early presence of an abundance of granular material in the hair itself, so that pigment would exhibit itself more readily than in the lesser colored stocks and cause the classification of follicles to be naturally of the higher type. Considering the entire range of possibilities it may be natural that the follicles of this particular color strain fall into this system of classification.

The chinchilla group of animals deviates from the average of the groups. The one-day age group reaches its maximum in follicle type 2 while in the average of the stocks, the maximum is reached in type 3. There are only 4 chinchilla animals studied.

We do not know why these strains differ. Ordinary fluctuating variability within small samples may, conceivably, explain the strain differences found.

The specimens of the 2-day-old class conform rather closely to the average or standard of the group. There is no striking deviation from this average in any case. The placement curve for this age shows 5% in follicle type 1; progresses to 10% for type 2; 25% type 3; and 57% type 4.

The most deviation in this age group is in the black and tan stock where follicle type 4 contains 69.7% of the follicles. No apparent reason for this deviation can be suggested other than that mentioned previously for this intense color type. Again the real solution remains an open question.

b- Discussion

Graphs 23 and 24 combine the data on the classification of follicles from the seven stock color strains of animals.

At the birth age color production is taking place and the follicles are mostly of type 1. Some are classed as type 2. A very few follicles are seen to be types 3 and 4.

Animals of the 1-day age period show hair follicle development so that only a few are classed as type 1. There are some classified as type 2. The majority have assumed type 3 classification. A goodly number are classed as type 4. A general impression of this aged specimen shows a progressive development, over the birth age, with the follicles centralizing in or about type 3.

Further development to the two-day age shows only a few follicles classified as type 1. Type 2 is less important than types 3 or 4. In the 2-day age we find the majority of follicles assuming type 4 proportions.

In general the development is rather constant with a maximum type for each being reached in the three age groups. Birth centers around follicle type 1. One-day centers near follicle type 3 and the two-day age grouping centers about follicle type 4.

4. MICROPHOTOGRAPHS OF OBSERVATION AREAS.

a- General Mechanics of Photographing.

Another system of presentation was decided upon which would display the hair follicles and structures in as near a natural setting as possible. This method was the photographing of the actual field as seen under the magnification of the microscope. It would depict the developmental structures associated with the hair as well as accurately display the follicle and the component granules in their exact relationship to the follicle structure.

The mechanics of photographing the follicles proved essentially to constitute fastening a compound microscope on an improvised platform of a photographer's camera and tilt it so that the barrel became parallel with the floor. A bulb was attached below the stage sending light through the microscope. The lens of a large photographer's camera was placed near the eyepiece of the microscope and the bellows adjusted so the image viewed became sharply focused on the ground glass plate. Sections containing the hair follicles were brought into position in the field and the projected image adjusted into sharp focus in the camera. Rapid speed film of the best type was used. The problem resolved itself into the selection of appropriate material and photographing.

The photographing, developing and printing of the pictures was done by the professional college photographer in his laboratory under the direction of the author of this thesis.

Appropriate material best suited for photographic work had to be selected from the material in the main body of the investigation. Material for photographic work had to be thin sectioned, contain a minimum of stain, yet be stained enough that the structural elements of the section were distinct. These sections had to be of maximum sharpness in outline to produce desired results.

b- Interpretation of Photographs.

A series of photographs was obtained from the Black and Tan stock for birth, 1-day and 2-day age stages. All of the photographs in this series were taken at X600 magnification. Plate A, Black and Tan, birth age, shows a follicle cut in longitudinal section through the papilla region. The darkened area at the apex of the papilla in the shape of a "u" contain the cells with their complement of pigment granules. This photograph reveals a representative stage of development for the birth age group. The granules present are confined to the upper portions of the papilla. The essential structures of the developing hair are in the initial stage of formation above the papilla.

Plate B, Black and Tan, 1-day age, shows a longitudinal section through the hair follicle and extending into the developing hair. This section is very lightly stained and hence does not show sharp cellular differentiation, but rather affords opportunity for the exhibition of the color granules. These granules are distributed completely around the papilla region and extend above the tip into the hair. The characteristic placement of individual granules and of the granule groups are evidenced in this photograph. At the base and along the sides of the papilla are seen circular granular arrangements around individual cell nuclei, whereas

at farther reaches of the follicle there is a tendency for the granules to become aggregate masses; they give the developing hair a ladder-like appearance which is easily discernable.

Plate C, Black and Tan, 2-day age, is a photograph through one follicle of hair showing the longitudinal region of the papilla and extending through the base of the developing hair. The section is deeply stained but color granules are evident in a "u" shaped cap over the sides of the papilla and into the hair structure. The dark masses of color granules in the apex of the papilla and on its lateral portions, as well as in the shaft, easily identify themselves. The nuclei of the cells in the follicles are readily distinguishable and are seen to be aligned with their long axis parallel to the axis of the hair shaft. Surrounding structure about the follicle is dimly outlined. The dark material in the papilla cells is stain and not color granules.

Plate D, Black and Tan, birth stage, is a photograph of a longitudinal hair follicle through the papilla. The particular follicle is in an early stage of development. The nuclei stain deeply and present a rather dark appearance. There is evidence of pigment granules in the region of the apex of the papilla but due to the presence of a large amount of stain these granules do not exhibit themselves to advantage.

Plate E, Black and Tan, 2-day-age is a photograph of the papilla cut through the central follicle structure of 1 hair follicle and two sagittal sections of adjacent hair follicles. There is evidence of the presence of color granules because a darkened "u" shaped area can be observed in the apex of the papilla region which without doubt is due to the granules. The section is darkly stained, which precludes its use for discovering the origin of color granules. But it affords an oppor-

tunity to examine the cellular structure of the follicle. The nuclei of follicular cells are seen to be large in relation to the entire cell, for the nucleus occupies the major portion of the cell. The cytoplasm is limited to a small area surrounding each nuclei. The cellular structures which surround the follicle are seen to be loose and irregular.

Plate F, Black and Tan, 6 days, is a photograph of a longitudinal section of the hair follicle which had been cut in a plane through the entire length of the papilla. The section exhibits a lightly stained follicle which allows the pigment granules to exhibit themselves in a "u" shape over the papilla, showing their characteristic locations and placement in relation to individual cell structures. These granules display circular alignments of granules around the individual cell nuclei. The later fact is particularly evident in the area along the sides of the papilla.

An interesting observation can be made in this section concerning the shape and placement of the cells of the papilla. Those at the tip are large and elongated with a tendency for them to become aligned parallel to the axis of the follicle. The cells at the basal region of the papilla are elongated but their major axis assumes a position at right angles to the surface of the follicle walls.

Plate G, Black and Tan, 6-day age, is a photograph of a longitudinal section of a hair extending from the papilla to a point in the body of the hair shaft above the papilla. This particular specimen contains no staining material hence no cellular structure is evident. The granules in places align themselves in a string-like manner and in other localities they are massed so compactly that they form opaque areas. Where the granules are sufficiently sparse it can be observed that they are packed around a clear space which evidently is the nucleus of the

cell which has produced them. This section illustrates the quantitative relationship of the granules in a single hair follicle and shaft.

Plates H, I, and J are photographs at birth of dark-eyed black agouti hairs. They show longitudinal sections through the hair follicles. The sections are lightly stained and reveal a minimum of individual cell structure but they show clearly the position of pigment granules. Plate H shows the granules extending into the developing hair. Along the sides of the papilla a dark area can be seen which is an accumulation of the granules. In the left portion of the follicle the granules are comparatively few in numbers and arranged in linear fashion. These masses conform to the shapes of the nuclei.

Plate I. The amount of granular material is increased over the amount in the previous specimen. In the apex of the papilla region the granules form dense opaque areas which photograph as black spots or bands. The masses of granules at the papilla apex extend upward into the medulla of the developing hair.

Plate J. The quantity of pigment present is comparatively small. The major portion is assembled adjacent to the upper half of the papilla. It presents rather a string or bead-like appearance which conform to the outline prescribed by the nucleus and limited by the boundaries of the cells. The papilla is not clearly evident but in general the area can be determined by the limits of the light central area.

In plates H, I, and J, the characteristic placement of granules is evident, the cells of the basal and lateral portions of the follicle are elongated and assume positions parallel to the surface; the cells near the papilla are more nearly round.

The remaining plates showing photographs of individual hair follicles were not selected to show particular characteristics of one strain. They were selected because of their photographic qualities and for some distinctive illustrative quality for their particular age or stage grouping.

Plate K, birth age, Light-bellied agouti, is a photograph of a hair follicle which displays the characteristic cell form of the papilla. The cells at the tip are large and slightly elongated, the nuclei of which have a tendency to assume a position parallel with the follicle surface. Those cells of the basal region are sharply elongated and are aligned perpendicular to the surface of the follicle. The cells of the entire papilla, illustrated by the light central portion, show a characteristic insertion into the basal region of the follicle. The general appearance of this plate shows rather poor focusing.

Plates L and M, Dilute Brown, 1-day age. These are photographs of follicles sectioned longitudinally through the papillary region. Plate L shows 6 aggregates of granules and several less distinct areas containing pigment granules. These areas completely surround the papilla. The lighter area in the center of the follicle is the papilla. The cell structure in the follicle is not in sharp definition. The purpose of this picture was to illustrate the shape of the follicle, the extent of granule production at this age, and the particular placement of granules and granule areas.

Plate M shows essentially the same features as described in the previous plate. There are 9 granule aggregates around the central papilla. Small amounts of pigment have appeared in the region of the developing hair. These to a slight degree take on linear arrangement parallel to the surface of the hair.

Plate N. Dark-eyed black agouti, 2 days of age. This is a photograph of a longitudinal section of a follicle through the tip region of the papilla. It reveals a darkened pigment producing band around the lighter papilla. It is sectioned in such a manner so it produces the visual effect of thickening of the granule band near the papilla base. This is not characteristic of the follicle but is due to the plane of sectioning. The amount of pigment present is of sufficient quantity in places to form dense massed areas. In other places it is in less quantity and presents a ringed or bead-like appearance about the cell nuclei.

Plate O is a photograph of a portion of a mature hair shaft from a follicle of a chocolate strain of mouse (6-day age). The original photograph was taken at X600 and the enlargement was made by projecting a part of this negative onto a new positive photograph. The section is lightly stained. The darkened areas are produced by the pigment contained in the hair shaft and in the cells associated with it. The main body of the hair contains pocket-like spaces filled with granular material. There are spaces between these granule filled areas which are devoid of pigment. This produces the characteristic ladder-like effect. The granules in the deeper layers of the hair assume a linear arrangement, forming broken lines parallel to the length of the hair. The cuticle, the cortex and the medulla region of the hair shaft are easily discernable.

c- Discussion.

Attempts were made to photograph hair follicles under highest power of magnification. This had to be abandoned as the results produced were undesirable. It proved of little value to attempt photographing material over X600 magnification.

The photographic film is sensitive to the material in the entire field. The human eye can be brought to focus on one single object. The photograph produced shows the general impression. Detail does not become an attribute of a high magnification photograph.

In photographing microscope material, all the objects in the field are sensitized on the film, those in sharp focus and those in comparatively indistinct outline. Material thinly sectioned as it may be, still possesses the depth dimensions. When it is brought into the field the light which shines through the material passing the near and distant surfaces of the cells creates a sensitive response on the photographic plate. The photograph not only records the near surface in sharp focus to the eye, but also the deeper surface of the cell not in focus with the eye. This produces a broadened perspective and a hazy cell outline on the photograph. The cell structures become doubly outlined.

Contrasts of structure secured by staining did not exhibit the same relationship in the photographs. These were transformed by the photograph to slight variations in light and dark areas. In the photograph color granules themselves and the stain both produce indiscernible gradations in dark and light areas.

The Black and Tan series of photographs does produce an accurate means of displaying the comparative differences between the age groupings. They show an accurate record of the relationship of the follicle with its component elements. They show the accurate association between the individual cells of the follicle with their relationship to the other structural elements. Exact area, size and space location of the pigment producing portion are shown. It indicates the outline of cellular structures

which comprise the follicle. Photographs give a good indication as to the detailed structural composition and location of the individual cells of the follicle which concern themselves with granule production. They give accurate place relationship of the granules in relation to the cytoplasm and nucleus of the cell which produces them.

The photographs give the exact placement of granule areas and the relative regions of the developing follicle. Developmental changes of follicles in the age classifications are more clearly expressed by photographs than any other means.

PART IV

DRAWINGS

a- Description of the color strains

b- Discussion

DRAWINGS

a- Description of color strains.

In drawing the follicles which are in the process of color production it was decided to draw characteristic follicles in the three age groups in order to exhibit the comparative differences or similarities and to accurately represent these qualities in the varying ages.

In like manner it was of importance to select individual granule producing cells in each of the three age groups to represent the structural relations within the cell, the relative sizes of the color granules, and the numbers and placement of the granules themselves within the cell structure. Thus cells from the follicles of the three different ages could be compared.

Drawings were made from selected follicles in the birth, 1-day, and 2-day stages. The general follicle drawings were made with the aid of a camera lucida (X600). The oil immersion objective (X1350) was used in making the enlargements of the individual cell drawings.

The nuclei were drawn as they were seen in the follicular cells. The pigment granules are exact representations of cellular constituents.

The drawings showing the detailed structures of the cells were made with great care. Representative, clearly defined granule producing cells in the papillary region were selected and carefully examined. The color granules were accurately drawn as to position and number. The individual granules were accurately represented as to size and shape. The drawings of the detailed cell structure and granular placement of necessity are greatly magnified and a statement as to the approximate degree of magnification can only be estimated. Knowing that the pictures obtain-

ed in the camera lucida drawings were magnified X600, it would be safe to say that the individual cell enlargements would be in the near vicinity of X1500. All were enlarged the same amount.

Black and Tan. The follicles of the black and tan stock in the three initial age classifications show progressive changes with increasing age. In the birth stage, figure 1, the granules more or less congregate in the region of the apex of the papilla. The quantity of pigment present is relatively small. The granules themselves seem more or less in a single layer around the nucleus, the majority being in close proximity to the nucleus.

In the follicle of the 1-day-age class of the same color stock, figure 3, there is an increase in the quantity of granules present. They assume positions along the lateral portion of the papilla. They tend to encircle the nuclei of the cells. The granules in this age are less string-like in arrangement in comparison to the birth stage. They become more freely arranged away from the central nuclear structure and appear to be in a process of divorcing themselves from the nucleus. Varying amounts of granular material are located in the region from which the future hair shaft will grow.

The 2-day-age classification, figure 5, shows rather remarkable developmental changes. The granules are now arranged along the entire length of the papilla. There has been a great increase in quantity of pigment to many times the amount present in the previous stage. Giant clusters of granules congregate in masses and form brown more or less semi-opaque areas. In between these semi-opaque areas are regions in which pigment granules are absent. The area producing the color granules has not alone increased in length along the lateral portions of the

papilla but also it has increased in width. In the region at the apex of the papilla the granule producing portion commands approximately half the width of the entire follicle. This band around the papilla fuses into one cluster at the tip. Near the base of the papilla the cells are well surrounded with color granules and are similarly arranged into clusters. Portions exhibit some of the loose or free granules which seem to be taking up their new locations.

The quantity of pigment present in this stage of development is relatively large. The increase in granules beyond the 2-day stage is so great that the granule producing parts of the cells are completely obscured. For this reason detailed study of the follicles beyond the three initial age stages seemed impractical.

Structural changes of the papilla and of the related portions of the follicle during these three age group stages of the black and tan are evident. The cells which make up the developing follicle comprise rather a loosely knit structure, the individual cells of which are rounded in the region adjacent to the papilla. To a slight degree cells near the edge of the follicle become elongated, the major axis assuming a position parallel to the direction of the growth of the hair. The cells of the follicle of the three age classes show these progressive changes of structure and enlargement coincident with the rapid increase of granular material.

The cells of the papilla region undergo changes in form and position. In the initial stage the papilla is large and bulb-like in shape, the individual cells are rounded, with some elongated and lying with their major axes perpendicular to the developing hair.

In the two-day age stage the cells at the apex of the papilla change their position, and the direction of elongation becomes parallel to the axis of the growing hair. The cells in the middle and basal papillary regions are oriented as before, being elongated at right angles to the axis of the hair follicle.

Drawings which were made of the detailed structures of the cells in the black and tan stock were from pigmented regions at the tip of the papilla. Figure 2 (birth stage) displays four cells taken from the region at the apex of the papilla, showing the detailed cell structure and the accompanying color granules. The nuclear structure is clearly defined. The chromatin material within the nucleus is readily distinguishable. It assumes a net-like form. The nucleolus appears as a slightly darkened area in the nuclear material.

The cytoplasm of the cell is comparatively transparent. The limits of the individual cells are not clearly defined. In the area between the adjacent cells one can not always distinguish exact cell boundaries. The limits of the cells in the drawings are indicated by dotted lines.

The color granules are found in the cytoplasm in close proximity to the nucleus. At first observation they seem to form chains about the nucleus, but on close examination no trace of the connections between the granules can be seen. Their arrangement, apparently, is one of free individual alignment.

The structure and form of the granules themselves exhibit variation. Some of them are large and rather irregular. Others are extremely small and appear more or less smooth. In fig. 2 approximately 30 granules are located in the cytoplasm adjacent to the sections of the nuclei of these cells.

In figure 4 (1-day stage) seven cells are shown which came from the region at the tip of the papilla. Four of these cells have a total of 45 color granules in them. These granules adhere rather closely to the nucleus. A few have separated a short distance from the nucleus and occupy free positions in the cytoplasm of the cell.

Figure 6 (2-day age) presents evidence of change from the two previous age groupings. The vast change in number of granules greatly alters the appearance. One of the cells has 65 granules in it. Near this cell, there is a mass of approximately 50 granules. These seem to be located rather as a unit and do not seem to be intimately associated with any individual cell nucleus. The large quantity of color granules and the tendency to grouping distinguishes this age classification from either of the two previous age groups. The structures of the nucleus and cytoplasm appear very similar to the two previous stages. The quantity and grouping of the color granules no doubt contribute to the intense color of this strain of mice.

Dark-eyed Black Agouti.

Individuals of the dark-eyed black agouti stock, figures 7-12, exhibit much the same progressive development as the black and tan stock.

Figure 7 (birth stage) shows a longitudinal section through the follicle revealing the placement of the granules in the apex of the papilla region and extending back to approximately half way along the sides. The granules are aligned in the proximity of the nuclei. The granules to a slight degree are grouped into small clusters.

The nuclei in the cells of the pigment producing area are slightly elongated. Those of the distal part of the papilla are elongated with the major axis pointing in the direction of hair development.

Figures 9 and 10 are from 1-day old specimens. They show longitudinal sections of the hair through the papilla. These sections were prepared as were the rest, but were not stained. This renders the cell structures of the follicle indistinguishable, allowing the granules present in the region to appear clearly. Figure 9 shows the outline of the follicle and of the pigment producing area. The pigment granules completely surround the papilla area. The individual granules show a rather interesting alignment, which is evidently determined by their proximity to nuclei. There is evidence of clustering of the granules, as two small clusters appear, one at the papillary tip and the other at the side.

Figure 10 shows essentially the same features as the previous specimen. This individual follicle taken from the same animal as the previous follicle illustrates the degree of variation which may exist among the follicles of the same individual. The degree of maturity of this follicle is easily observed. The granules outline the various nuclei. They have clumped into thick clusters which extend from the distal tip of the papilla out into the axis of the developing hair. This follicle represents the furthest extreme of development at this age.

Figure 11 is a specimen of the two-day age. It reveals the granules at the apex of the papilla and extending over the lateral portions to a point about half way to the papillary base. The granules are again closely associated with the nuclei. To a certain degree we find them making clusters at the apex of the papilla. Some of the granules are seen to be in what will become the base of the new hair.

In Figures 7 and 11 (birth and two-day stages) we notice the characteristic change in the cells of the papilla. In the initial stage the cells at the tip are large oval structures, with those at the

base tending to be elongated in a plane perpendicular to the surface of the follicle. In the two-day stage the cells in the apex of the papilla are assuming elongated shapes, the elongation pointing to the tip of the developing hair, and the cells at the papilla base taking a more pronounced right angle position.

Figure 8 (birth stage) shows 3 complete cells taken from the tip of the papilla region. The nucleus is clearly defined, and its nuclear contents reveal a darkened area, the nucleolus. Some seventy color granules are seen to be associated with these cells, a number of which are near the nuclear surface and others are further out in the cytoplasm region. A slight tendency toward grouping of the granules is exhibited at certain points in the cell. The boundaries of the individual cells are not clearly defined.

Figure 12 (two-day age) shows three complete cells with their 125 associated color granules. These cells were drawn from a region approximately at the tip of the papilla. The nucleus is clearly outlined, and the internal nuclear structure is apparent with the nucleolus, which is a darkened area. The nuclei are oblong, the shape of the entire cell more or less conforming to the various positions afforded it. The cell boundaries are not always clearly outlined.

The granules are to a considerable extent aligned near the nuclear surface. There is evidence of clustering of granules in some locations.

The difference between the two stages, i.e., birth and two-day, is in the quantity of granules produced; the older age has about two times the number of granules of the initial stage.

Light-Bellied Agouti.

The follicles of the light-bellied agouti strain of mice, of the birth, 1-day and 2-day groups are drawn as figures 13-18.

Figure 13 (birth age) is a drawing of a longitudinal section through the papilla of the follicle. Granules are present and extend from the papillary tip over the lateral surface of the papilla to a point approximately half way to the base. In places they form broken granular rings around the nuclei. Some of the granules appear loose in the section but remain in the general pigment producing area. A few of the granules have assumed positions distal of the papilla tip. There is a tendency for the granules to bunch, as is shown by three or four clusters in this section. The nuclei at the sides of the papilla are elongated in a direction parallel to the developing hair.

The papilla in the section is rather large, the cells at the base being elongated in a transverse direction while those at the tip are aligned more or less parallel to the future hair shaft.

Figure 15 (1-day age) exhibits a sagittal section through the papilla. Only a small portion of the papilla is evident, and this from the region of the tip. The granules lie over the papilla throughout its entire length, some even being in the region above the papillary apex. They form definite clusters in proximity to the sides of some of the nuclei. Other individual nuclei possess but a few granules which form their characteristic ring. The quantity of pigment present is greatly increased over that of the initial stage. The nuclei of the cells of the outer edge of the follicle are oriented with their long axes parallel to the hair shaft.

Figure 17 (2-day age stage) shows a longitudinal section through the papilla. The granules surround most of the papilla, and extend distally from the apex to the region of the future hair. Advancing toward the papilla tip, the granules can be seen to be formed into clusters in the proximity of the nuclei. Passing to the region above the apex of the papilla we find definite large masses of granules and a few isolated granules lined in somewhat parallel rows extending toward the end of the developing hair. The nuclei resting on the lateral regions of the papilla are somewhat elongated, extending at right angles to the papilla while those of the edge of the follicle assume a position with their longitudinal dimensions parallel to the follicle. The papilla nuclei in the majority of instances have their major axis perpendicular to the long axis of the hair.

Figure 14 (birth stage) is an enlarged drawing of five cells of the follicle from the apex region of the papilla. The nuclei are intensely outlined and exhibit the internal structure quite clearly, the darkened area being the nucleolus. These cells have 42 granules associated with them which are aligned in the near proximity of the rounded nuclei presenting a bead-like appearance. The cell outlines are indistinguishable at certain parts and faintly outlined in other portions.

Figure 16 (1-day-age) shows an enlargement of three complete cells with ring like associations of 75 granules in proximity to the individual nuclei. The nuclei are elongated and contain differentiated nuclear material, the darkest portion of which is the nucleolus. The cell outlines are not always discernible but in some places are faintly visible. The majority of the granules are near the nuclei, but a few are seen to be in the cytoplasm a little distance away from the nucleus. Individual

granules vary in size, one in this section being many times the size of its accompanying members. This age does not exhibit bunching of the granules. However, we do notice an increase in number of granules present in comparison with the birth stage (Fig. 14).

Figure 18 (2-day-age) shows an enlarged drawing of two cells from the tip of the papilla region with their association of about 150 color granules. The nuclei are distinctly outlined and the nucleolus is evidenced by a darkened portion in the nuclear structure. The nuclei are oval-shaped and at various points in their circumference there are aggregates of the color granules. The increase in the quantity of pigment granules in this stage over the preceding age is evident.

Dilute Brown stock.

Figures 19-24 are drawings made from the dilute brown strain of mice, consisting of the birth, 1-day, and 2-day age classes. Fig. 19 (birth stage) is a drawing of a longitudinal section through the entire papilla region. It displays the color granules around the tip of the papilla, and extending along the sides throughout half the papilla length. The granules themselves are aligned in a string-like band about the individual nuclei. The nuclei are irregularly elongated, those along the edge of the follicle being parallel to the surface.

The cells of the papilla are arranged in positions with the long axis of the nuclei at right angles to the surface of the follicle.

Figure 21 (1-day age) is a drawing of a longitudinal section through the follicle traversing the papilla area. The granules are evident in the follicle throughout the entire length of the papilla. They are massed in groups and form opaque areas, four being found in

this section. Some of the nuclei have few granules about them. Granules are found in a region above the tip of the papilla. The two masses there are occupying the area which in the future would have been the central portion of the mature hair. The cells of the papilla are parallel to the developing hair shaft at the tip, whereas in the basal region the cells are transverse to this original position. The increase in quantity of pigment is very noticeable at this age over that of the birth age group.

Figure 23 (2-day age) shows a drawing of a longitudinal section of a hair follicle through the papilla region. The granules are seen along the sides of the papilla and at its tip. There are eight pronounced groupings with areas in between which are comparatively devoid of granules. A great number of nuclei have the granules about them in a linear arrangement. The area at the very tip of the papilla contains a number of loosely aggregated granules among which can be noticed several very large ones. The nuclei of the external portion of the follicle are aligned in general parallel to the surface of the follicle. There is an increase in the number of granules at this age over the birth and 1 day stages.

Figure 20 (birth age) shows a drawing of three complete follicle cells which were taken from the apex region of the papilla. The cells are outlined in part by the dotted lines, and in part the exact determination of cell limits was not possible. The nuclei are well defined with their contents revealing a darkened portion, the nucleolus. Many of the granules are close to the peripheries of the nuclei. The central cell exhibits granules around a major portion of the nuclear circumference. The granules themselves are arranged in some of the regions in layer-like order. There are about 74 granules in association with the three cells in question.

Figure 22 (1 day-age stage) is a drawing of seven follicle cells taken from the lateral region of the papilla. The cells are outlined in part, but a portion of the cell boundaries can not be seen. The nuclei are well defined, and their contents reveal a darkened area, the nucleolus. Four of the cells drawn have only a few color granules associated with the nuclei. The major per cent of the granules are associated with two of these cells. These granules are located in the cytoplasm between the two nuclei. Near one end of a third cell can be seen a small aggregate of granules, and two apparent linear arrangements of granules, one being near the nucleus and the other near the outer limits of the same cell. Individual associations of small numbers of granules are seen in the other cells. The total quantity of pigment present is greater than in that of the initial birth age.

Figure 24 (2-day age) is a drawing of five cells taken from the follicle in the lateral region of the papilla. The cells are fairly distinct in outline as indicated by the dotted lines, but in some portions the boundaries cannot be accurately defined. The nuclei are distinct. The nucleolus is seen as a darkened area. The cells exhibit varying quantities of granules. Three of the cells drawn exhibit granules throughout the circumferences of the nuclei and in addition are accompanied by large masses of granules in the outer cytoplasm of their cell structures. There are about 200 granules associated with the cells in this drawing.

In noting the individual cell enlargements of the three age groups, one can notice an orderly increase in the amount of pigment present in each succeeding stage until in the two day age stage there is a grouping or massing of granules associated with the cells.

Chinchilla.

Figures 25-28 are drawings of chinchilla specimens obtained at 1 day and 2 day age stages.

Figure 25 (1-day age) is a drawing of a longitudinal section through the hair follicle traversing the major portion of the papilla. It reveals the presence of granules covering the papilla from the tip along the side to a point about half way to its base. The granules appear to be rather intimately associated with the nuclei of the various cells, presenting in some places a chain-like appearance around these nuclei. At other positions in the section the granules seem somewhat independent. Some granules are found associated with the nuclei of cells which are three cells away from the tip of the papilla, in the direction of the growth of the hair. There is a relatively small amount of pigment in the entire section. The nuclei of the follicle cells have a tendency toward elongation; the direction of their major axis extended parallel to the surface of the follicle. The nuclei of the cells of the papilla appear elongated at right angles to the walls of the follicle.

Figure 26 (1-day age) shows an enlarged drawing of three cells of the follicle taken in the region adjacent to the side of the papilla. These cells reveal poorly delineated cell boundaries. The nuclei are well defined and display a darkened nucleolar area. The color granules are located in close proximity to the nuclear wall and form small rows at various loci on the circumference. Near the outer limits of the cell similar lines of granules, 7 or 8 granules in an area, appear. The sizes of the individual granules vary. There are some in this cell group which are three or four times as large as the others. One of the cells here

drawn has three such giant granules. There is a total of 53 granules associated with these three cells.

Figure 27 (2-day age) is a drawing of a longitudinal section through a follicle cutting the papilla region. Color granules are exhibited along the sides of the papilla extending some distance past the tip into the region to be occupied by the future hair shaft. The granules are associated with the nuclei and form a broken band around it. In the region next to the papilla the cells have granules around approximately one-fourth their circumference. The nuclei of the cells along the edge of the follicle are elongated with their major axis extending parallel to the surface. The cells of the papilla are oval in shape.

Figure 24 (2-day age) shows an enlarged drawing of five cells taken from the region near the tip of the papilla. The cell boundary is lightly outlined as indicated by the dotted lines. The boundaries of adjoining cells are also similarly indicated. The body of the nuclei are distinct. The nuclear contents are well defined, the nucleoli appearing as darkened areas. On the outside circumference of the nuclei are broken bands of granules which are aligned in this type of locations. There are approximately 50 granules lying in close proximity to the nuclear structure. An additional 50 granules are found in the cytoplasm region lying near one end of the cells.

Chocolate.

Figures 29-32 are drawings of follicles and cell enlargements for the individuals of the chocolate strain of mice whose age groupings are for the 1-day and 2-day periods.

Figure 31 (1-day age) is a longitudinal section through the follicle of a hair cut at a plane running through the major axis of the

papilla. It shows granules extending along the entire length of the side of the papilla and above the tip to a point roughly 1/3 the length of the papilla. The granules are found to be spaced in broken band-like rings around the nuclei of the cells from the base of the papilla past the tip. In some instances these granules extend around half the circumference of the nucleus; in other cells the granules appear at the nuclear surface for a smaller fractional part of the distance around the circumference. The nuclei of the cells of the follicle are elongated, extending mostly in the direction of the major axis of the follicle.

Figure 29 (2-day age) is a drawing of a longitudinal section through a follicle of hair striking the papilla. The cells of the papilla have not been drawn in this section. Some of the nuclei in the heavy pigment production area have purposely been omitted to show granule placement rather than nuclear position. The granules have massed themselves into groups. There are spaces between these masses which are devoid of pigment. In the region at the base of the papilla the follicular cells are longitudinal in their alignment. The medullary cells of the future hair assume the ladder-like arrangement found in the mature hair. The nuclei at the edges of the follicles are aligned parallel to one another and with the surface of the follicle. The quantity of pigment is relatively very large and causes the entire follicle to appear very mature. Production of the granules in the follicle from the 1-day age to the 2-day age as here revealed is rather rapid.

Figure 32 (1-day age) is an enlarged drawing of two follicle cells adjacent to the tip of the papilla. The outlines of the cells, indicated by the dotted lines, are partially defined. The nuclei are

deeply outlined and appear as elongated structures which show their nuclear content as strands or nets of material. A dark portion is the nucleolus. Adjacent to the circumference of the individual nuclei, and in close proximity to them, can be seen broken arrangements of color granules. Some of the granules have assumed positions a small distance away from the nuclear circumference, but well within the limits of the cell. There are eighteen granules associated with one of the cells and approximately thirty in association with the second cell. Observation of these drawings reveal rather a typical location for the granules of this age, i.e., some in close association to the nuclear structure and some in regions further away from the nucleus.

Figure 30 (2-day age) is an enlarged drawing of three cells of the follicle taken in the region adjacent to the papillary tip. The cells themselves lack clearly defined outlines. These boundaries are indicated by dotted lines. The nuclei are deeply outlined and contain clear net-like arrangements of nuclear material with a darkened portion, the nucleolus. Near the edge of the nuclei are seen broken band-like rings of granules partly surrounding the nuclei. In the cytoplasm region of the cells granules are seen which seem more or less free. Some appear to assume line or string-like alignment but others are evidently free. A variation in the size of the granules is clearly seen. Some are relatively large while others are conspicuous for their smallness. On the average there are between forty to fifty granules associated with each of the cells. In comparison to the previous day age, it can be noticed that there is an increase in the number of pigment granules. These granules show the tendency to become free and leave the vicinity of the nucleus.

b- Discussion of the Drawings.

We are now able to view the entire problem and form conclusions concerning the origin of the color granules. Let us first discuss the birth age group. The amount of granule production is similar for all of the strains. An examination of the drawings proves this. The similarity is so close that even though one knew the source of material to be from two different colored stocks this fact could not be detected by any observed difference in the quantity of pigment present.

The various color strains of individuals show approximately the same degree of follicle development for the birth age group. This observation was based upon a study of the entire group of follicles for the age, and not upon the drawings alone. These drawings were made from selected material and hence in themselves might not be fair samples of the type and age. However, viewing the drawings alone it can be observed that there is a similarity at this stage of development in the various colored strains.

Reverting to the classification of the follicles previously devised in another section of the thesis, it can be seen that the birth age follicles just described conform to types 1 and 2. This result was obtained previously for this particular age. The follicles of this age show the granules to be first formed above the apex of the papilla and in the region extending along the sides of the papilla. The quantity of pigment is not great in comparison with the older ages. This indicates that pigment production is beginning at birth and is continued from then on. The quantity and arrangement of pigment is substantially the same at this age for all strains of mice.

A most careful examination reveals, in all of these granule producing cells of the follicle, there is an identity in the origin of the color granules. In all of the strains, these color granules are first seen in the cytoplasm at the surface of the nucleus. At this point the granules appear at first in comparatively small numbers. They align themselves in broken ring-like formation about the outer nuclear surface.

Let us now turn our attention to the one-day-age group. At this age all the strains show an increase in pigment over the birth-age-stage. Following the classification system devised in the previous section it can be seen that the follicles fall into the higher divisions as would be expected. They belong to types 2 and 3, with some advancing to type 4. The individual granules can be seen to be more thickly placed around the nuclei, and some can be seen occupying the area between the limits of the cell and the margin of the nucleus. The point of origin of the granules appears the same for this age as for the previous age, that is, near the nuclear surface.

Reference to the 2-day-age grouping reveals a continuation of the processes initiated in the previous ages. There is a continued increase in the amount of pigment in the large follicles. The granules themselves are assuming mass groupings around the papilla and in the region occupied by the developing hair. The type 4 follicle is characteristic for this age. The developing hair has a distinctly mature appearance. The quantity of granules had greatly increased over the amount present at birth. The massed granules have assumed such proportions that they now give a definite color to the hair. They become

located farther away from the nucleus, so are more independent of the place of their origin. Though these masses ultimately give the visible color to the particular strain of mouse, yet a person can not distinguis h one color from another at two days of age.

Viewing the entire group of drawings of color strains of mice in the various age classifications, it can be seen that there is a steady increase in the quantity of pigment in each succeeding age. At birth a comparatively small amount is present and in most cases is located at the region near the apex of the papilla. In the succeeding age stages the quantity increases and extends along the sides of the papilla, almost completely surrounding it. In the two-day-age stage the pigment has not only increased over the preceding age, but it has become implanted in the medulla of the developing hair. Further advance beyond these stages involves merely a continuation of the processes described above. In general it conforms to an increase of the structures already formed in the early stages. The layers of the hair follicle become more clearly defined and the pigment granules become greatly increased and are characteristically placed in spaces in the medulla, which are provided for them. This gives the hair a mature appearance.

In summary, the granules originate in the cytoplasm at the circumference of the nucleus of the cells near the birth age, and as the tissue develops, the granules increase in number and find new positions in the cell nearer the boundaries. As development continues these granules become aggregated and form characteristic groups which are very evident in the cytoplasm of the cells. As further development is continued these form pocket-like masses in the medulla of the hair, and cause it to possess the characteristic ladder-like appearance of the fully developed hair.

Each of the questions of how, when and where melanin forms has been given the individual attention of scientific study. It is an established fact that melanin formation is an oxidation process, probably that of an enzyme or enzymes.

The facts presented in this thesis are highly suggestive and lend weight to the solution of the problem as to where the granules originate. The facts suggest that there is some type of chemical action between the substances derived from the nucleus and from the cytoplasm shortly before and after birth, so that melanin pigment granules are precipitated at the outer surface of the nucleus.

It is known that color is due to the action of a gene, or genes, in the chromosome. Wright (31) suggests that melanin is produced by oxidation of protein through the action of enzymes. The reaction occurs in the cytoplasm, probably by enzymes secreted from the nucleus.

The evidence of the author's investigation strongly suggests that the "C" and other genes for color located in the chromosomes give up some material, or enzyme, which produces its effect by a chemical reaction taking place at the nuclear surface. At this point the materials come in contact with other chemicals of cytoplasmic origin and result in the production of melanin granules. It appears as though the enzyme released from the nucleus meets the materials produced in the cytoplasm in the presence of oxygen, the oxidation reaction results in the production of melanin.

Three distinct features were observed which bear out the correctness of the above contention. (1) No granules were ever seen to appear in the nucleus of the cell. (2) The first appearance of granules is at the

nuclear surface in the cytoplasm. (3) After the initial appearance of granules, these are observed to move to new positions nearer the walls of the cell. The granules formed at the nuclear surface shift their positions to the outer parts of the cell where they later tend to become massed. Newly formed granules appear continously at the periphery of the nucleus.

The process of melanin formation appears to be a colloid surface phenomenon taking place at the limits of the nucleus in the cytoplasm. The resulting oxidation process forms melanin in this area.

There is no doubt that oxygen gains entrance into the nuclei, yet we do not observe granule formation within the nuclei. The inference is that the nuclear membrane is not penetrated by the cytoplasmic substances, perhaps an amino acid, but the nucleus produces an enzyme which is brought into contact in the cytoplasm, with material produced by the same, resulting in the oxidation process producing melanin.

In brief, the matter resolves itself into the contention that the color gene, or genes "C", in the chromosomes produce a material which finds its way through the nuclear membrane into the cytoplasm oxidizing chemical components already present to form the melanin granules.

PART V

CONCLUSIONS

CONCLUSIONS

The information secured in this investigation seems to justify certain conclusions.

1. Color granules are present in the follicles of hair at birth.

2. The color granules appear to originate in the cells of the hair follicle adjacent to the papilla.

3. The melanin color granules appear to originate in the cytoplasm on the surface of the nuclear boundary in the individual granule producing cells.

4. Though the color granules appear to originate at the surface of the nucleus they later assume positions in the more peripheral parts of the cell.

5. The color granules are of various sizes.

6. The color granules first appear in the region at the apex of the papilla.

7. There are reasons for believing that the color genes in the chromosomes form or cause to be formed substances, perhaps enzymes, which pass outward through the nuclear wall and assist in precipitating the melanin granules at the boundary between the nucleus and cytoplasm.

8. It seems likely that oxygen diffuses inward from the surface of the cell meeting the nuclear secretions at the nuclear wall with the result that an oxidation process occurs producing the melanin granules.

9. An understanding has been obtained which deals with the progressive changes experienced by the developing hair follicles.

10. Hair begins to erupt on mice on the second day of life, by the third day 87% have erupted hair, and by the fourth day all have erupted hair from the skin surface.

11. A system of classification of hair follicles has been devised and carefully described.

PART VI

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PART VII

DIAGRAMS

GRAPHS

DATA

MICROPHOTOGRAPHS

PHOTOGRAPHS OF DRAWINGS

Diagrams of

Follicle Type

Classification based on placement of granules.

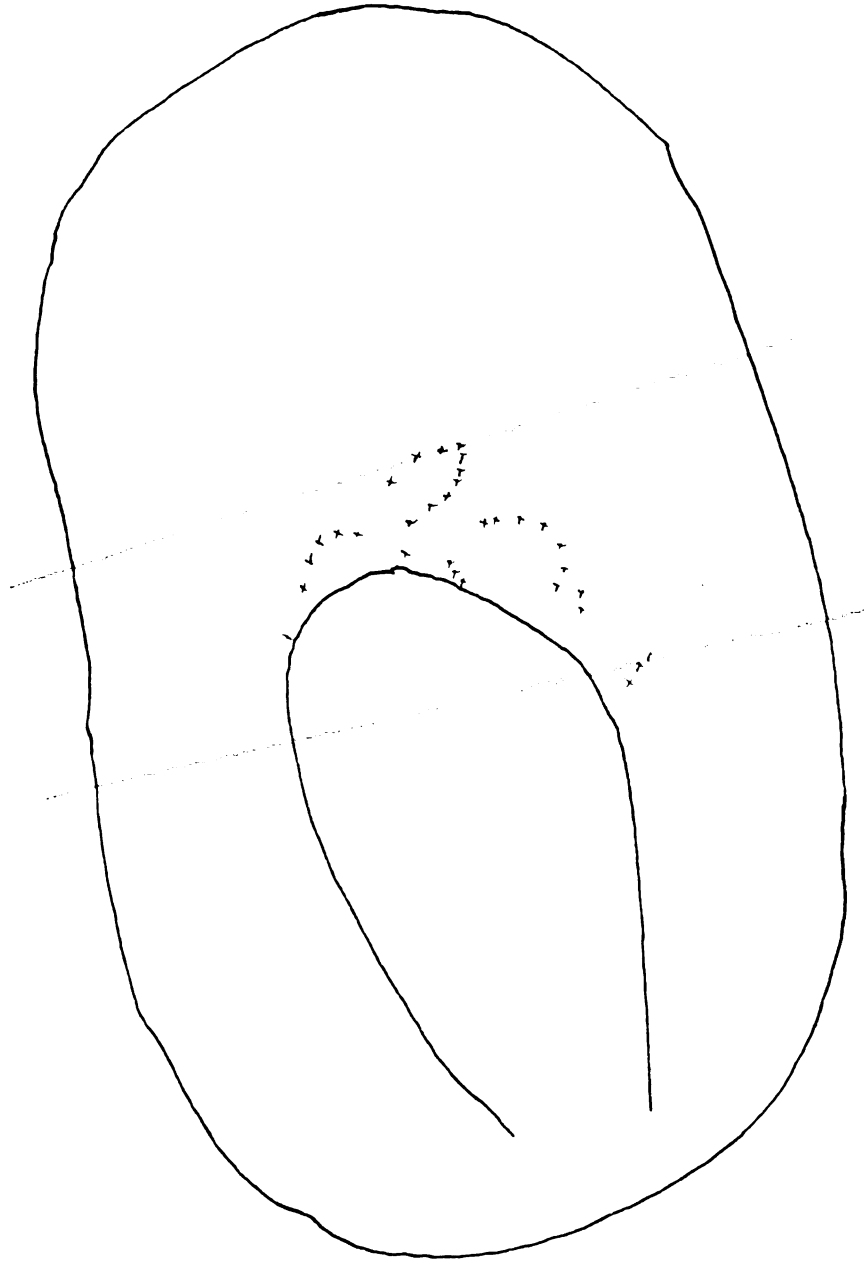
Type #1

Type #2

Type #3

Type #4

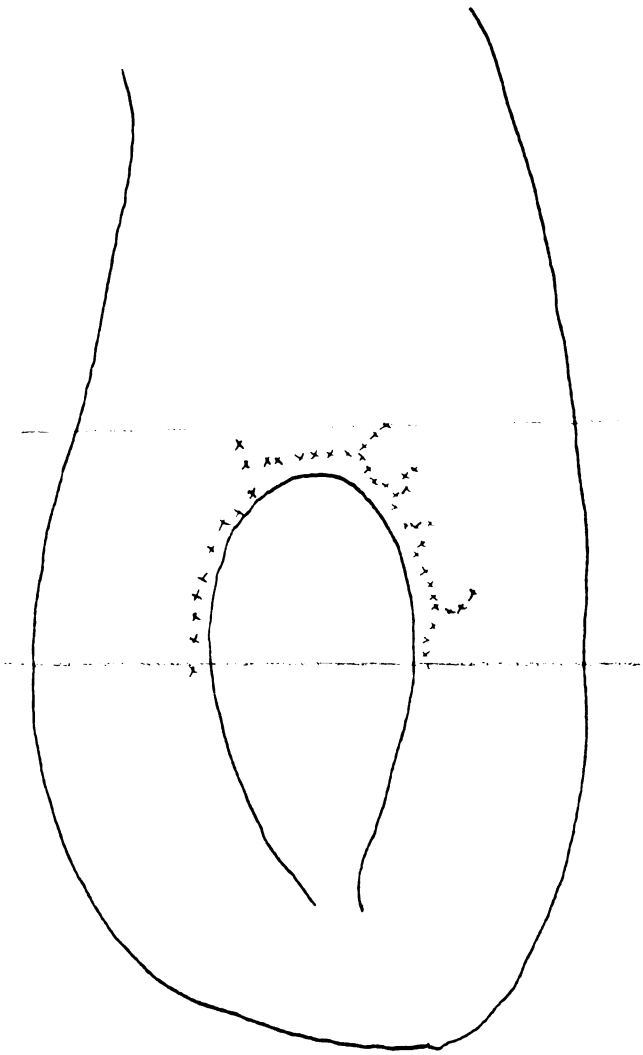
Follicle type #1



219A $\frac{13.1}{70.1}$

Diagram 1.

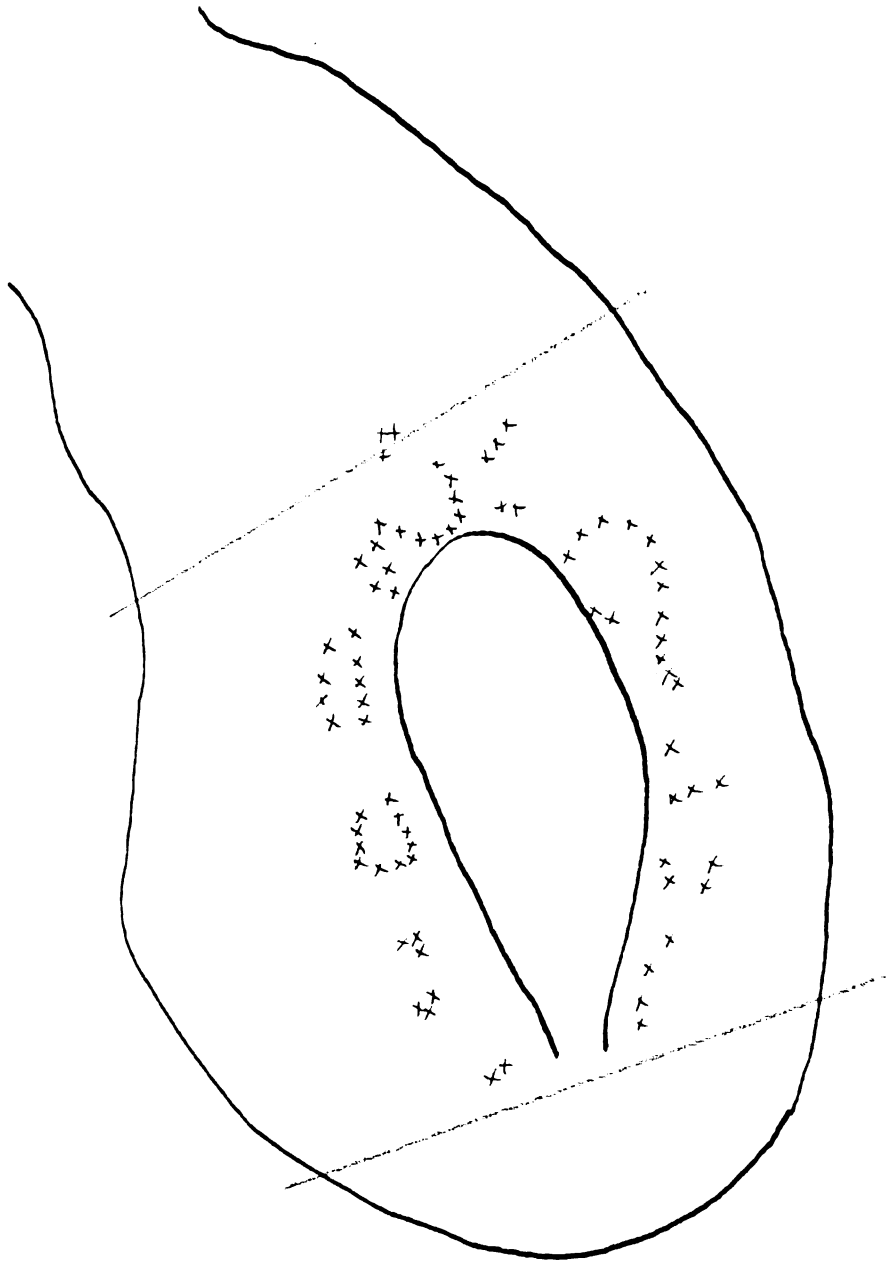
Follicle type #2



219A $\frac{20.5}{68}$

Diagram 2.

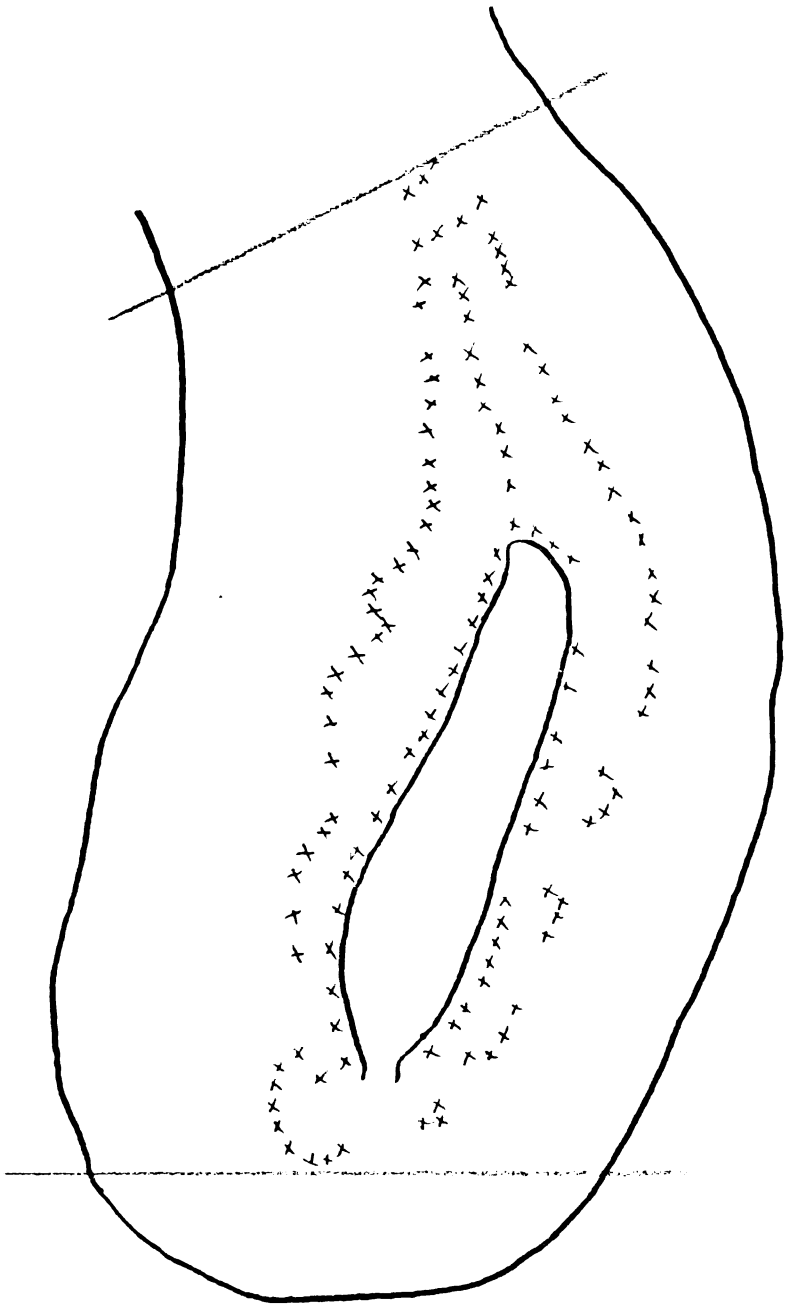
Follicle type #3



228S 19/52

Diagram 3.

Follicle type #4



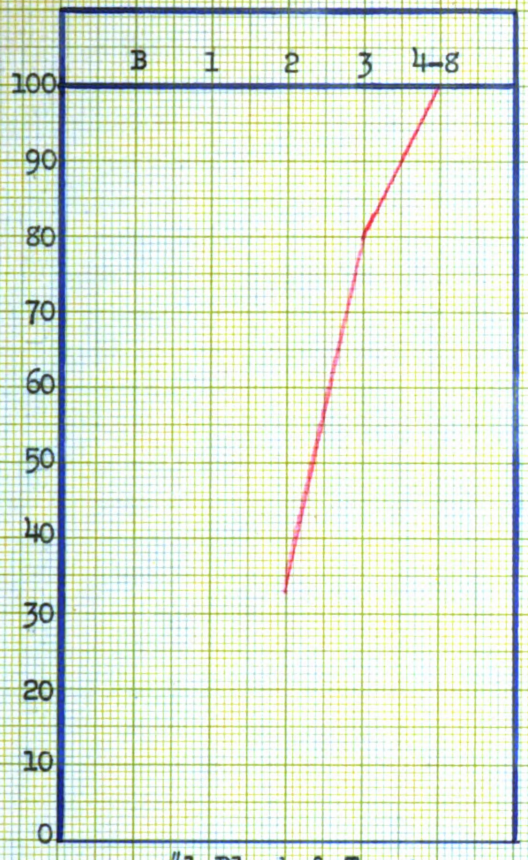
134A $\frac{22.8}{75.3}$

Diagram 4.

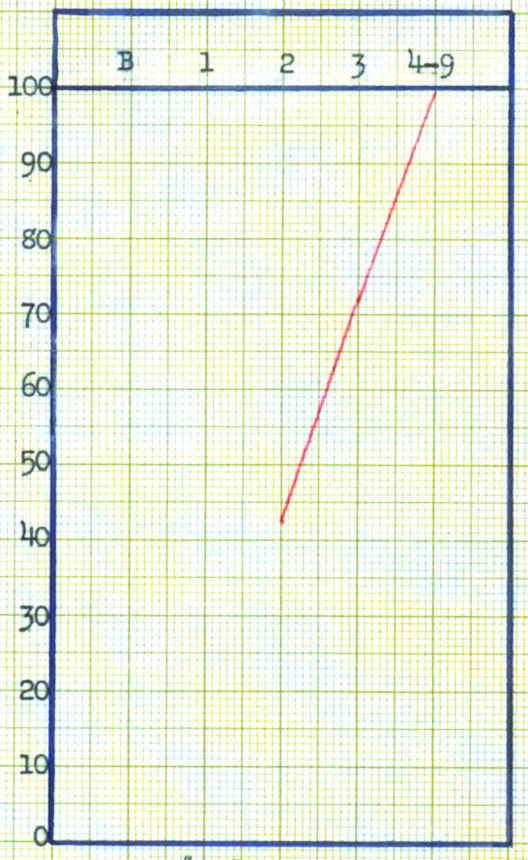
ERUPTION OF HAIR

Graphs - Table

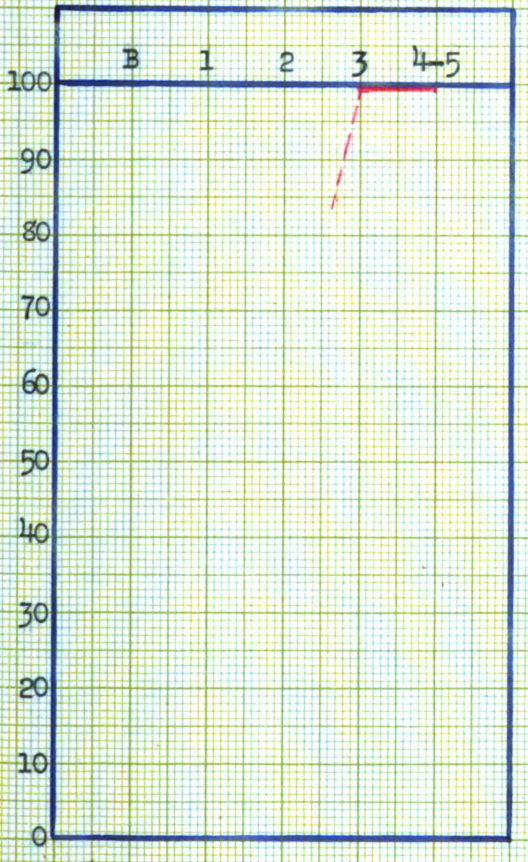
GRAPHS Showing time and per cent of eruption of hair.



#1 Black & Tan



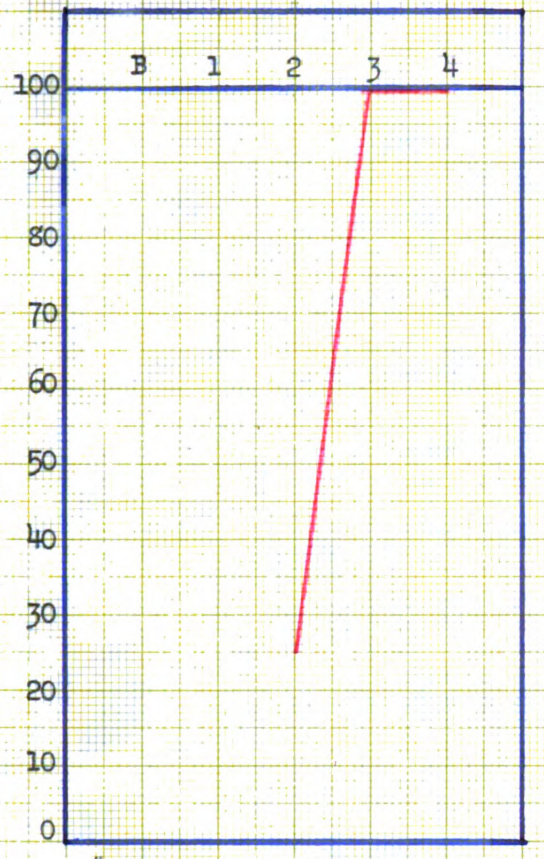
#2 Chocolate



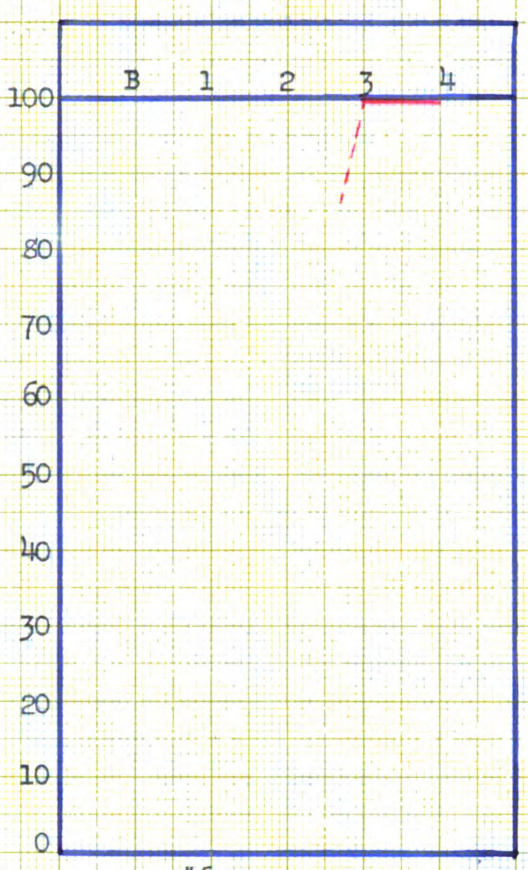
#3 Light-bellied Agouti

#4 Dark-eyed black Agouti

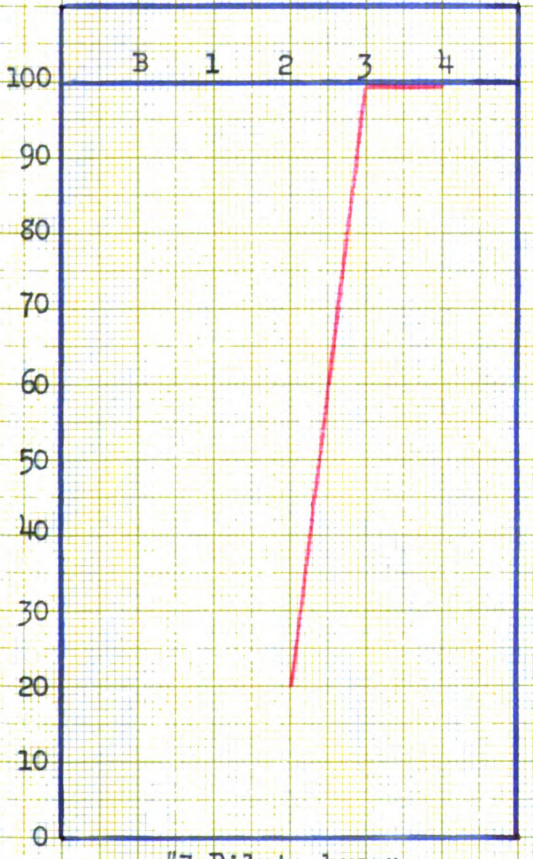
GRAPHS Showing time and per cent of eruption of hair.



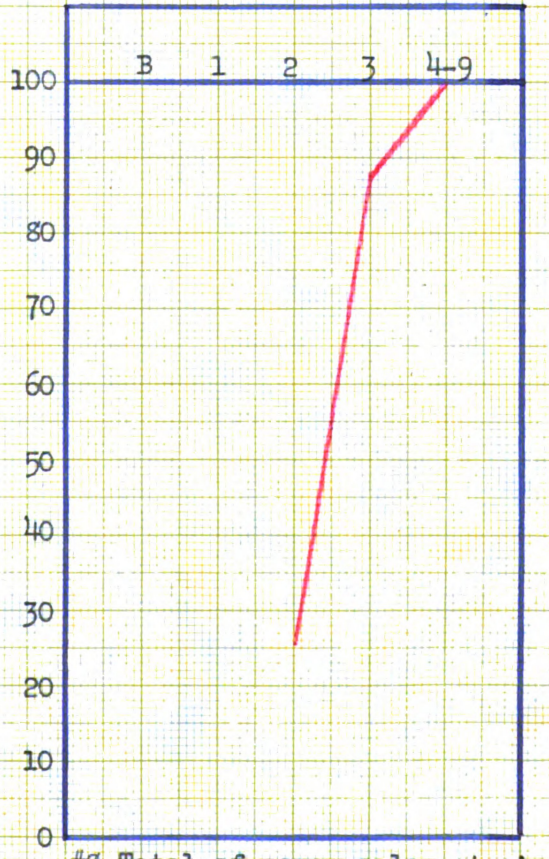
#5 Pink-eyed black agouti



#6 Chinchilla



#7 Dilute brown



#8 Total of seven color strains

Data on eruption of hair.

Strains of Mice	Age: Birth			1 day			2 days			3 days			4-9 days		
	Number of Animals	Number showing eruption of hair	Per cent showing eruption of hair	Number of animals	Number showing eruption of hair	Per cent showing eruption of hair	Number of animals	Number showing eruption of hair	Per cent showing eruption of hair	Number of animals	Number showing eruption of hair	Per cent showing eruption of hair	Number of animals	Number showing eruption of hair	Per cent showing eruption of hair
Black and Tan	5	0	0	9	0	0	9	3	33 1/3%	10	8	80%	25	25	100%
Chocolate	4	0	0	9	0	0	7	3	42.8%	7	5	72%	19	19	100%
Light-bellied agouti	3	0	0	5	0	0	2	0	0	2	2	100%	3	3	100%
Dark-eyed Black Agouti	4	0	0	5	0	0	5	1	20%	5	5	100%	13	13	100%
Pink-eyed Black Agouti	0	0	0	4	0	0	4	1	25%	3	3	100%	1	1	100%
Chinchilla	0	0	0	2	0	0	2	0	0	2	2	100%	2	2	100%
Dilute Brown	3	0	0	13	0	0	10	2	20%	4	4	100%	4	4	100%
Totals	19	0	0	47	0	0	39	10	25.6%	33	29	87.8%	67	67	100%

Table #3

DATA ON THE CLASSIFICATION OF THE FOUR FOLLICLE TYPES

Black & Tan

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
136	birth	47	29	17	1	0
216	"	25	11	12	2	0
217MH	"	25	15	8	1	1
218A	"	25	17	7	1	0
219C	"	25	16	7	2	0
46	1 day	25	3	7	13	2
150C	"	25	2	8	12	3
105	"	25	1	4	5	15
96	"	25	1	4	8	12
98	"	25	2	3	4	16
87	"	25	1	4	5	15
86	"	25	1	2	10	12
71	"	25	1	3	13	8
82	"	25	0	2	12	11
160A	2 day	25	1	2	2	20
159A	"	25	2	0	3	20
108	"	25	3	3	4	15
102	"	25	1	2	8	14
99	"	25	1	2	3	19
88	"	25	2	1	6	16
84	"	25	2	2	4	17
75	"	25	3	2	3	17
50	"	25	0	2	4	19

Chocolate

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
23	birth	0	0	0	0	0
24	"	0	0	0	0	0
42	"	0	0	0	0	0
41	"	0	0	0	0	0
7	1 day	2	2	0	0	0
25	"	0	0	0	0	0
26	"	0	0	0	0	0
30	"	0	0	0	0	0
34	"	25	2	11	9	3
37	"	0	0	0	0	0
44	"	25	0	6	13	6
43	"	14	0	2	9	3
53	"	25	1	6	14	4
27	2 days	5	0	1	4	0
28	"	25	2	3	6	14
33	"	25	2	2	5	16
40	"	25	2	5	4	14
48	"	25	2	1	4	18
49	"	25	0	2	6	17
57	"	25	2	3	7	13

Light-bellied Agouti

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
220	birth	25	15	7	3	0
222A	"	25	8	13	4	0
221	"	25	13	9	3	0
223	1 day	25	2	6	14	3
224	"	25	2	4	16	3
225	"	25	2	6	9	8
163	"	12	2	3	5	2
164S	"	25	2	7	10	6
169	2 days	16	2	2	5	7
170	"	25	0	2	9	14

Dark-eyed Black Agouti

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
127	birth	25	3	11	11	0
211	"	25	5	12	7	1
212	"	25	6	13	6	0
213S	"	25	2	16	7	0
16	1 day	0	0	0	0	0
59	"	25	2	12	11	0
134	"	25	1	7	14	3
135	"	25	0	5	15	5
214	"	25	2	8	9	6
1	2 day	0	0	0	0	0
17	"	25	2	1	6	16
63	"	14	0	0	5	9
76	"	25	1	2	8	14
152	"	25	0	1	6	18

Pink-eyed Black Agouti

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
No	birth	0	0	0	0	0
171	1 day	3	3	0	0	0
172	"	7	5	2	0	0
182	"	25	2	9	9	5
183	"	25	4	7	11	3
177	2 days	25	2	8	10	5
178	"	25	0	3	6	16
193	"	25	1	3	7	14
194	"	25	1	3	8	13

Chinchilla

Specimen number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
190	1 day	25	5	9	9	2
191	"	25	4	11	7	3
197	2 day	25	0	3	6	16
198	"	25	1	4	6	14

Dilute Brown

Specimen Number	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
121	birth	18	18	0	0	0
124	"	25	22	3	0	0
125	"	25	25	0	0	0
122	1 day	6	6	0	0	0
123A	"	25	5	11	9	0
130	"	25	2	7	10	6
131	"	25	1	3	13	8
128	"	25	2	7	10	6
129	"	25	1	4	18	2
184	"	25	1	5	12	7
185	"	25	0	4	16	5
188	"	25	2	5	14	4
189	"	25	0	7	14	4
226	"	25	3	9	11	2
227	"	25	2	9	12	2
228	"	25	3	13	7	2
132	2 day	25	0	5	8	12
133	"	25	1	3	9	12
142	"	25	4	3	7	11
143	"	25	2	0	8	15
140	"	25	0	4	7	14
141	"	25	3	5	7	10
186	"	25	2	3	7	13
187	"	25	2	3	6	14
195	"	25	2	6	8	9
196	"	25	0	5	8	12

Total of Strains Studied

Strains	Follicle types			
Birth	1	2	3	4
Black and Tan	88	51	7	1
Chocolate	0	0	0	0
Light-bellied Agouti	36	29	10	0
Pink-eyed Black Agouti	0	0	0	0
Chinchilla	0	0	0	0
Dark-eyed Black Agouti	16	52	31	1
Dilute Brown	65	3	0	0
390 total	205	135	48	2
1 day				
Black and Tan	12	37	82	94
Chocolate	5	25	45	16
Light-bellied Agouti	10	26	54	22
Pink-eyed Black Agouti	14	18	20	8
Chinchilla	9	20	16	5
Dark-eyed Black Agouti	5	32	49	14
Dilute Brown	28	84	146	48
total 944	83	242	412	207
2 days				
Black and Tan	15	16	37	157
Chocolate	11	17	36	92
Light-bellied Agouti	2	4	14	21
Pink-eyed Black Agouti	4	17	31	48
Chinchilla	1	7	12	30
Dark-eyed Black Agouti	3	4	25	57
Dilute Brown	16	37	75	122
total 911	52	102	230	527

Grand total Follicles 2245

TOTAL NUMERICAL DATA

ON FOLLICLE TYPES.

TOTAL PERCENTAGE DATA

ON FOLLICLE TYPES

Black & Tan

Total of data on follicle types

Number of specimens	Age	Number of follicles classified	Follicle types			
			1	2	3	4
5	birth	147	88	51	7	1
9	1 day	225	12	37	82	94
9	2 days	225	15	16	37	157

Total of data on follicle type percentages

Number of specimens	Age	Number of follicles classified	Follicle types			
			1	2	3	4
5	birth	147	59.8%	34.7%	4.8%	.09%
9	1 day	225	5.3%	16.4%	36.4%	41.7%
9	2 days	225	6.6%	7.1%	16.4%	69.7%

Chocolate

Total of data on follicle types

Number of Specimens	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
4	birth	0	0	0	0	0
9	1 day	91	5	25	45	16
7	2 days	155	11	17	36	92

Total of data on follicle type percentages

Number of Specimens	Age	Number of follicles classified	Follicle types			
			1	2	3	4
4	birth	0	0	0	0	0
9	1 day	91	5.5%	27.4%	49.4%	19.7%
7	2 days	155	7%	10.9%	23.7%	59.3%

Light-bellied Agouti

Total data on follicle types

Number of specimens	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
3	birth	75	36	29	10	0
5	1 day	112	10	26	54	22
2	2 dats	41	2	4	14	21

Total data on follicle type percentage

Number of specimens	Age	Number of follicles classified	Follicle types			
			1	2	3	4
3	birth	75	48%	38.6%	13.3%	0
5	1 day	112	8.9%	23.2%	48.2%	19.6%
2	2 days	41	4.87%	9.7%	34.1%	51.2%

Dark-eyed Black Agouti

Total data on follicle types

Number of specimen	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
4	birth	100	16	52	31	1
5	1 day	100	5	32	49	14
5	2 days	89	3	4	25	57

Total data on follicle type percentage

Number of specimen	Age	Number of follicles classified	Follicle types			
			1	2	3	4
4	birth	100	16%	52%	31%	1%
5	1 day	100	5%	32%	49%	14%
5	2 days	89	3.3%	4.5%	28.1%	64.4%

Pink-eyed Black Agouti

Total data on follicle types

Number of specimen	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
No	birth	0	0	0	0	0
4	1 day	60	14	18	20	8
4	2 day	100	4	17	31	48

Total data on follicle type percentage

Number of specimen	Age	Number of follicles classified	Follicle types			
			1	2	3	4
No	birth	0	0	0	0	0
4	1 day	60	23.3%	30%	33.3%	13.3%
4	2 day	100	4%	17%	31%	48%

Chinchilla

Total data on follicle types

Number of specimen	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
2	1 day	50	9	20	16	5
2	2 days	50	1	7	12	30

Total data on follicle type percentage

Number of specimen	Age	Number of follicles classified	Follicle types			
			1	2	3	4
2	1 day	50	18%	40%	32%	10%
2	2 days	50	2%	14%	24%	60%

Dilute Brown

Total data on follicle types

Number of specimen	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
3	birth	68	65	3	0	0
13	1 day	306	28	84	146	48
10	2 days	250	16	37	75	122

Total data on follicle type percentage

Number of specimen	Age	Number of follicles classified	Follicle types			
			1	2	3	4
3	birth	68	95.6%	4.4%	0	0
13	1 day	306	9.1%	27.45%	47.7%	15.68%
10	2 days	250	6.4%	14.8%	30.0%	48.8%

Total of 7 color strains

Total data on follicle types

Number of specimen	Age	Number of follicles classified	Follicle type classification			
			1	2	3	4
19	birth	390	205	135	48	2
47	1 day	944	83	242	412	207
39	2 days	911	52	102	230	527

Total data on follicle type percentage

Number of specimen	Age	Number of follicles classified	Follicle types			
			1	2	3	4
19	birth	390	52.56%	34.6%	12.3%	.51%
47	1 day	944	8.7%	25.6%	43.6%	21.9%
39	2 days	911	5.7%	11.2%	25.24%	57.8%

GRAPHS SHOWING

PERCENTAGE OF FOLLICLE TYPES

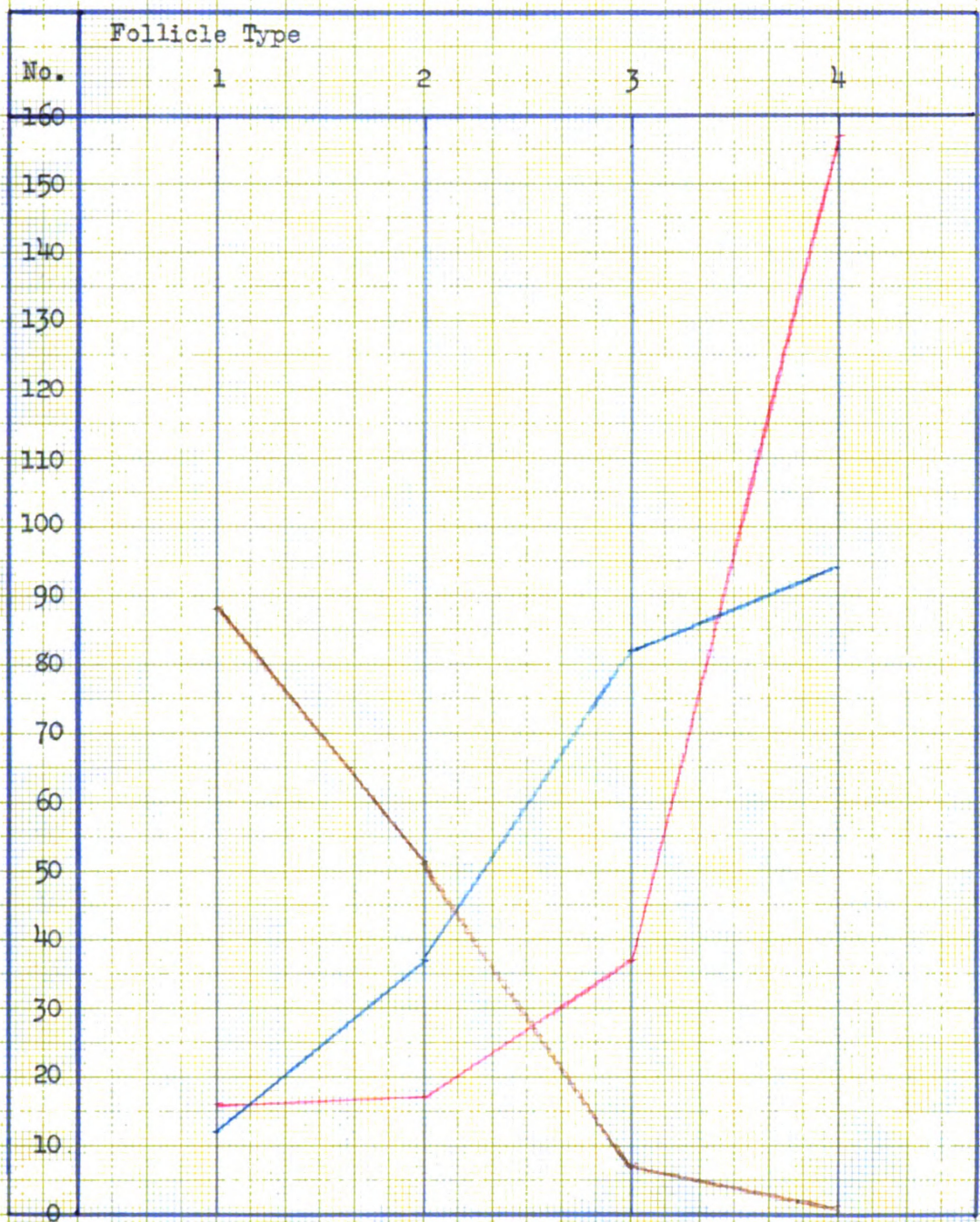
GRAPHS SHOWING

NUMBER OF FOLLICLES

IN EACH OF THE FOUR TYPES

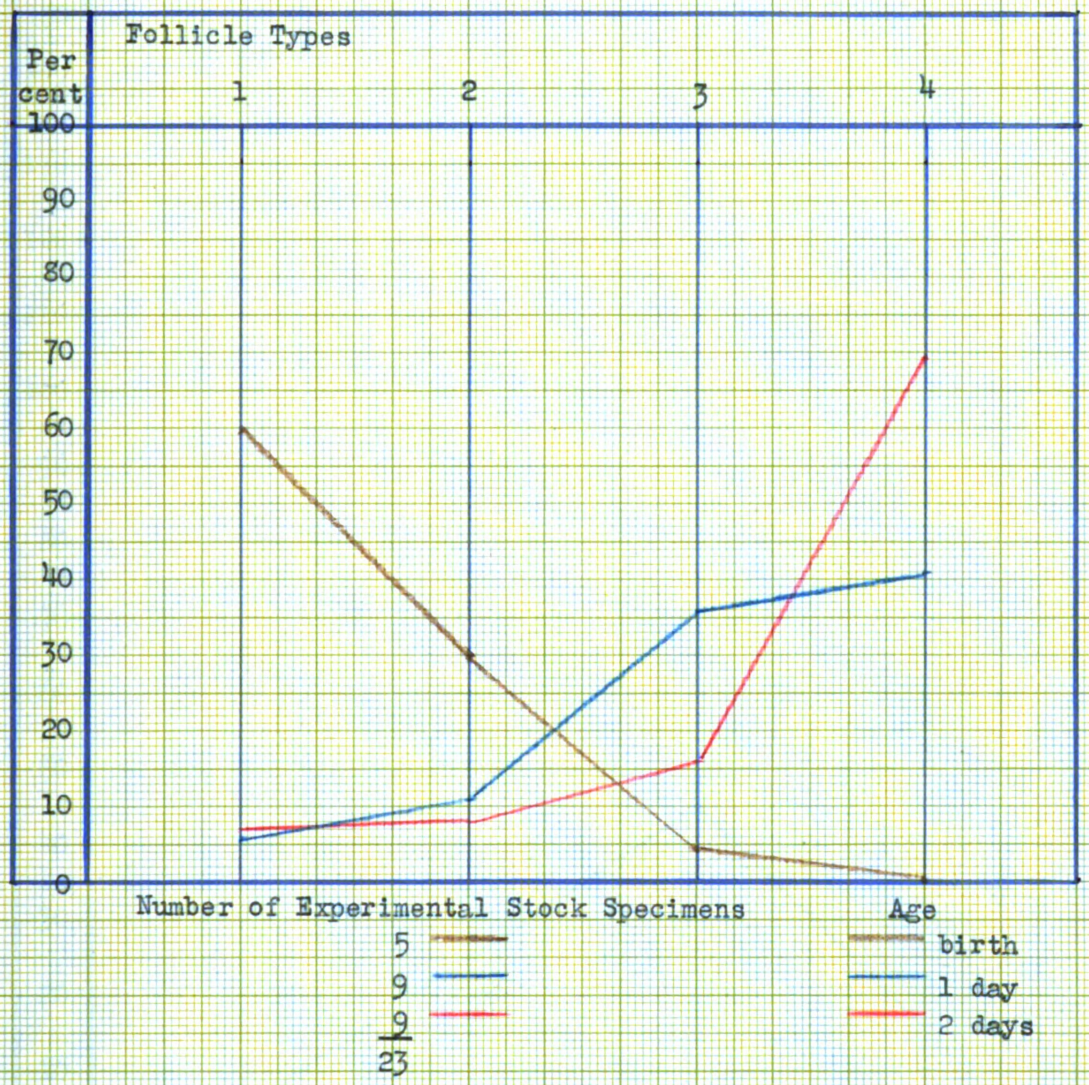
Graph #9

Black & Tan



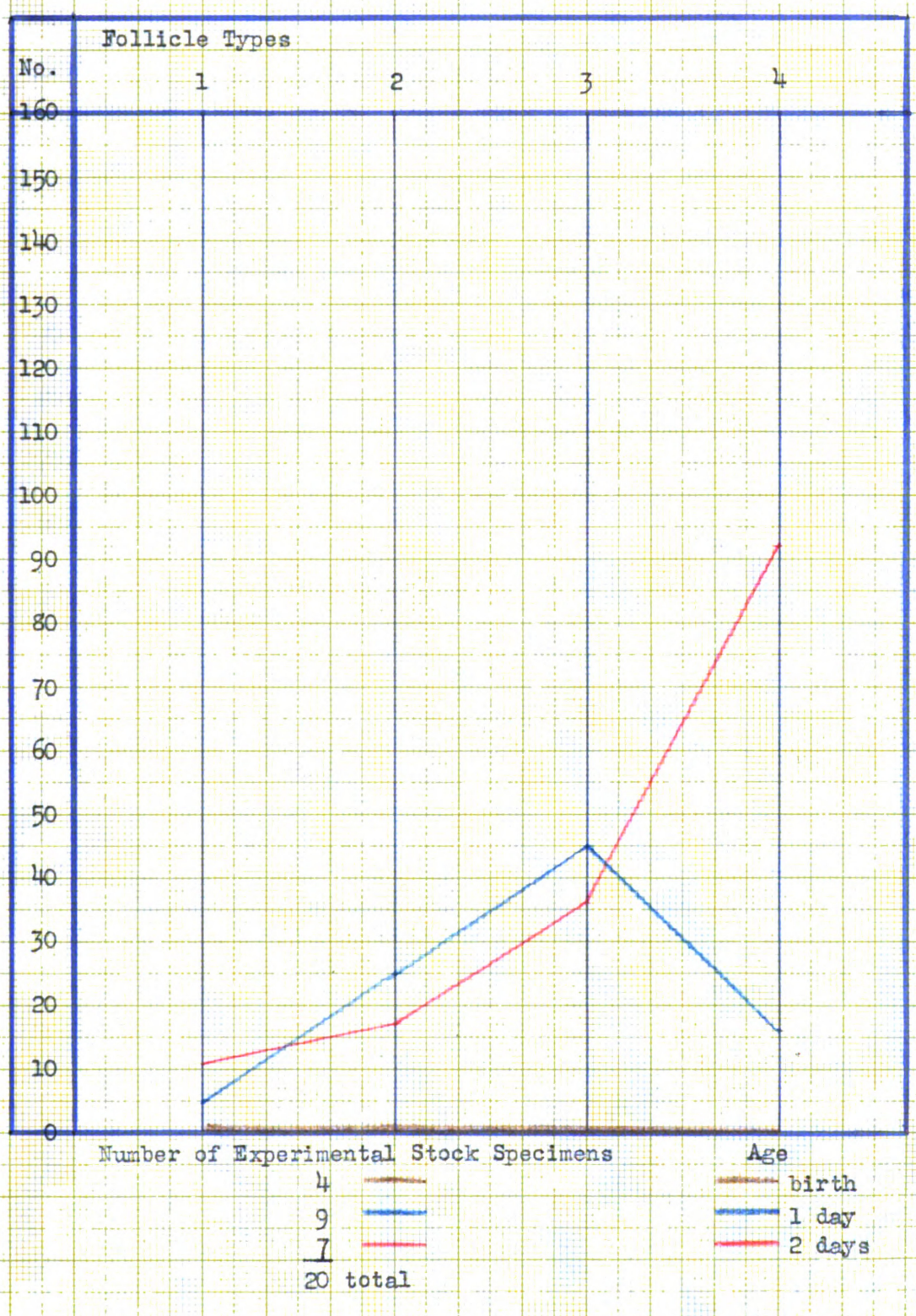
Graph #10

Black & Tan



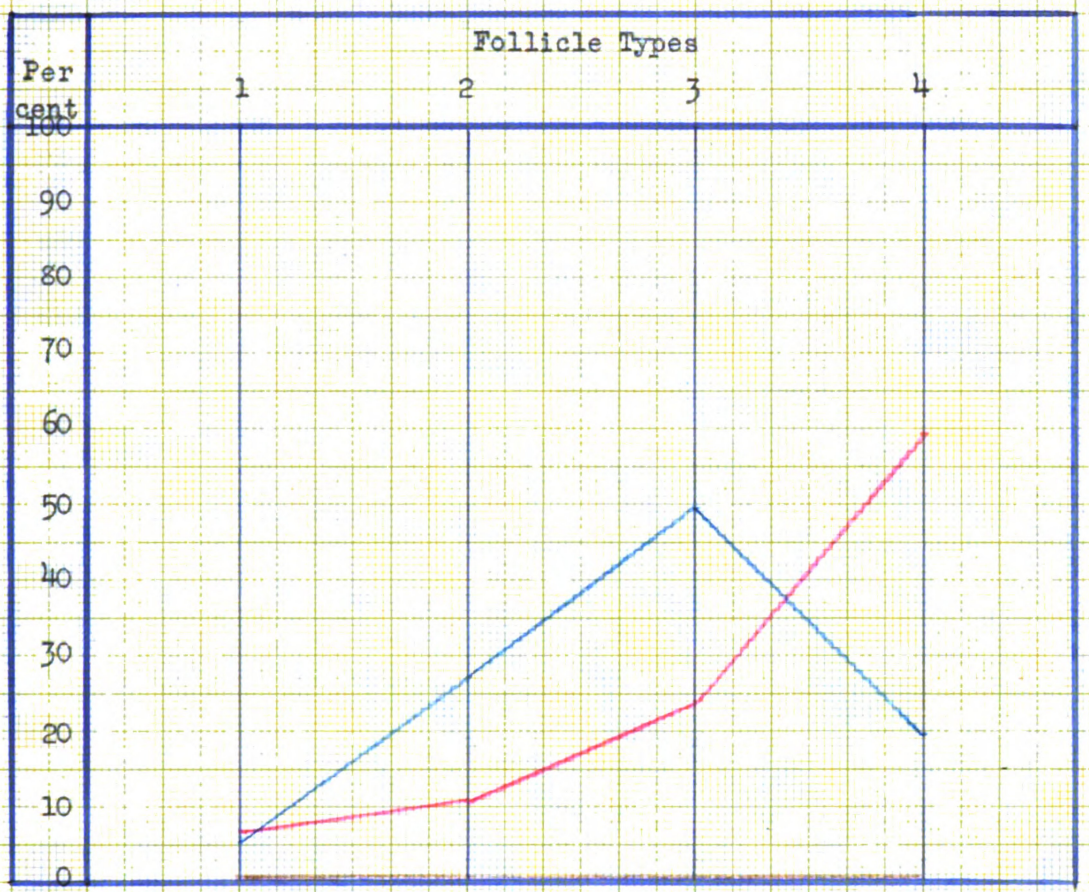
Graph #11

Chocolate



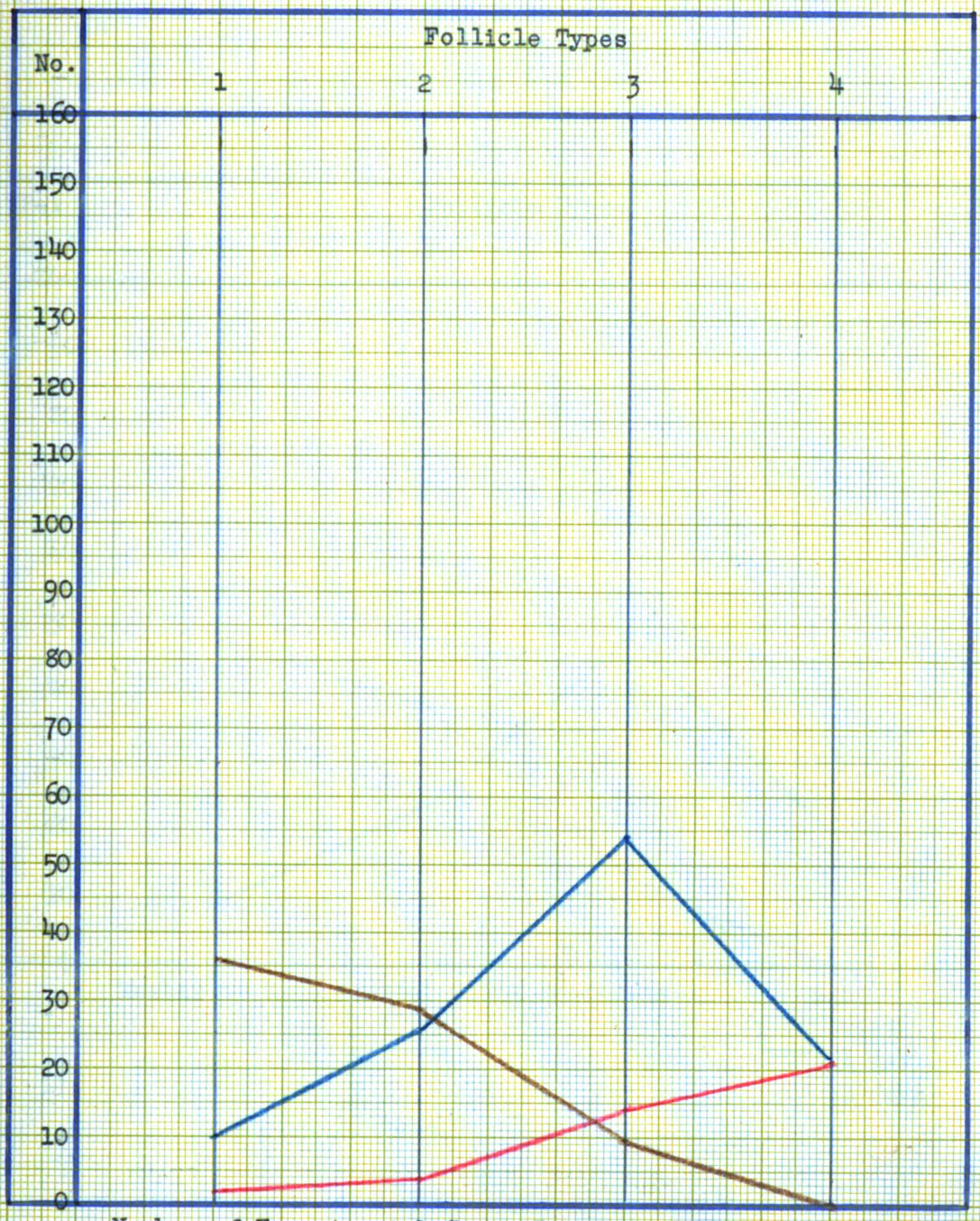
Graph #12

Chocolate



Graph #13

Light-bellied Agouti



Number of Experimental Stock Specimens

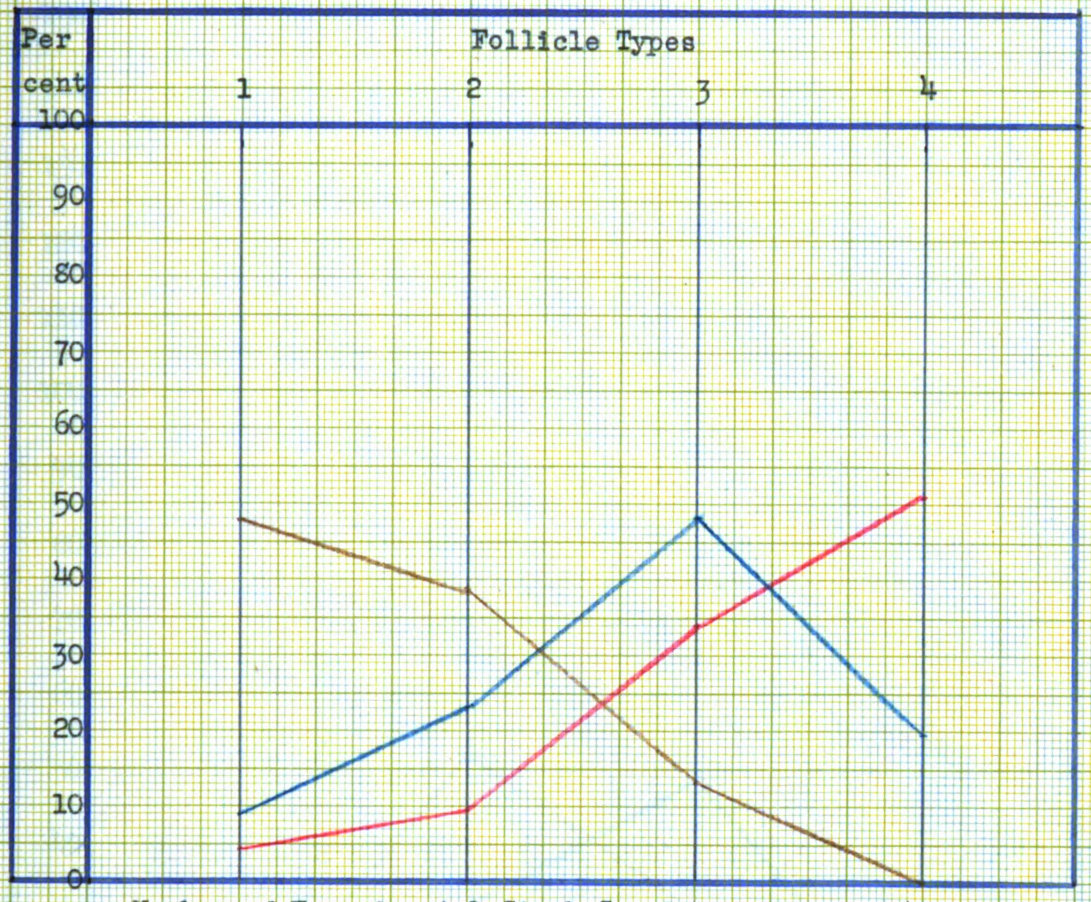
3
5
2
10 total

Age

birth
1 day
2 day

Graph #14

Light-bellied Agouti



Number of Experimental Stock Specimens

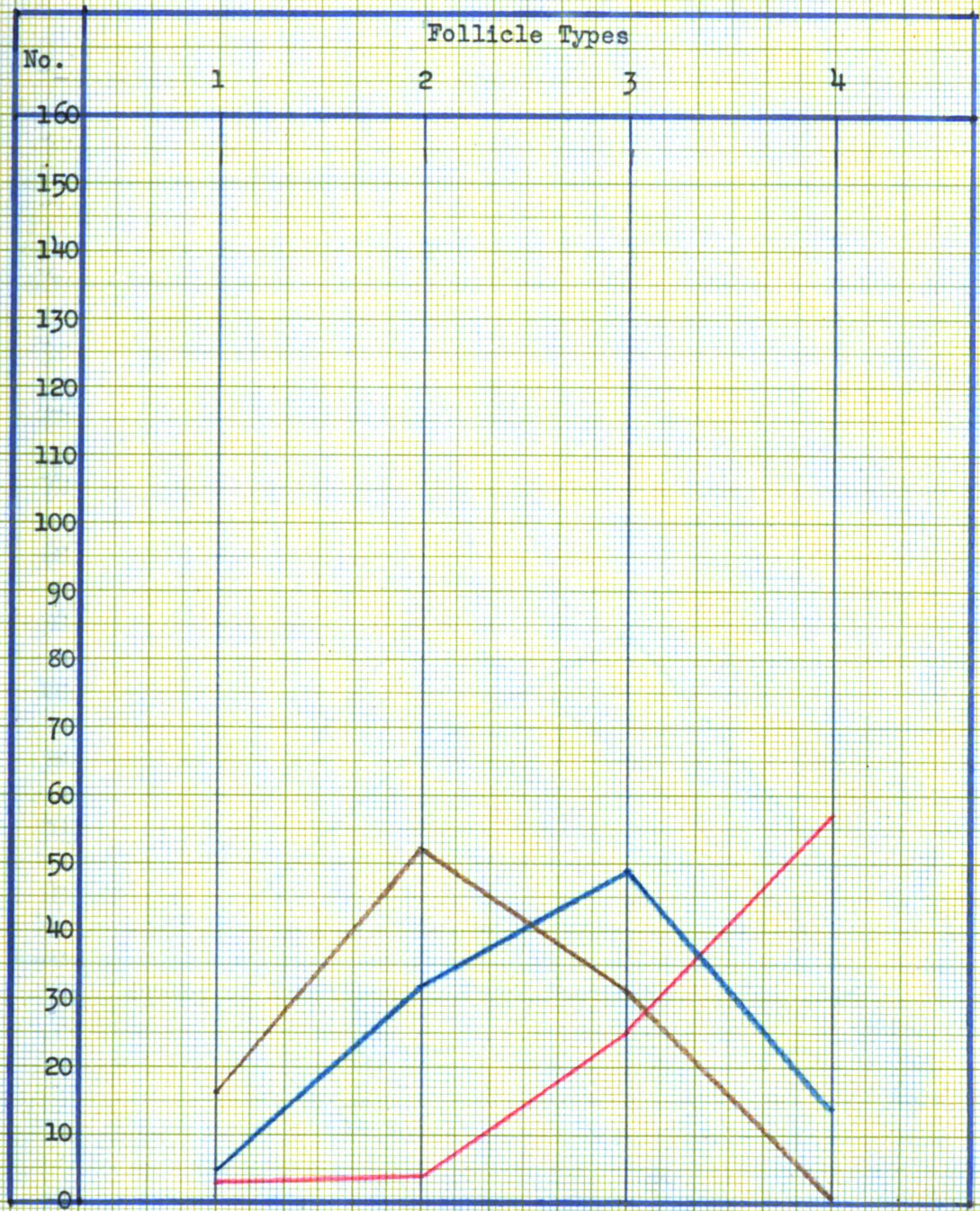
3
5
2
10 total

Age

birth
1 day
2 days

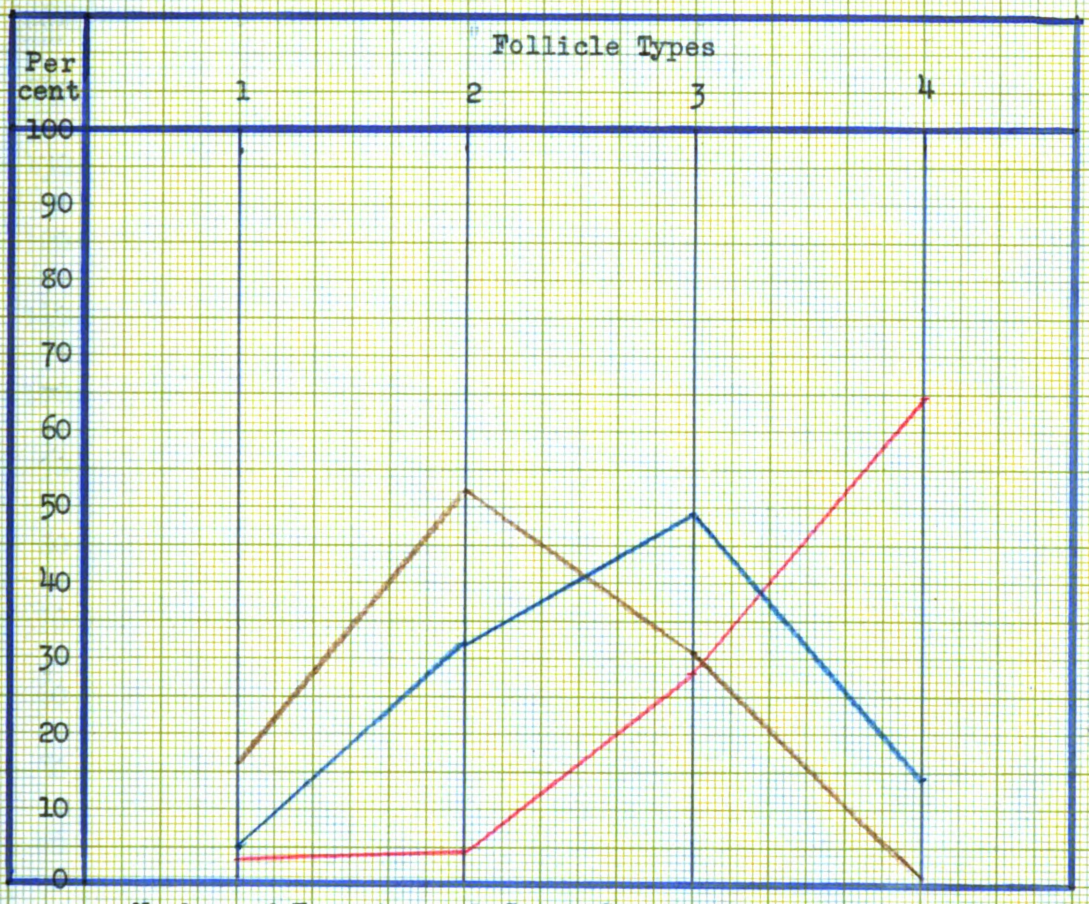
Graph #15

Dark-eyed Black Agouti



Graph # 16

Dark-eyed Black Agouti



Number of Experimental Stock Specimens

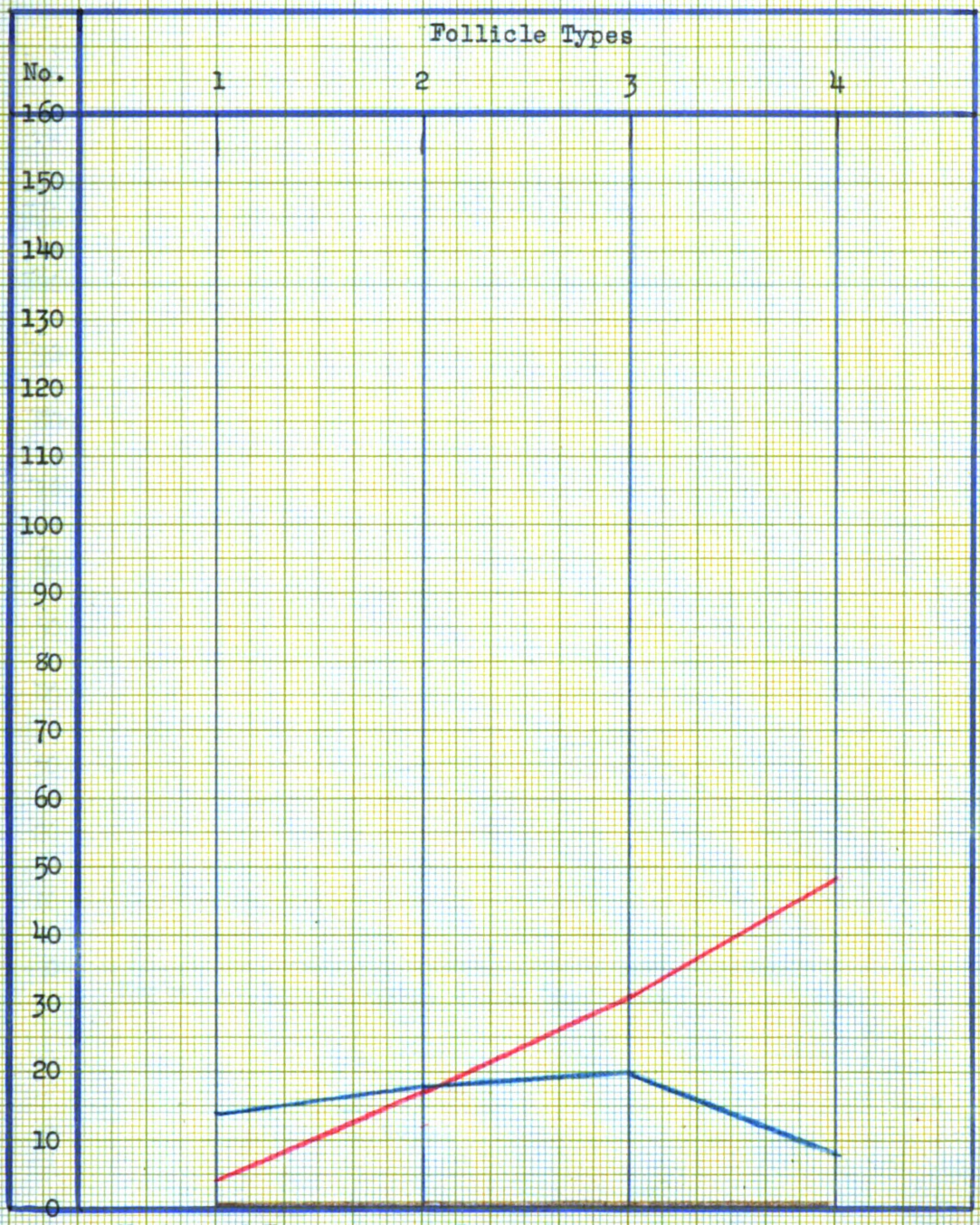
4
5
5
14 total

Age

Birth
1 day
2 days

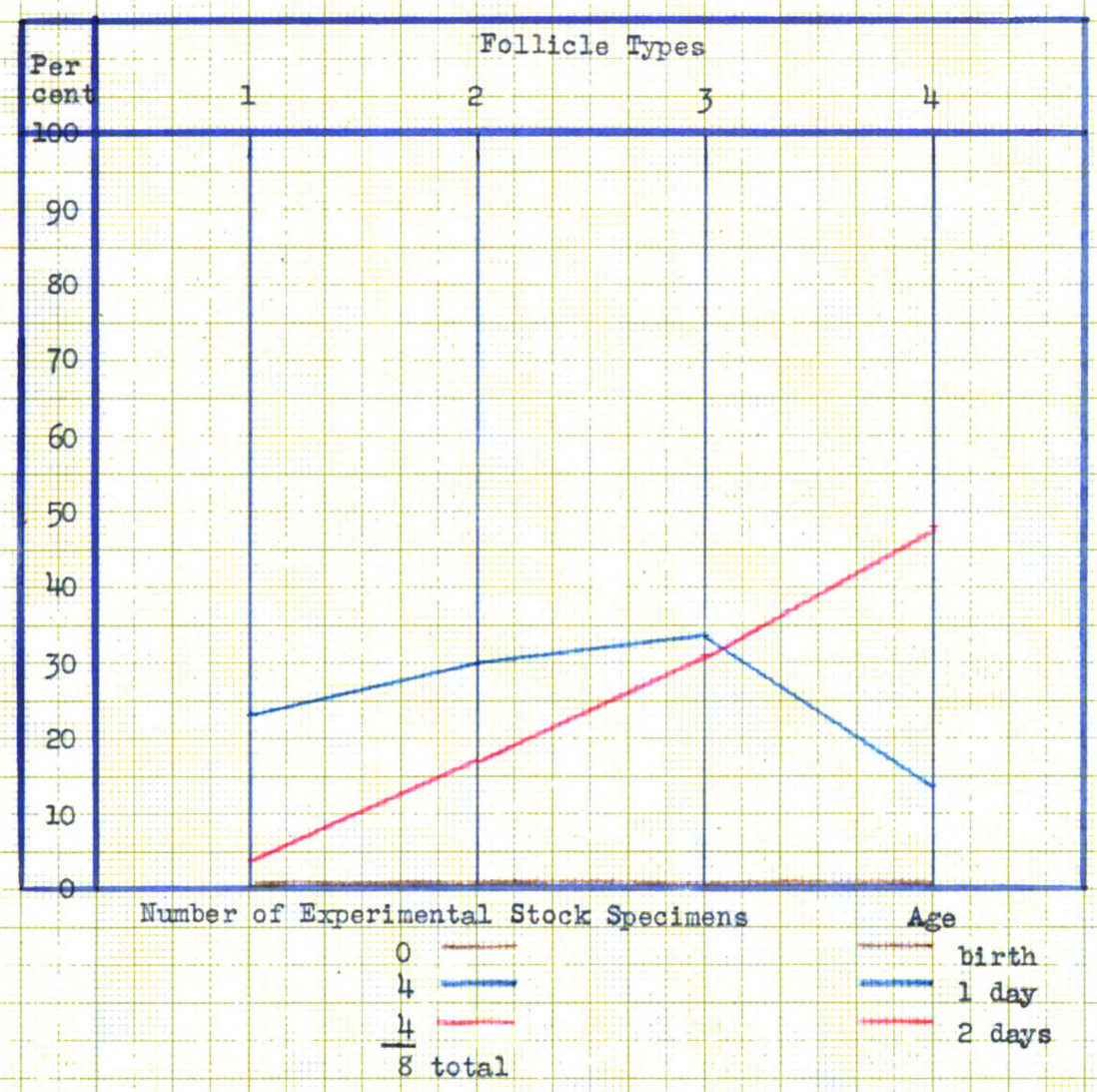
Graph #17

Pink-eyed Black Agouti



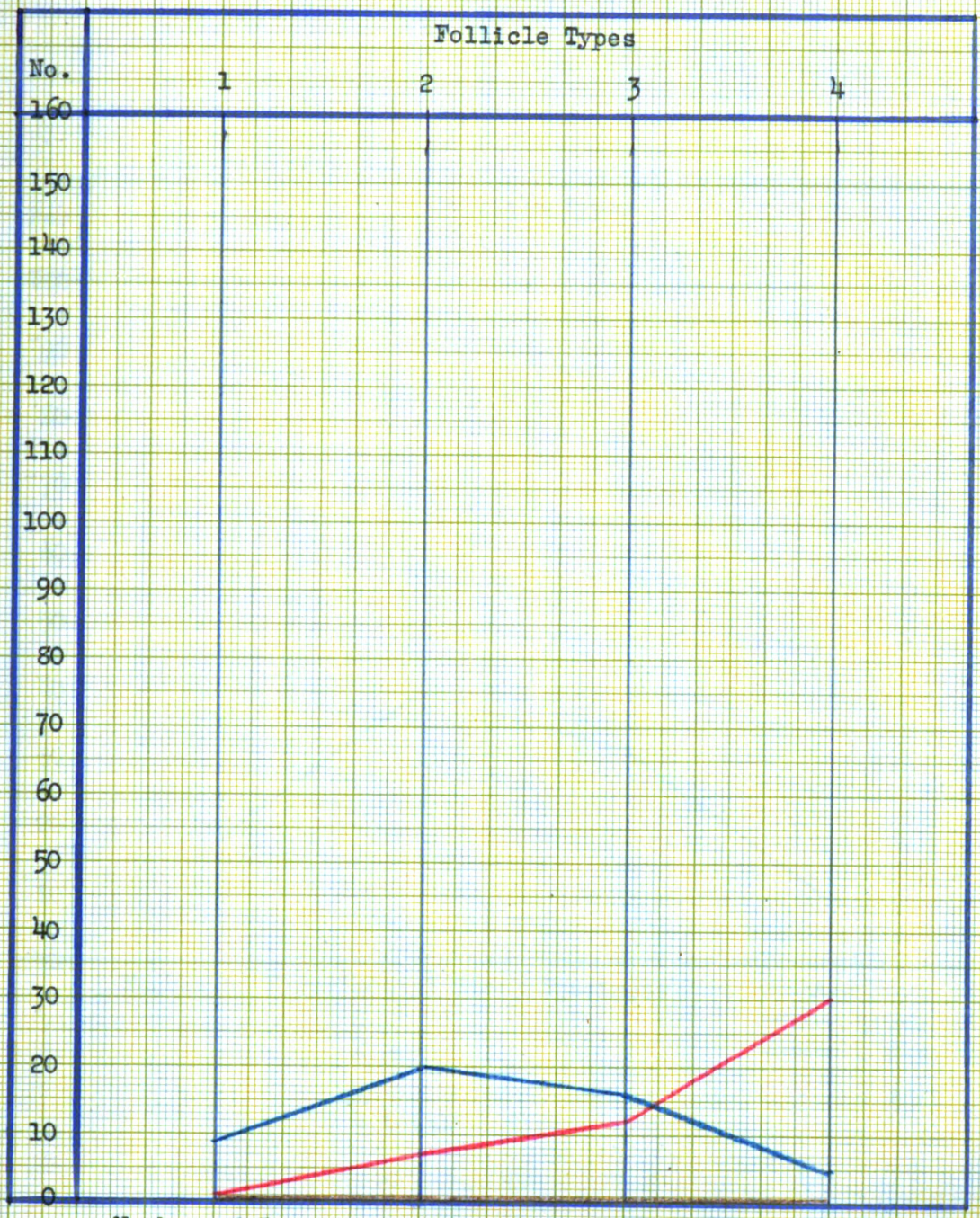
Graph #18

Pink-eyed Black Agouti



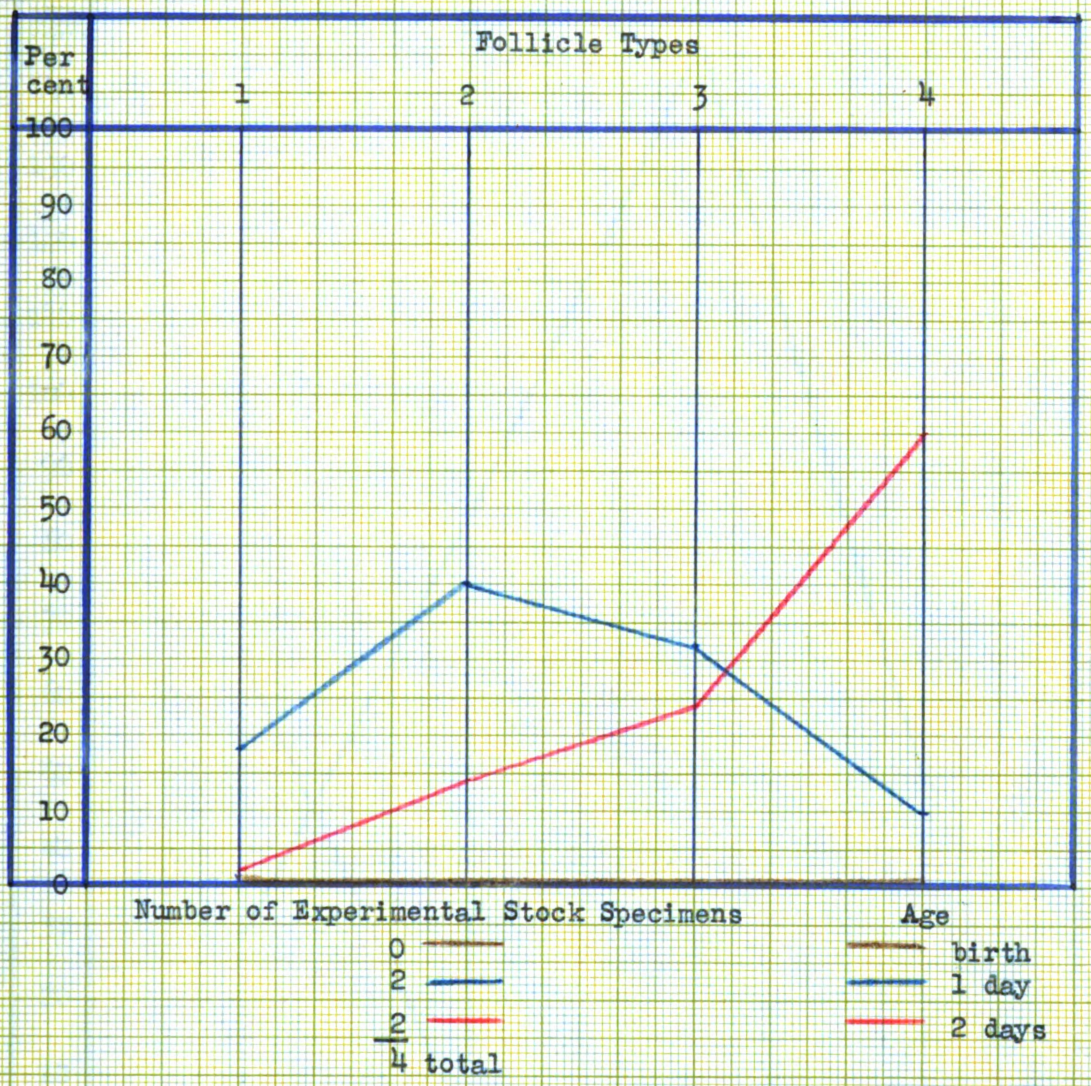
Graph #19

Chinchilla



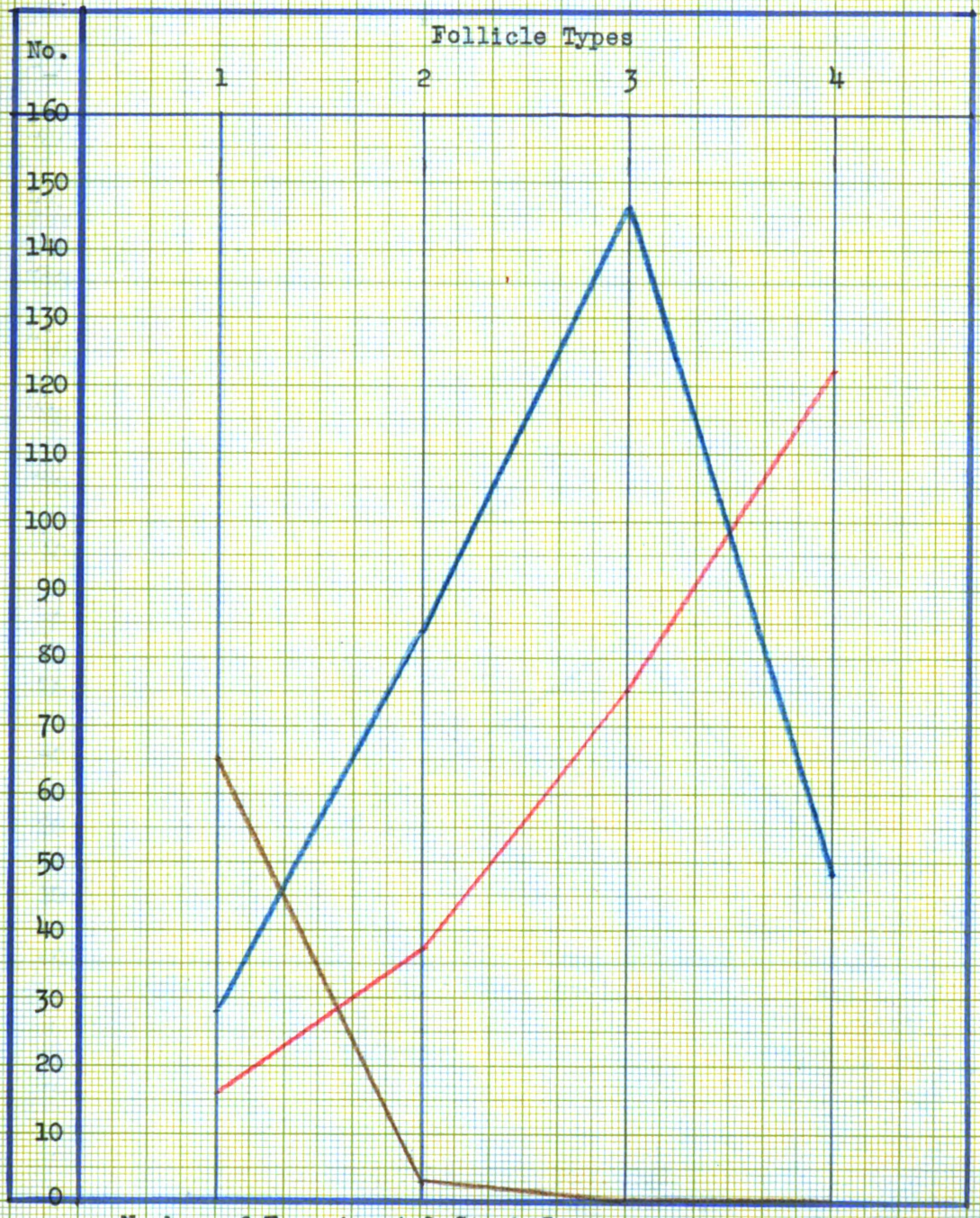
Graph #20

Chinchilla



Graph #21

Dilute Brown



Number of Experimental Stock Specimens

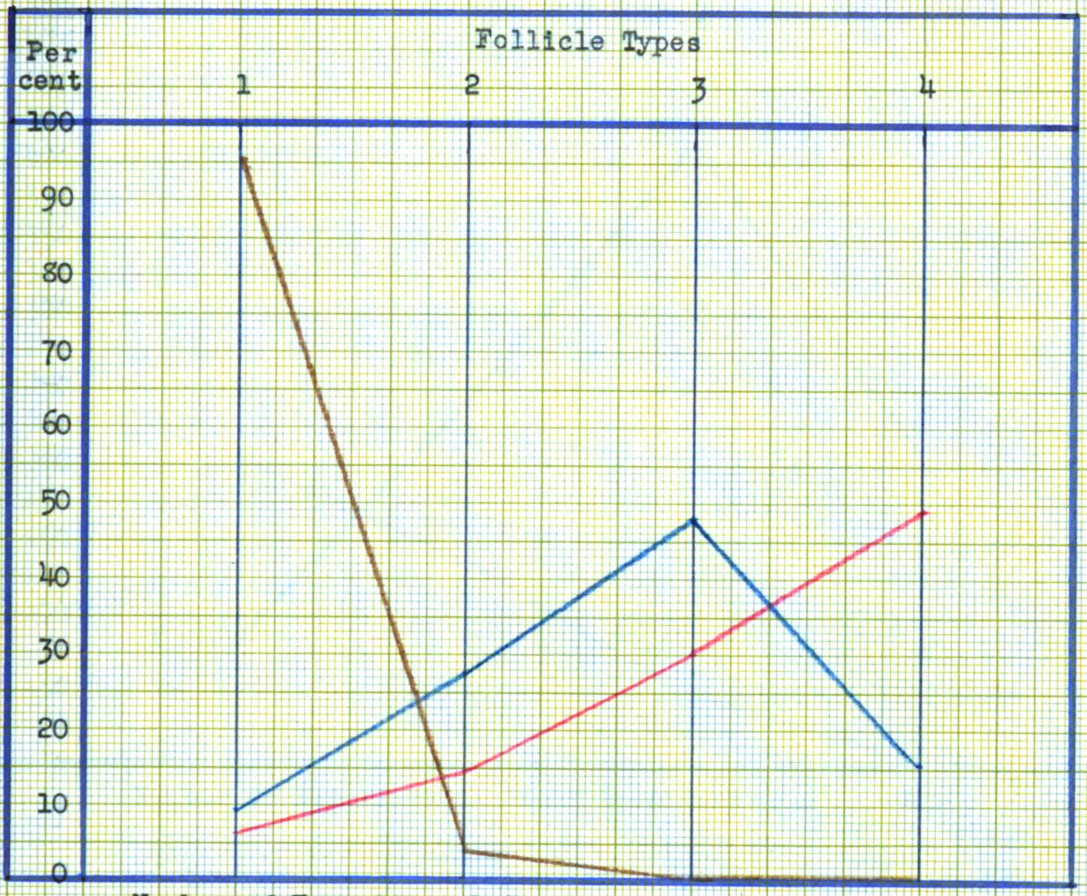
3
13
10
26 total

Age

Birth
1 day
2 day

Graph #22

Dilute Brown



Number of Experimental Stock Specimens

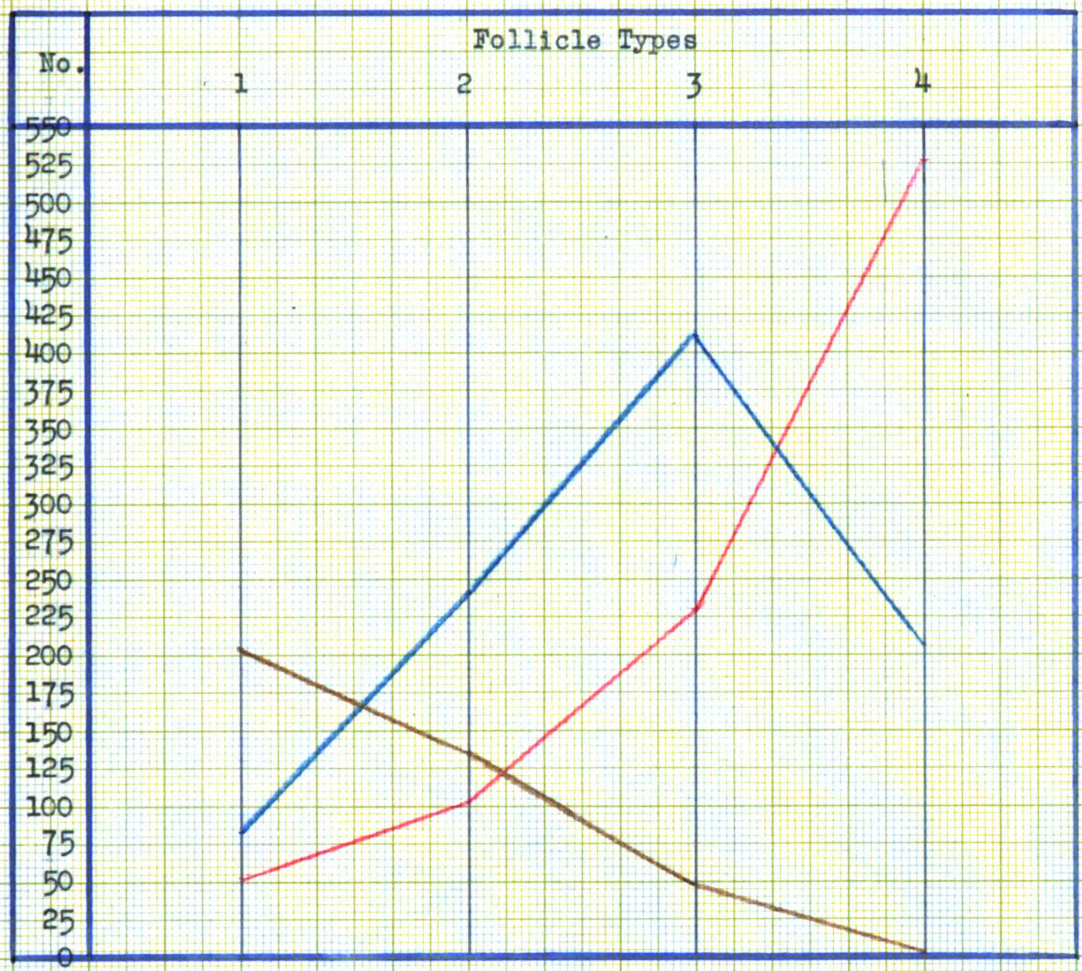
3
13
10
26 total

Age

birth
1 day
2 days

Graph #23

Totals of Strains Studied



Number of Experimental Stock Specimens

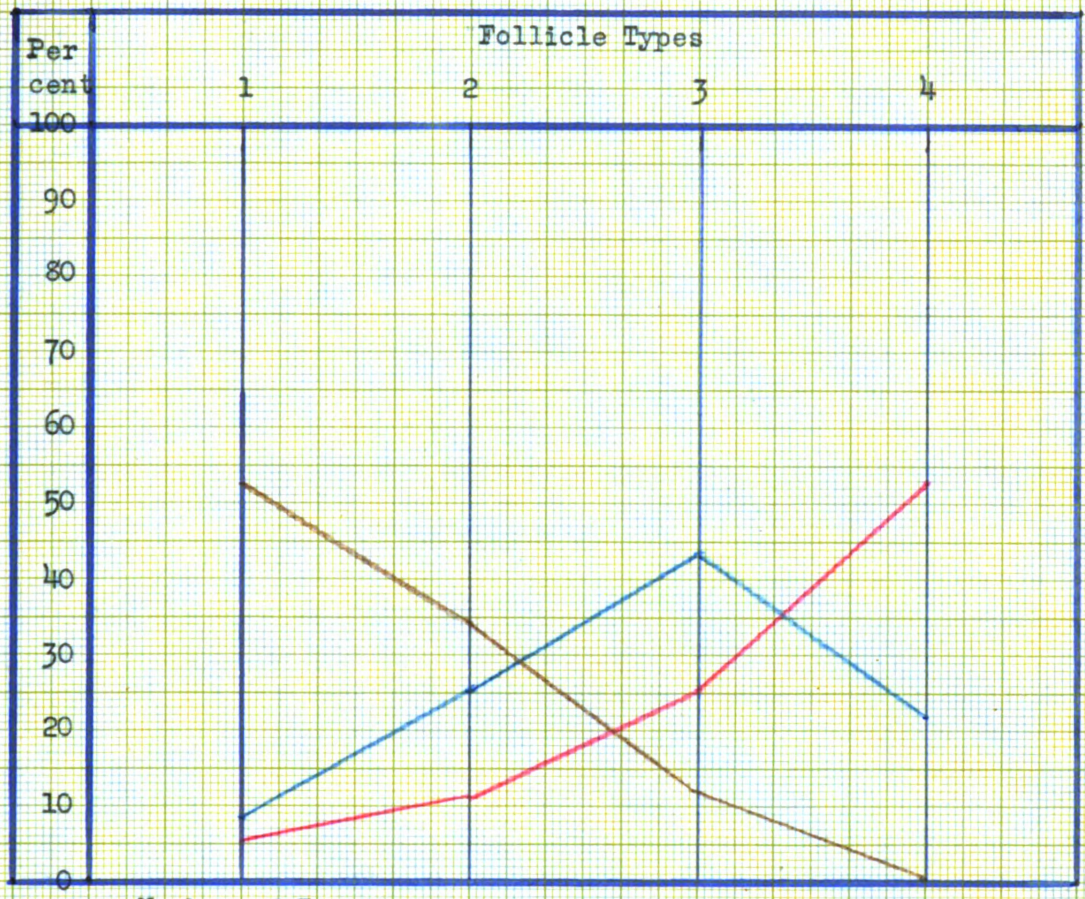
19
47
39
105

Age

birth
1 day
2 days

Graph #24

Total of Strains Studied



Number of Experimental Stock Specimens

19
47
39
105

Age

birth
1 day
2 days

KEY TO

MICROPHOTOGRAPHS

C = Cortex of hair

F = Follicle

G = Pigment granules

M = Medulla of hair

P = Papilla

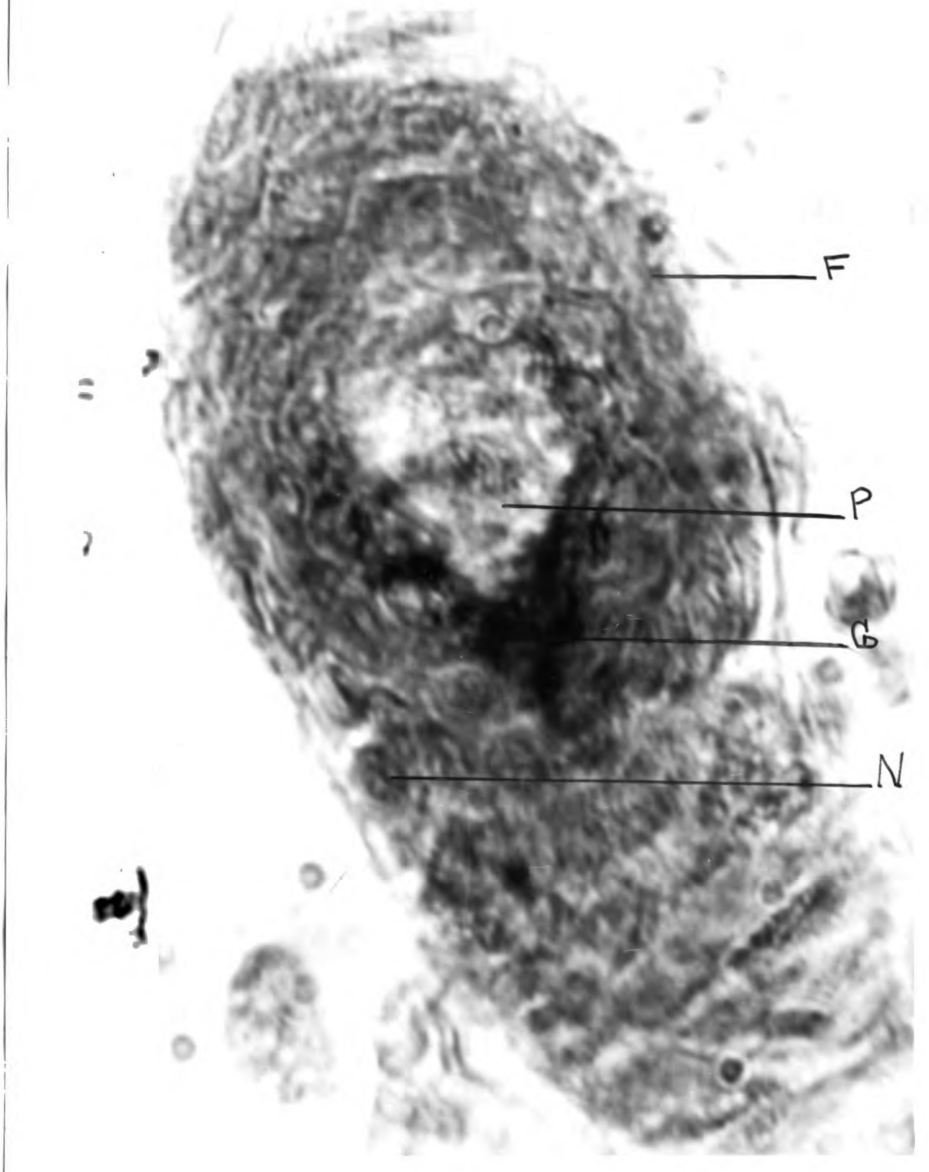


Plate A

Black and Tan Birth

219A 20.5/68 X 600

PLATE B

Black and Tan 1 day

150D 10/62 X600

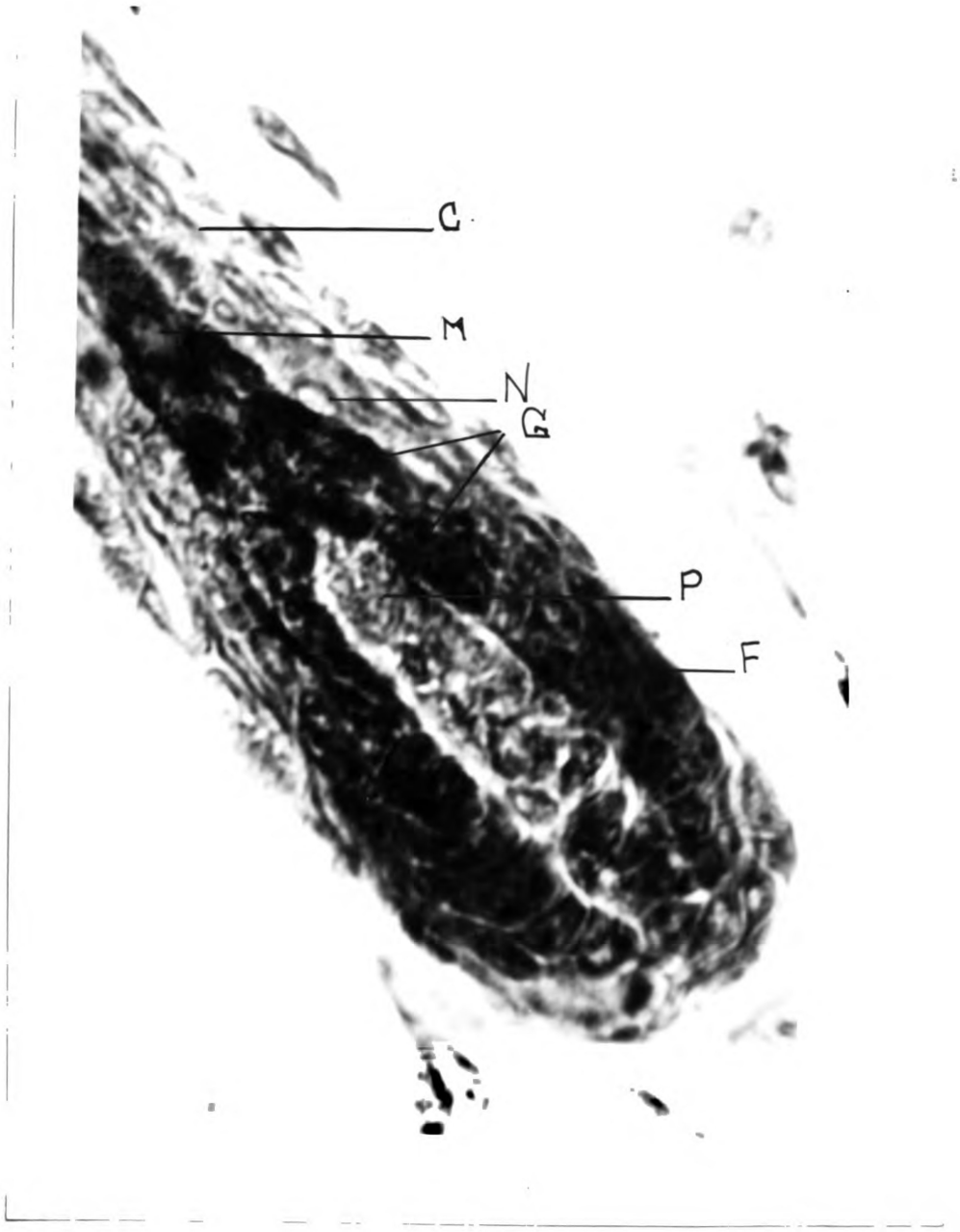


PLATE C

Black and Tan 2 day

160Sp 22.5/76.2 X 600

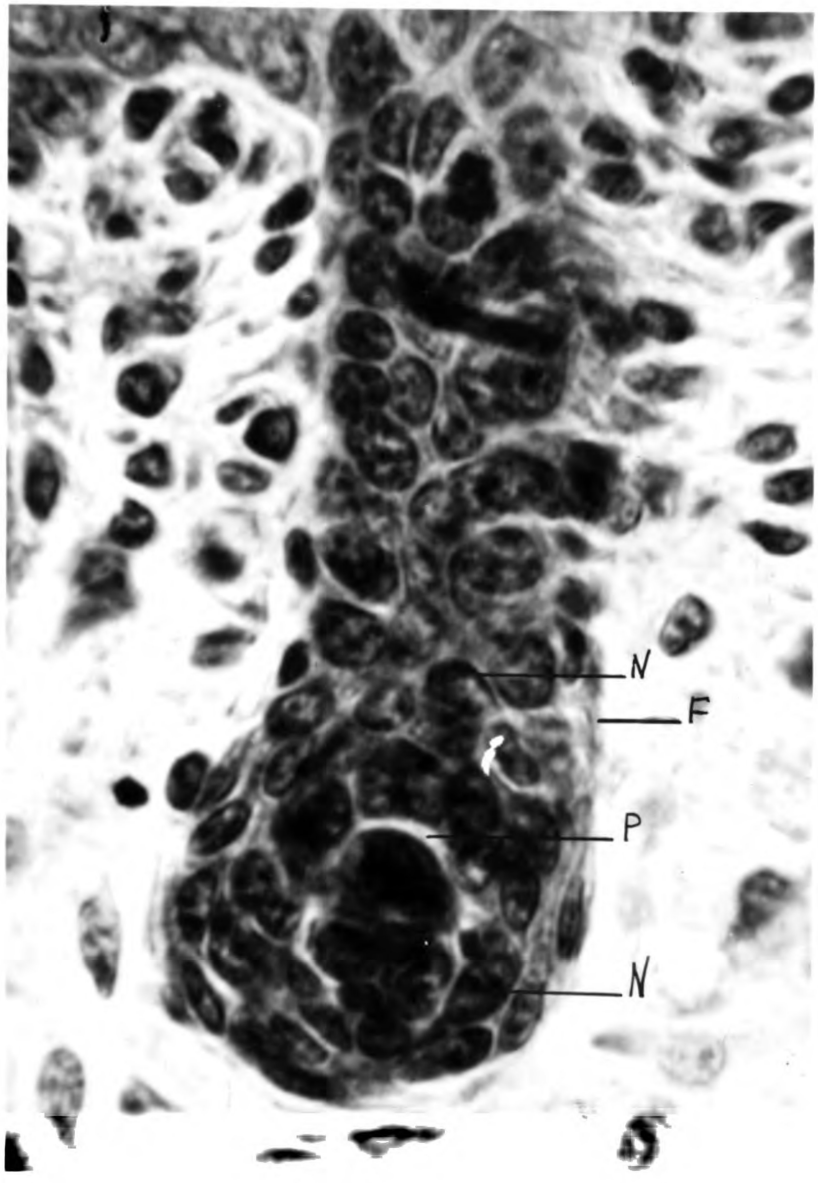


PLATE D

Black and Tan Birth

217MH 16.1/72.7 X 600

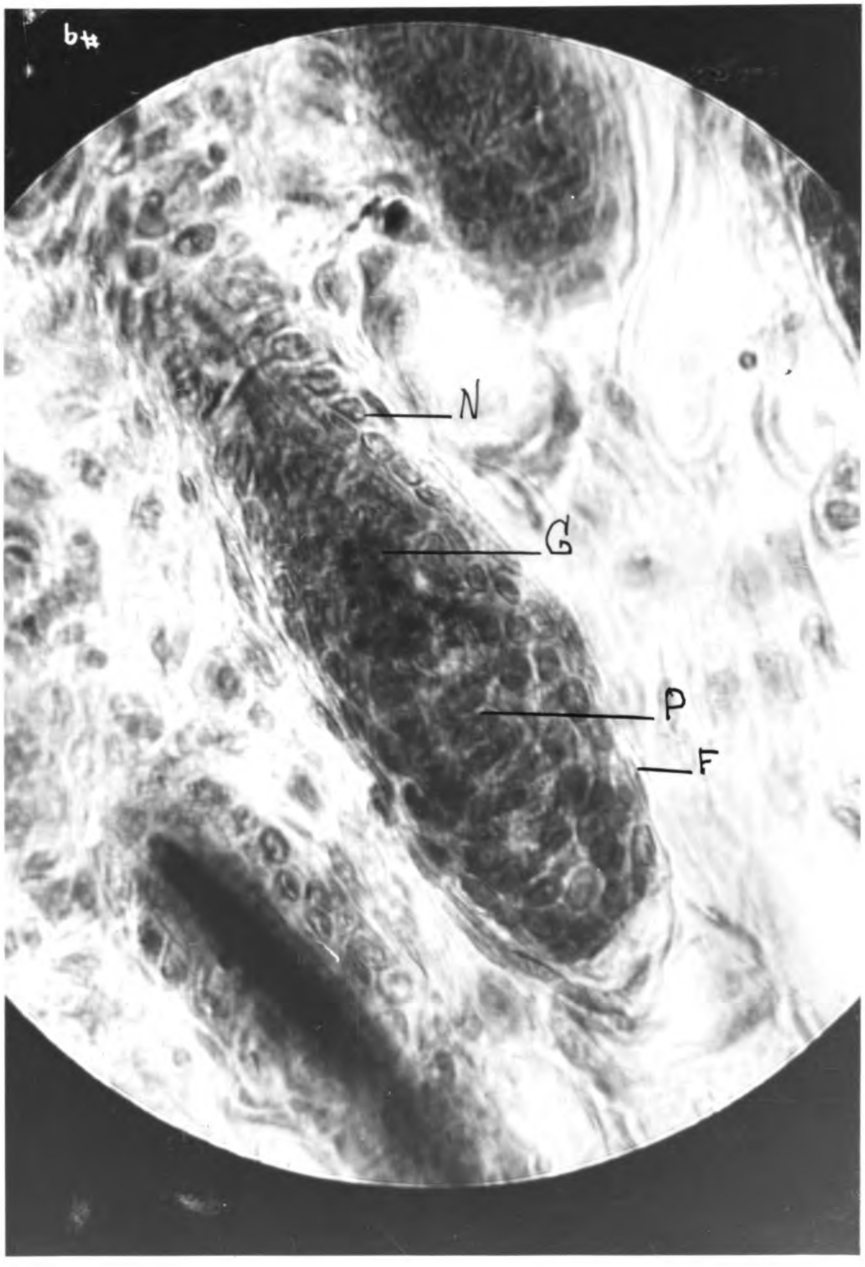


PLATE E

Black and Tan 2 day

160A 19.5/74.7 X 600

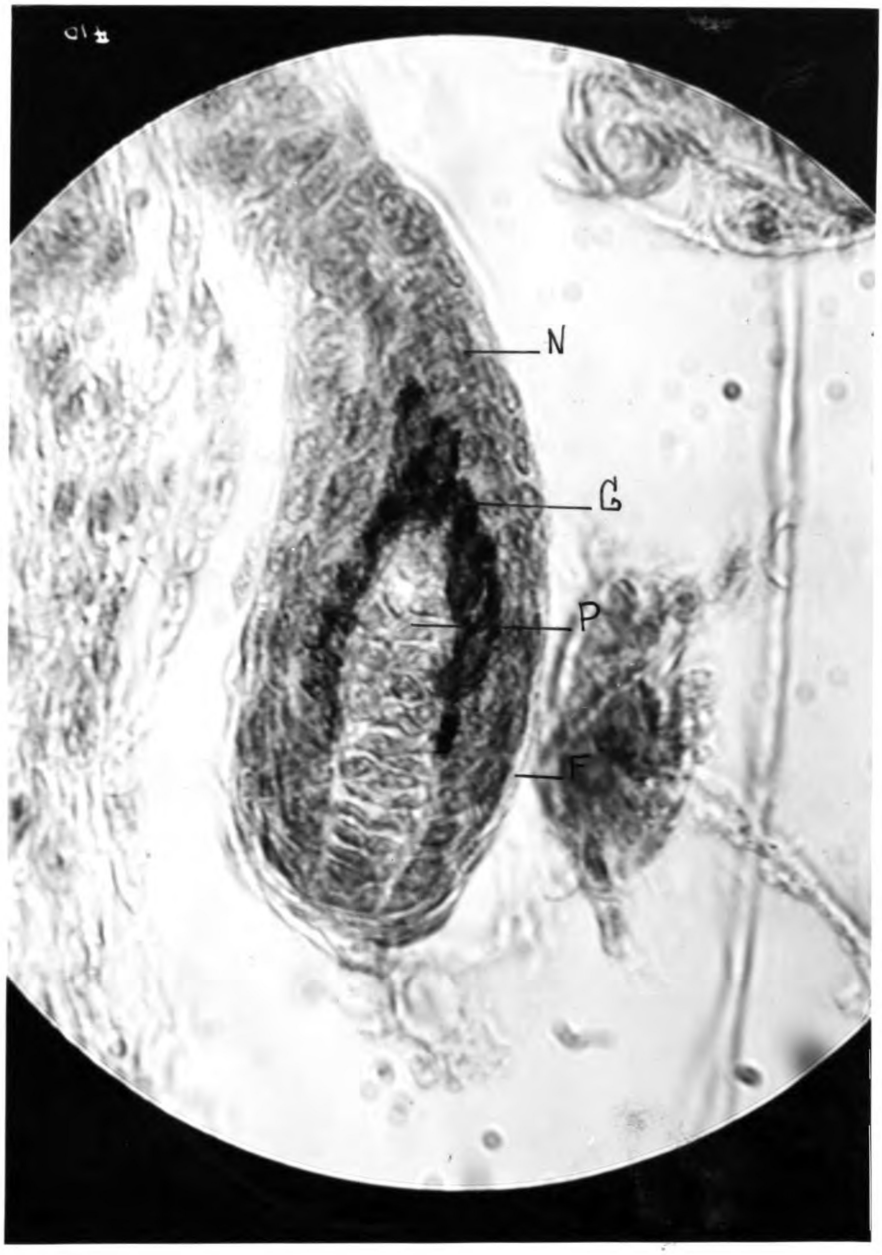


PLATE F

Black and Tan 6 days

113 19/64.5 X 600

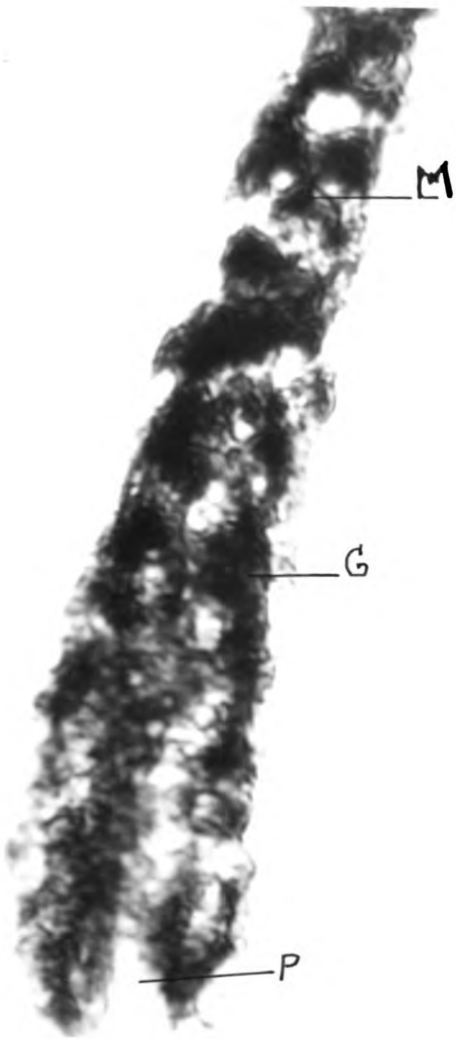


PLATE G

Black and Tan 6 days

113 20.5/80 X 600

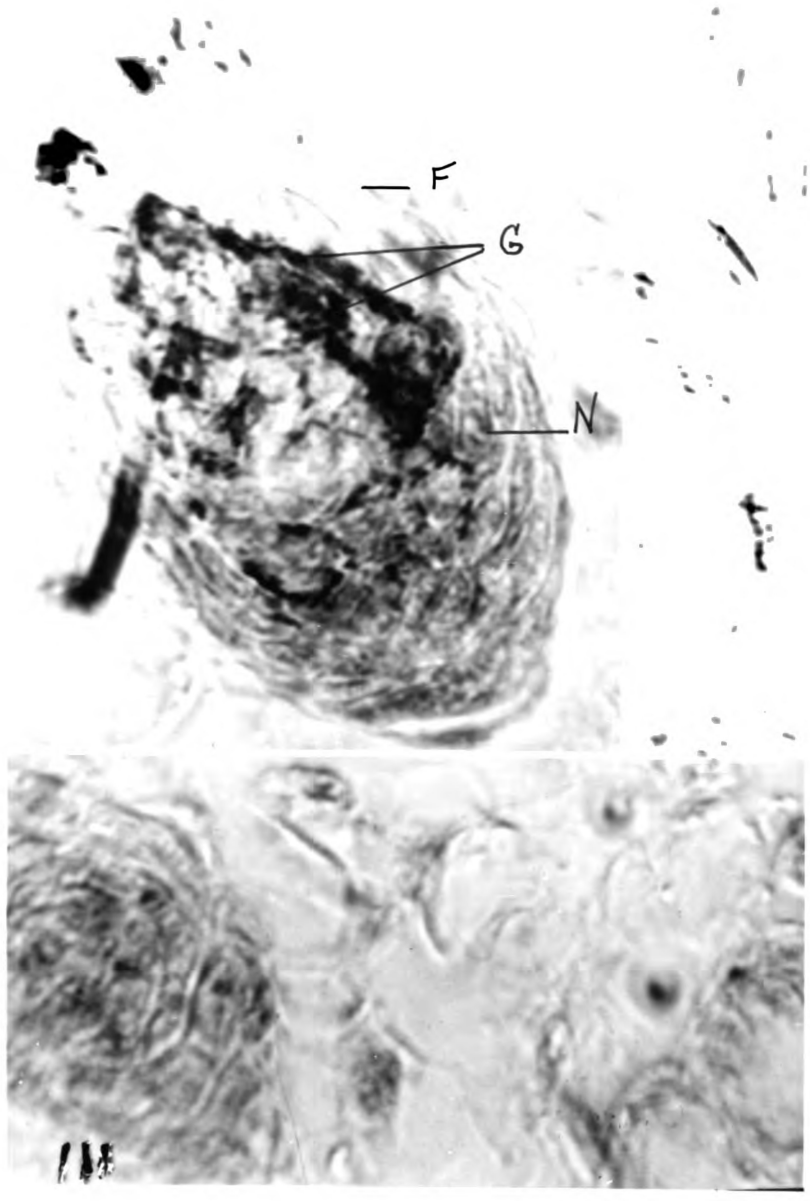


PLATE H

Dark-eyed Black Agouti Birth

127A 20/78.5 X 600

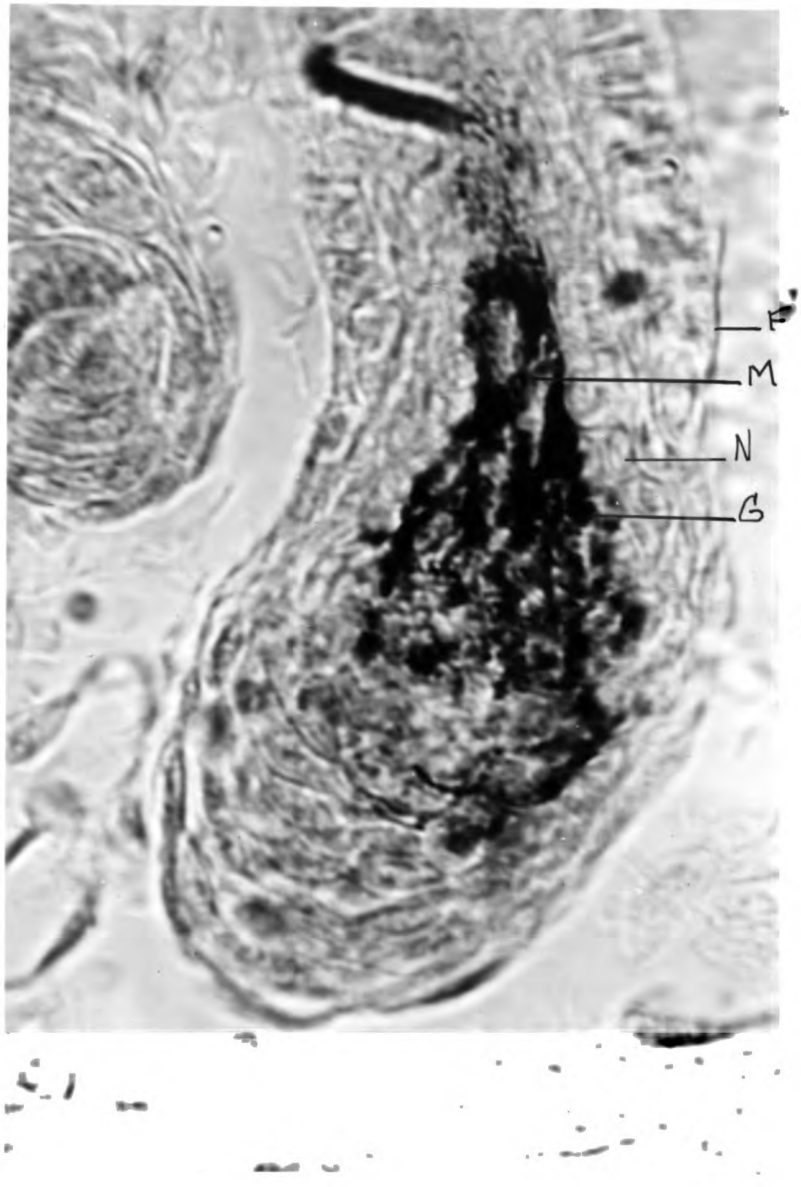


PLATE I

Dark-eyed Black Agouti Birth

127A 20.5/78.7 X 600

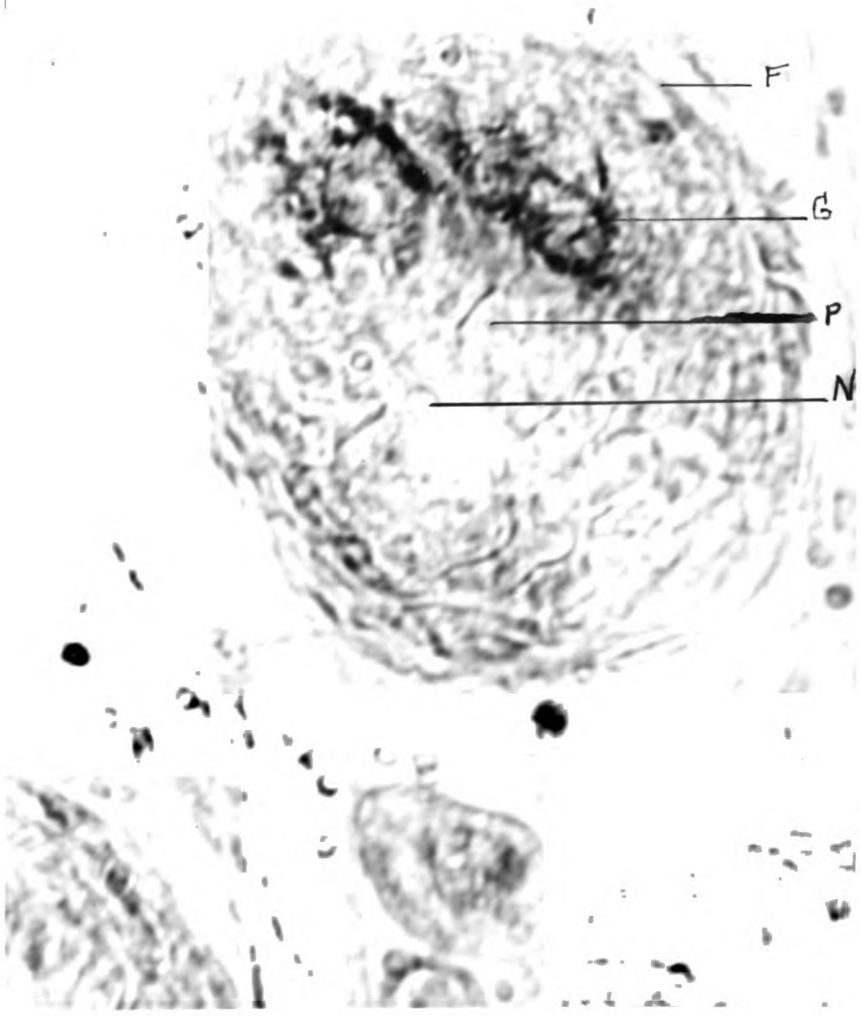


PLATE J

Dark-eyed Black Agouti Birth

211s 14/56 X 600

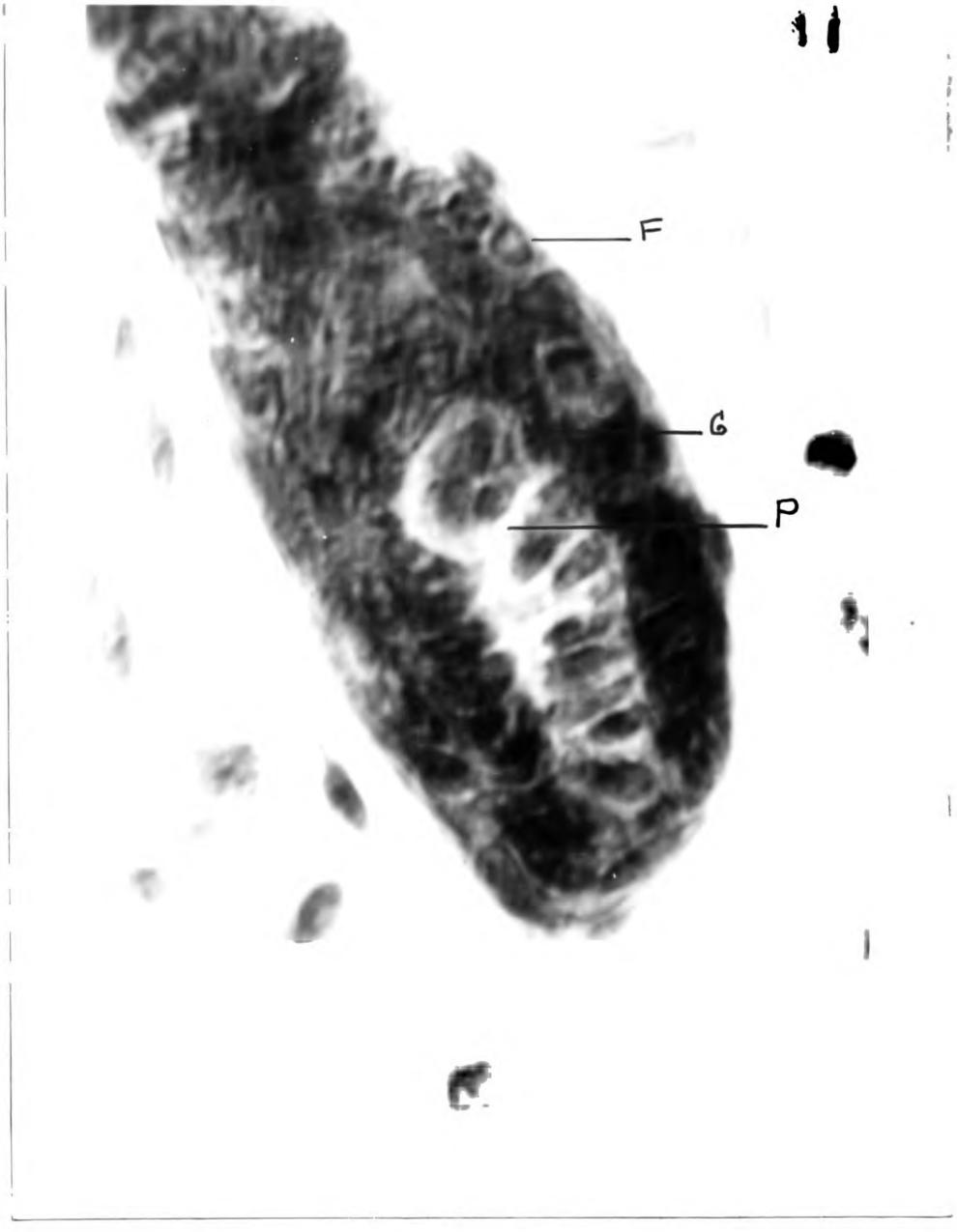


PLATE K

Light-bellied Agouti **Birth**

220A 23.5/45.7 X 600

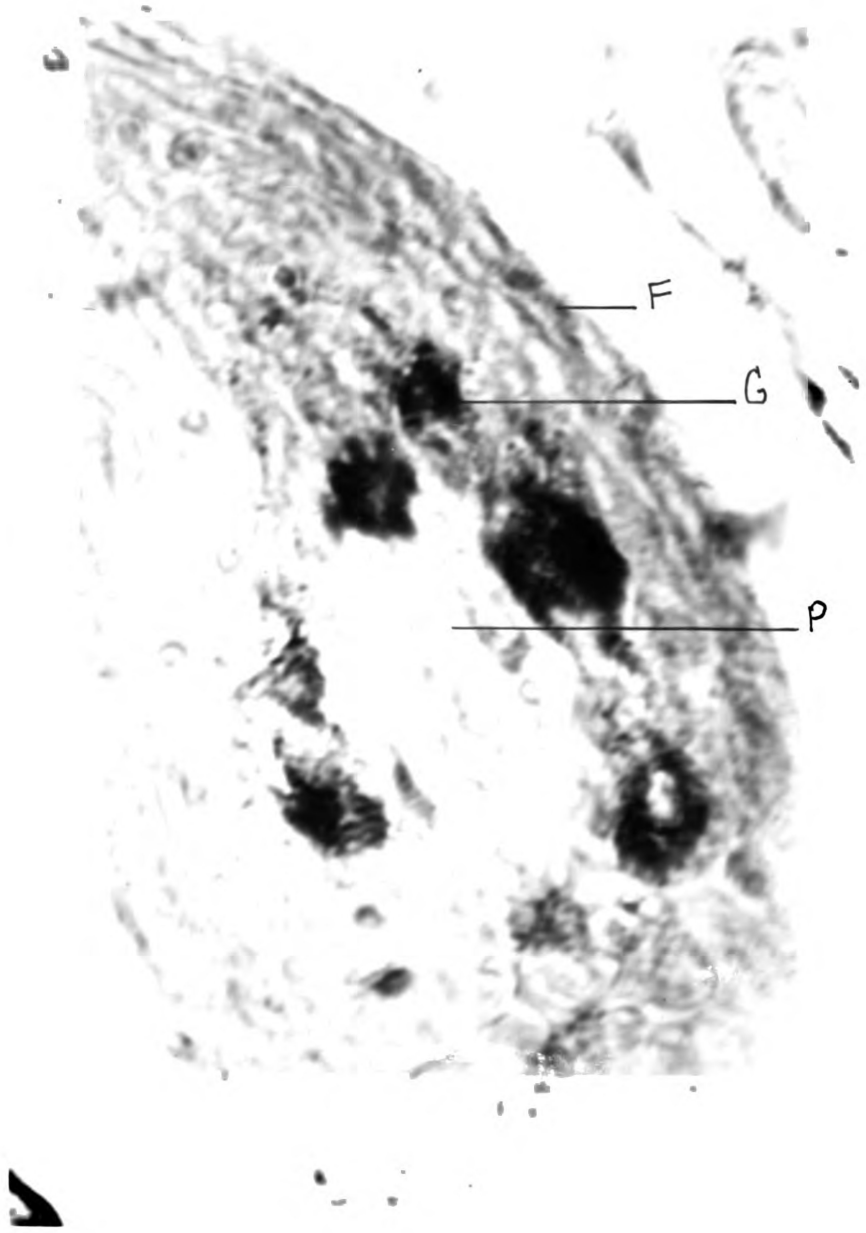


PLATE L

Dilute Brown 1 day

228S 19/52 X 600

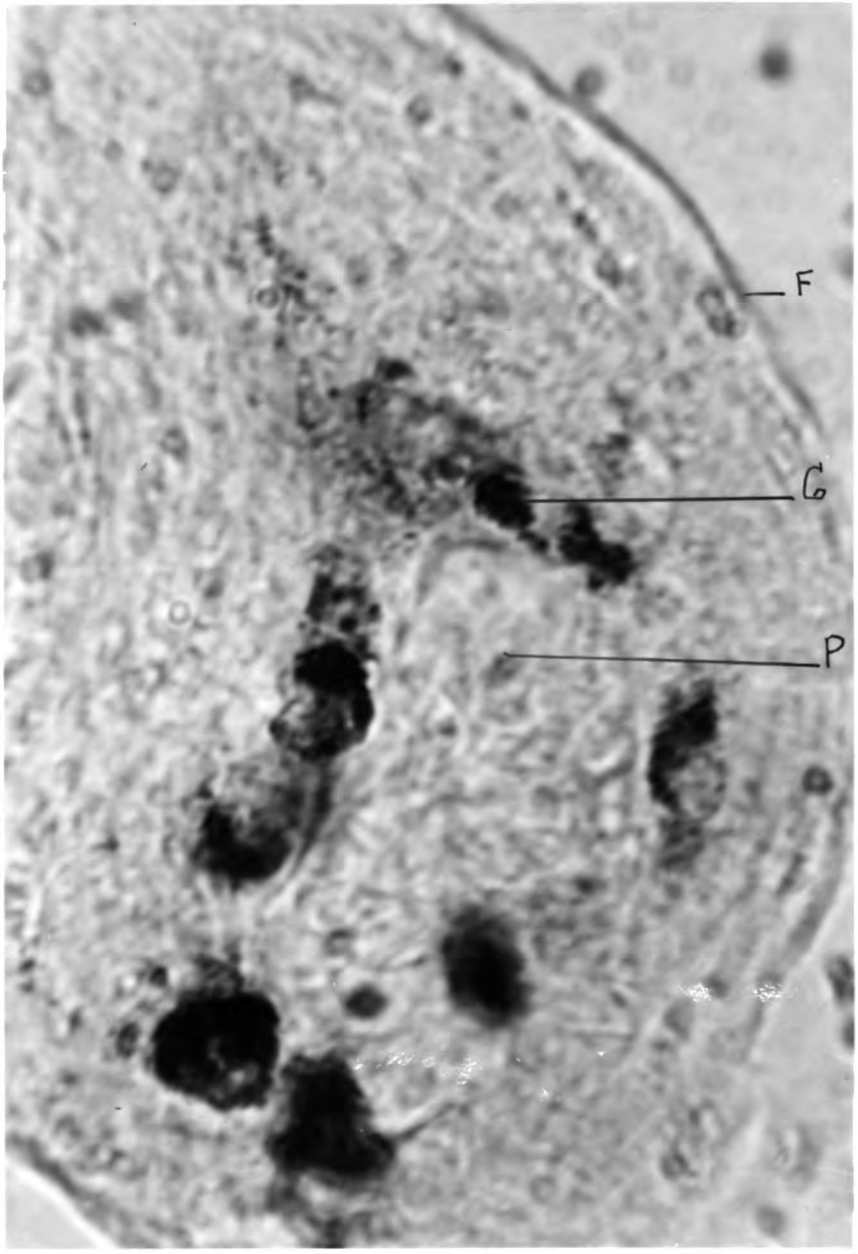


PLATE M

Dilute Brown 1 day

131C 14/69.5 X 600

PLATE N

Dark-eyed Black Agouti 2 days

152B 14.5/63 X 600

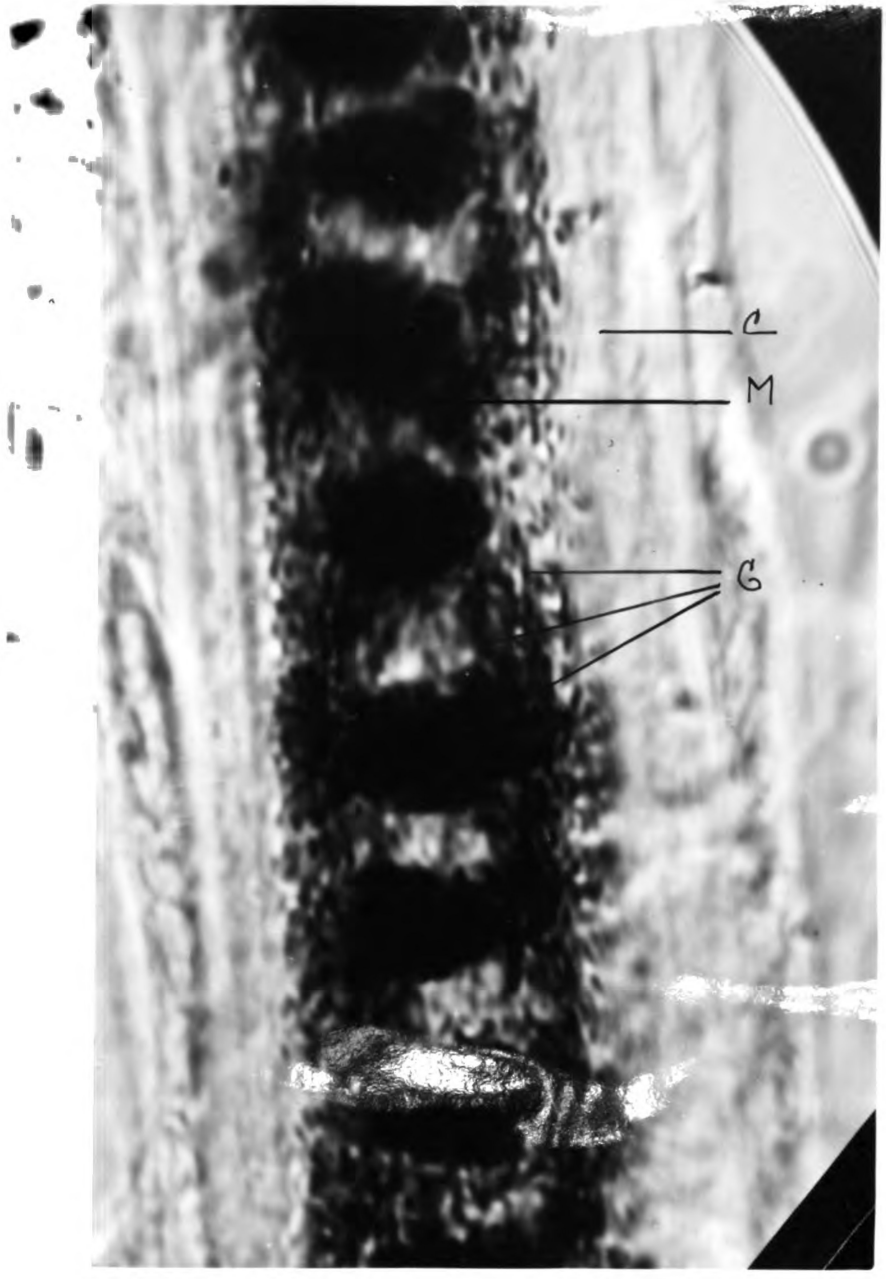


PLATE O

Mature Hair Enlargement

Chocolate Stock

KEY TO

DRAWINGS

Cb = cell boundary

Ch = Chromatin

Cy = Cytoplasm

F = Follicle

G = Pigment granules

N = Nuclei

Nu = Nucleolus

P = Papilla

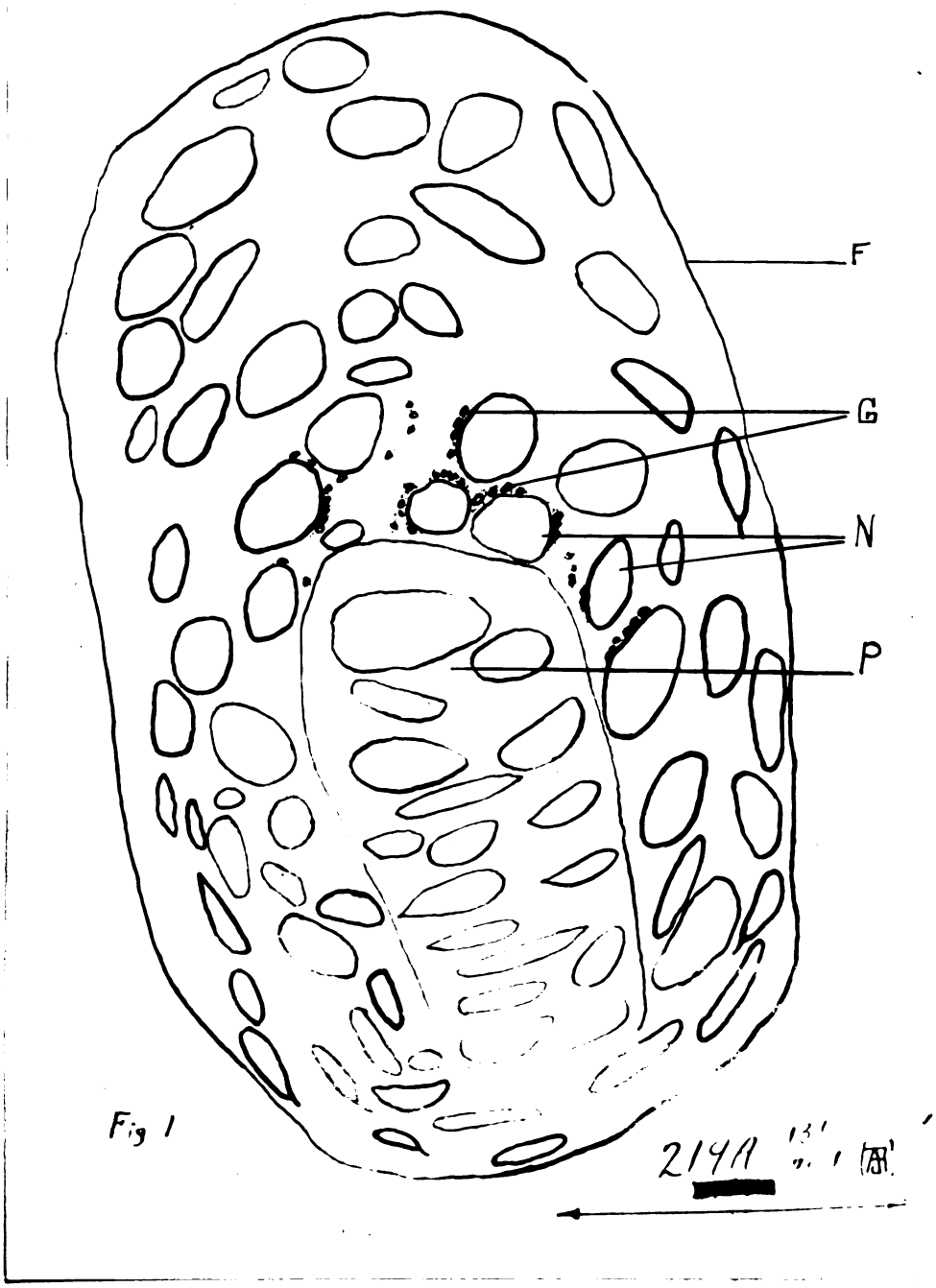
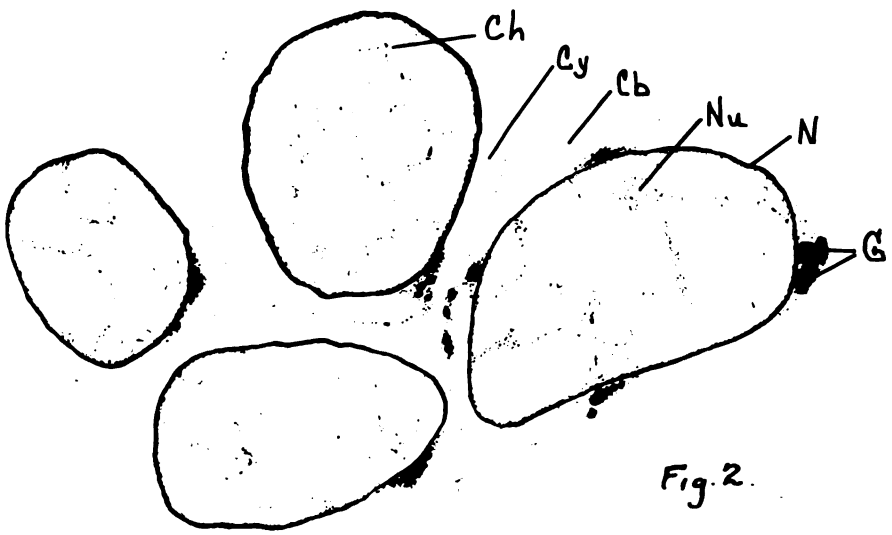


FIGURE 1

Black and Tan Birth

General View X 600



217 B 4.1 / 53.5

FIGURE 2

Black and Tan Birth

Enlargement X 1500

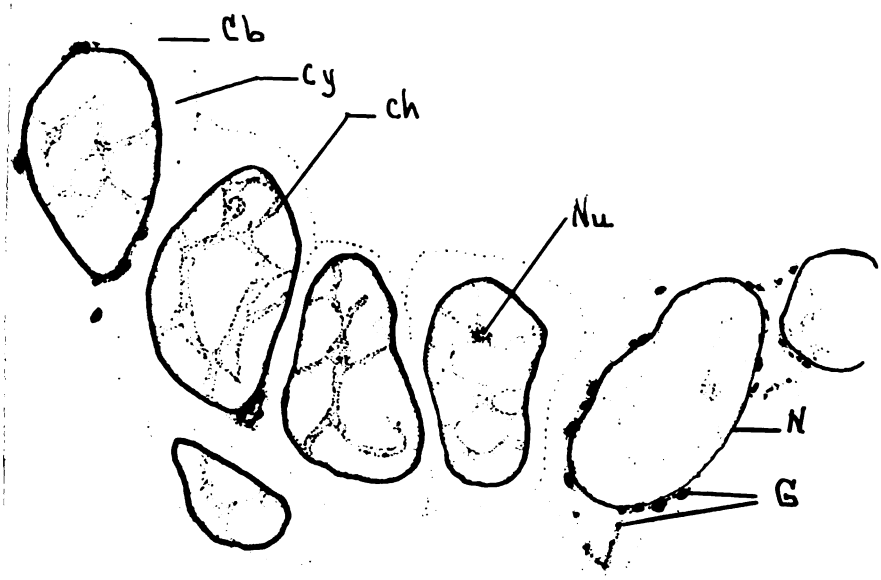


Fig. 4

150 C 3.9/104 (AR)

FIGURE 4

Black and Tan 1 day

Enlargement X 1500

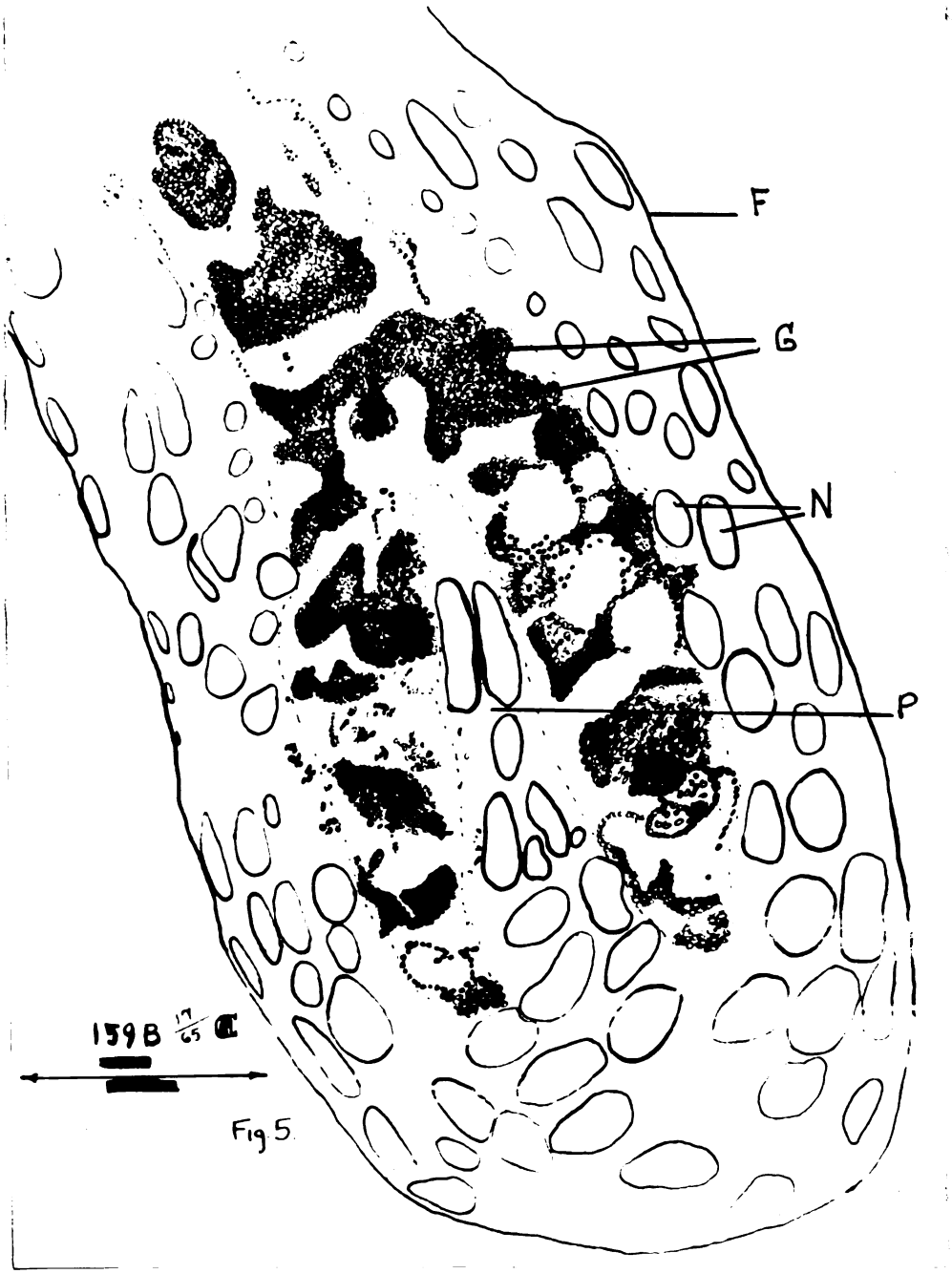


FIGURE 5

Black and Tan 2 days

General View X 600

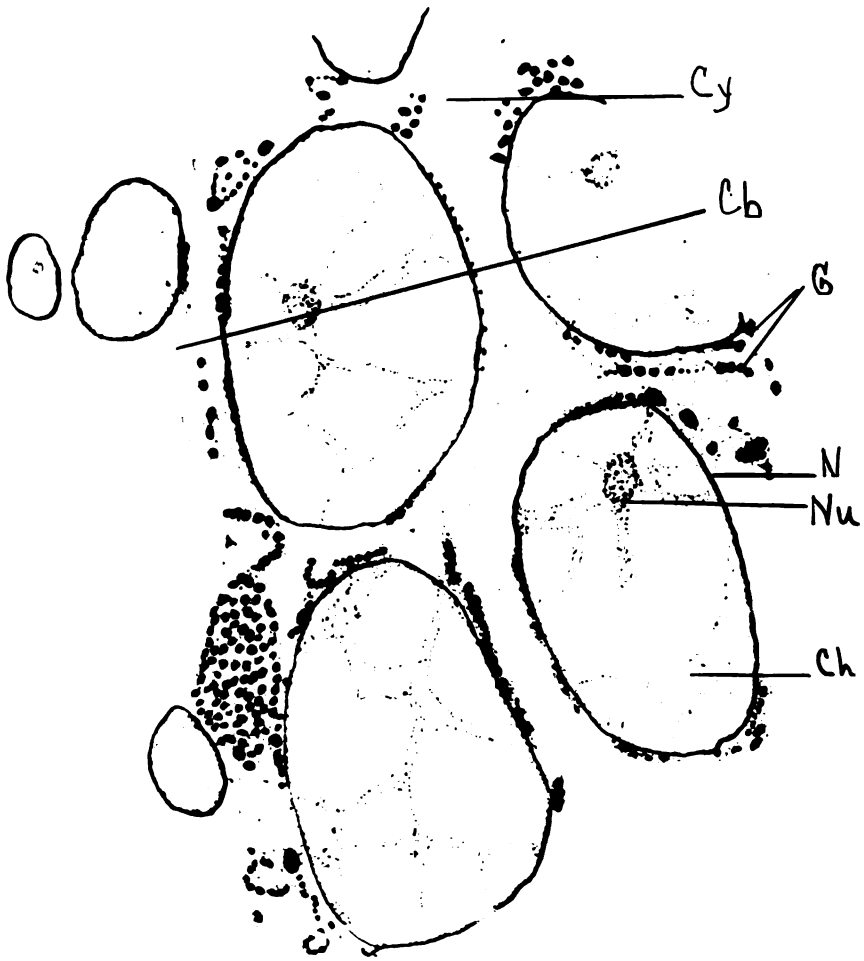


Fig. 6.

1578

FIGURE 6

Black and Tan 2 days

Enlargement X 1500

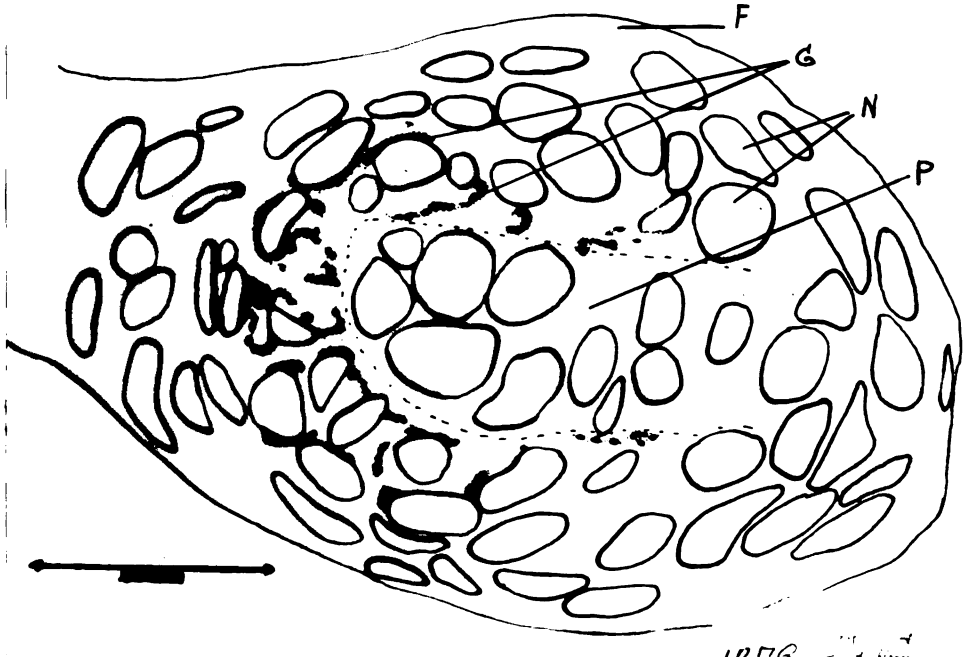


Fig. 7.

1276

FIGURE 7

Dark-eyed Black Agouti Birth

General View X 600

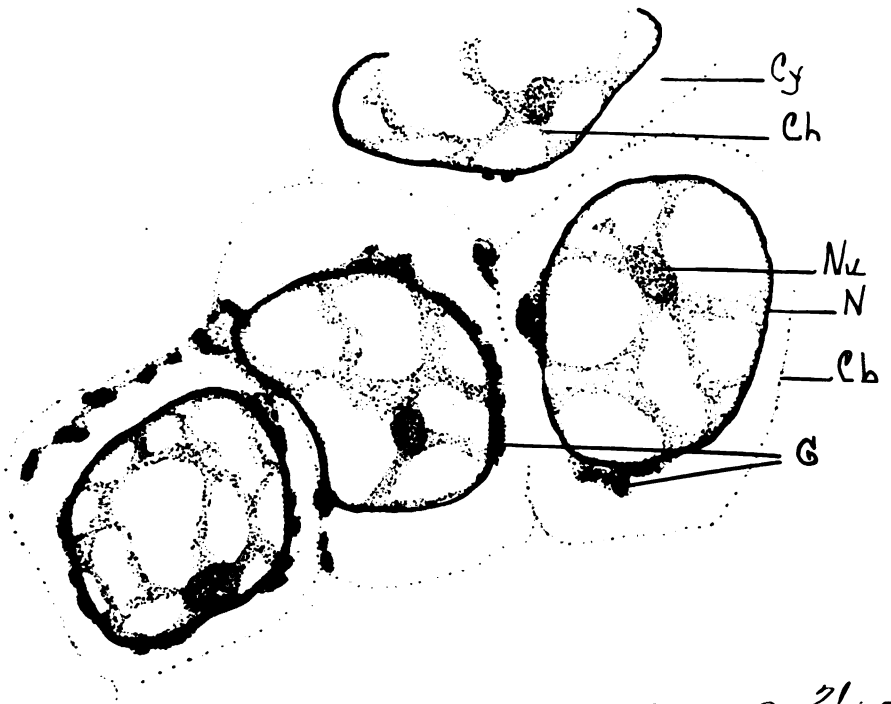


Fig. 8

127β 21/16.1

FIGURE 8

Dark-eyed Black Agouti Birth

Enlargement X 1500

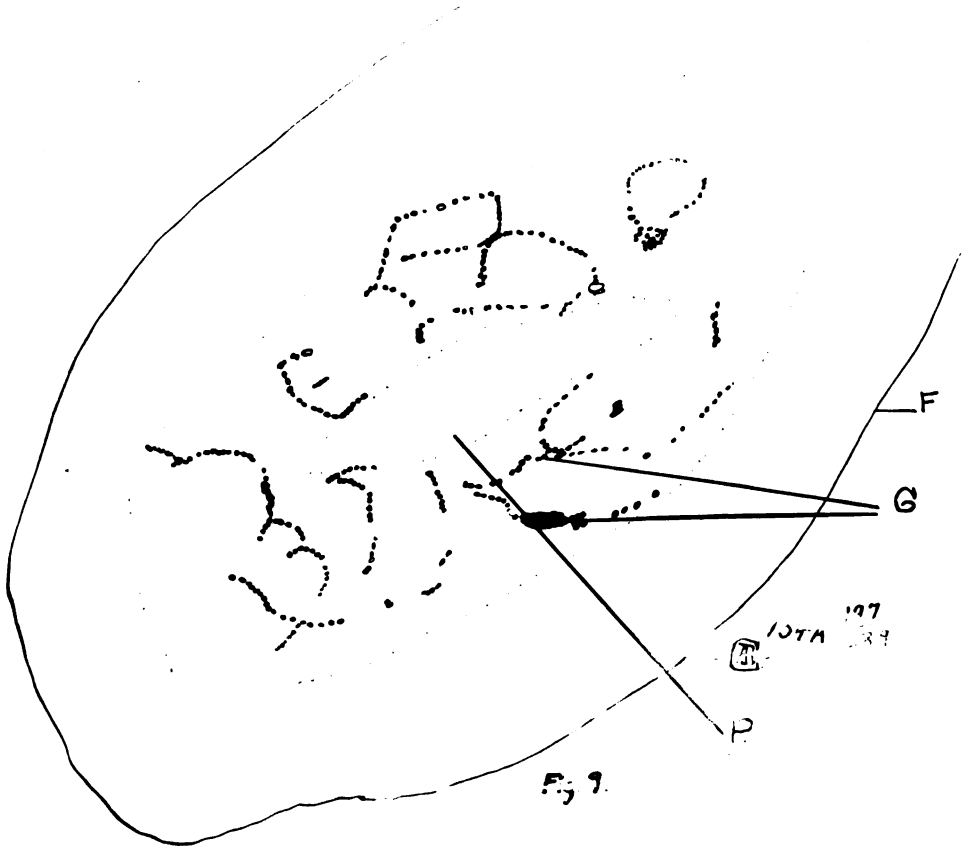


FIGURE 9

Dark-eyed Black Agouti 1 day

General View Unstained X 600

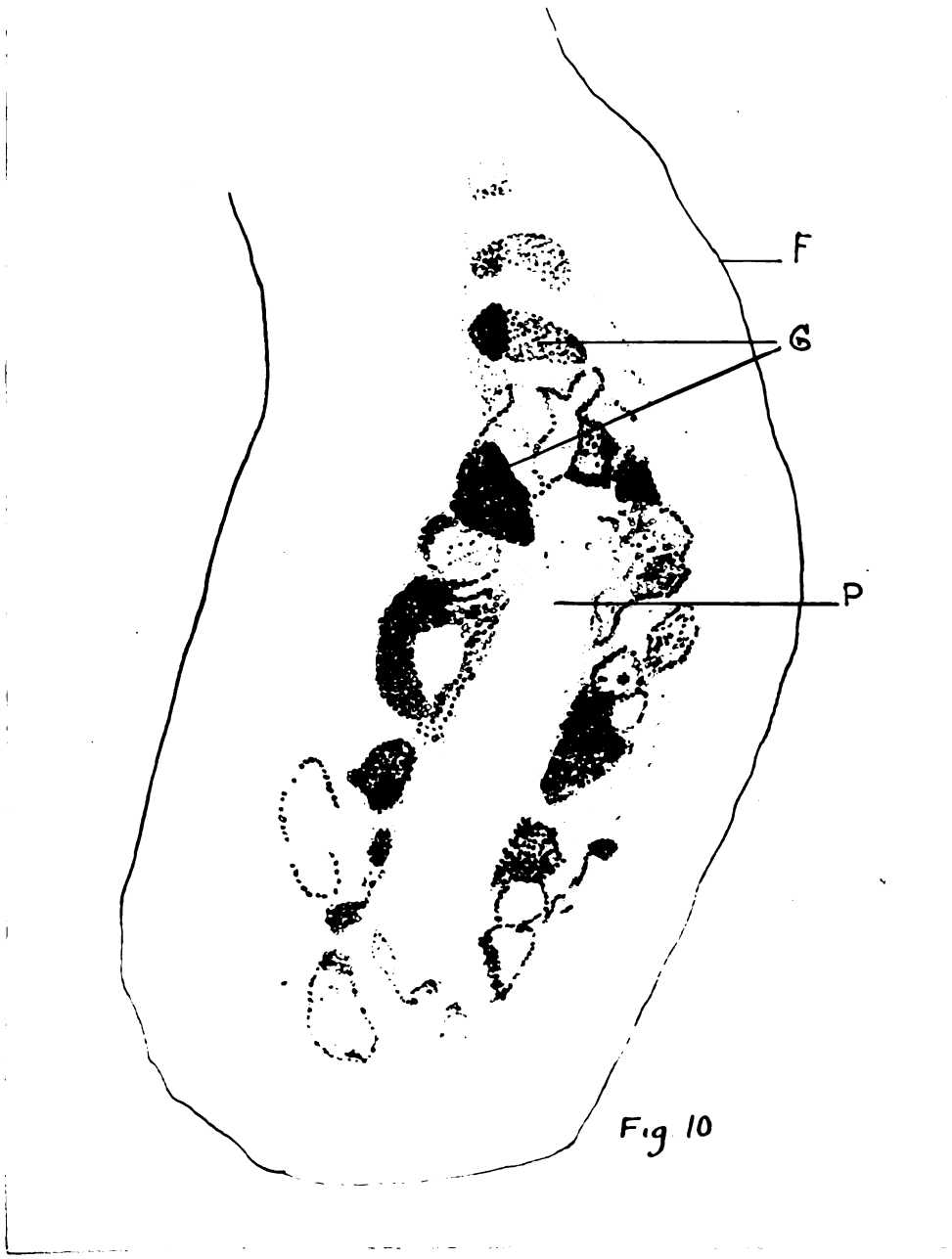


FIGURE 10

Dark-eyed Black Agouti 1 day

General View Unstained X 600

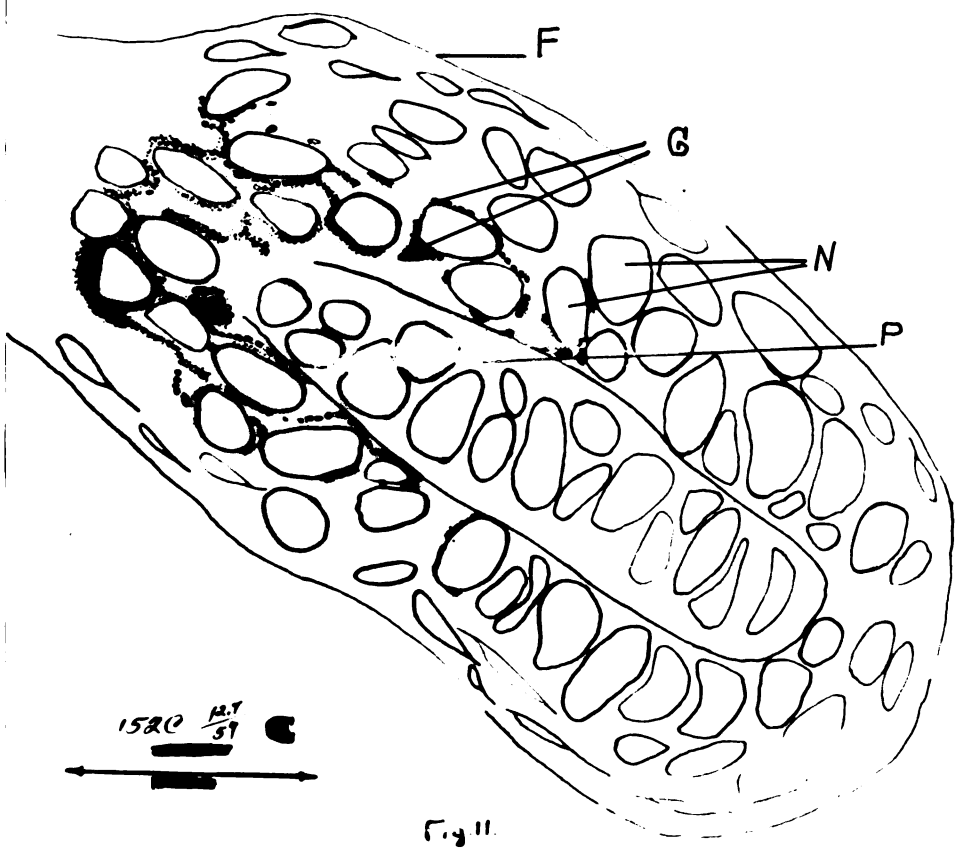


FIGURE 11

Dark-eyed Black Agouti 2 days

General view X 600

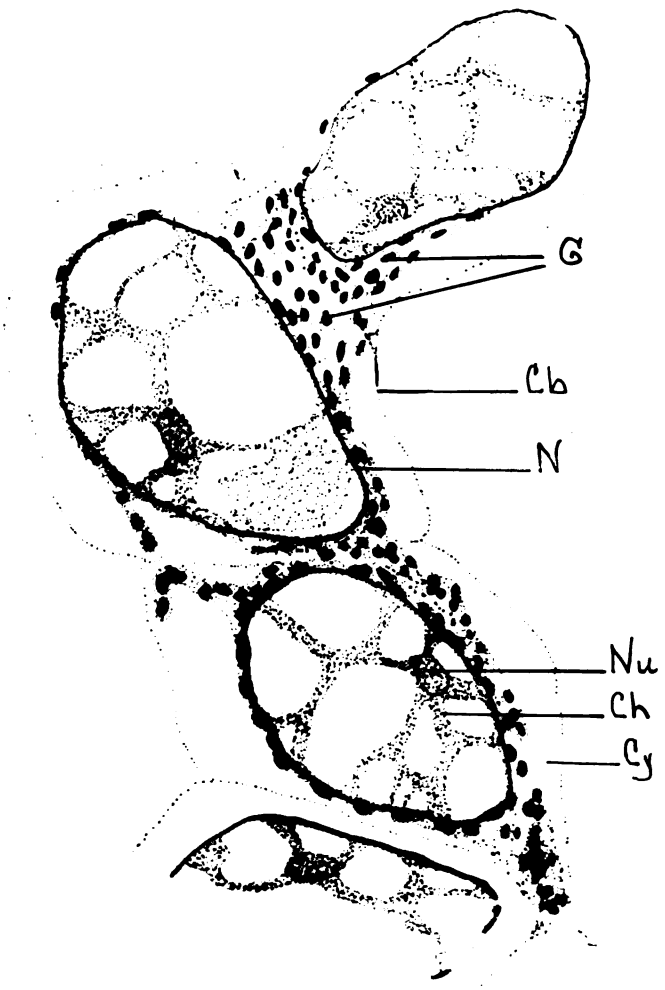


Fig. 12. E. 153 C 12?/73.8

FIGURE 12

Dark-eyed Black Agouti 2 days

Enlargement X 1500

FIGURE 13

Light-bellied Agouti Birth

General View X 600

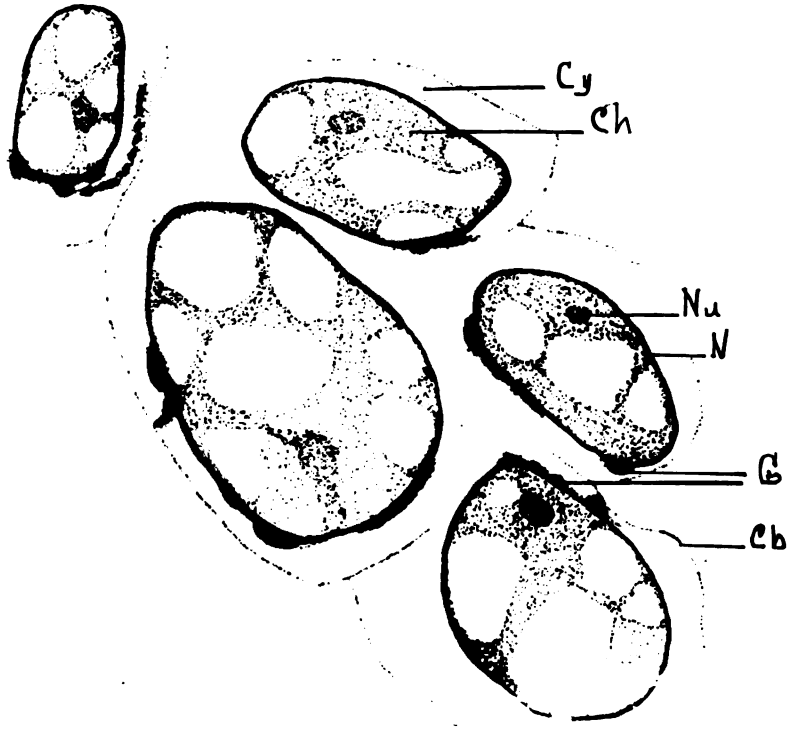


Fig. 14

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FIGURE 14

Light-bellied Agouti Birth

Enlargement X 1500

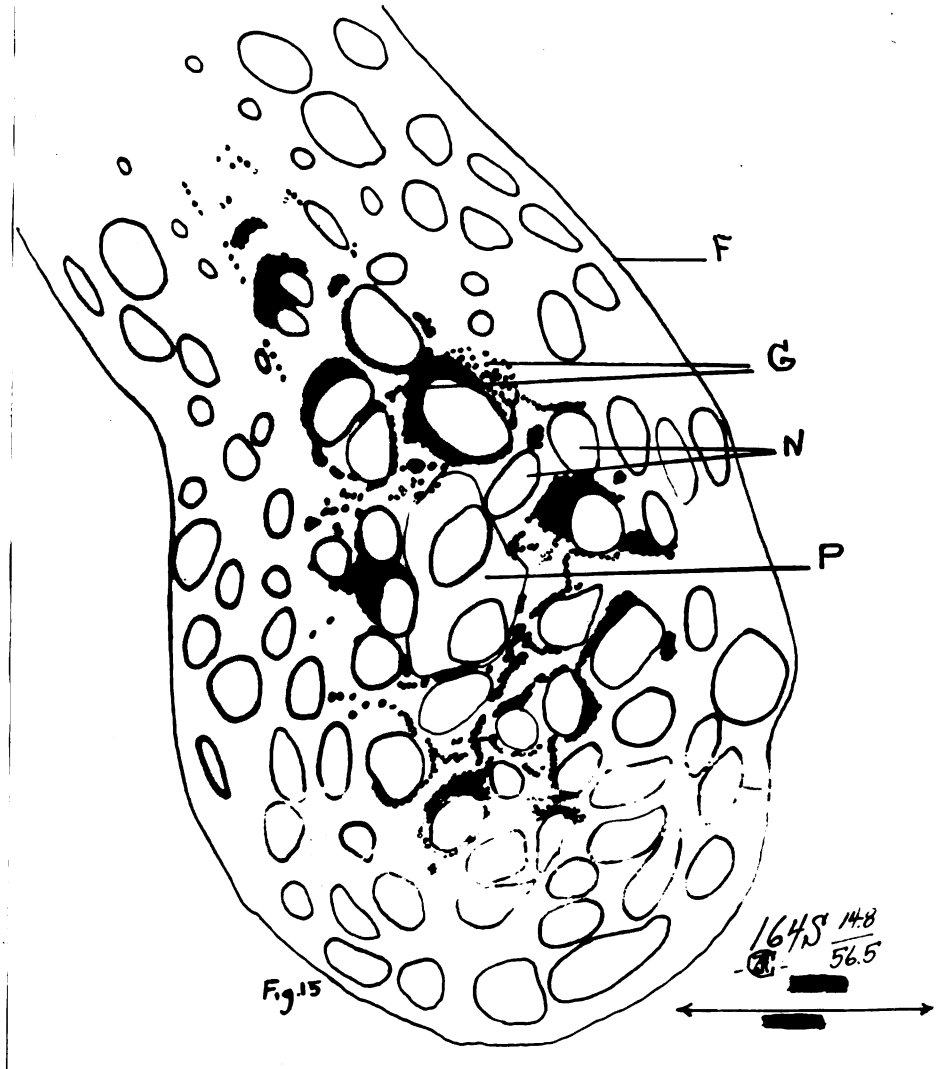

FIGURE 15

Light-bellied Agouti 1 day

General View X 600

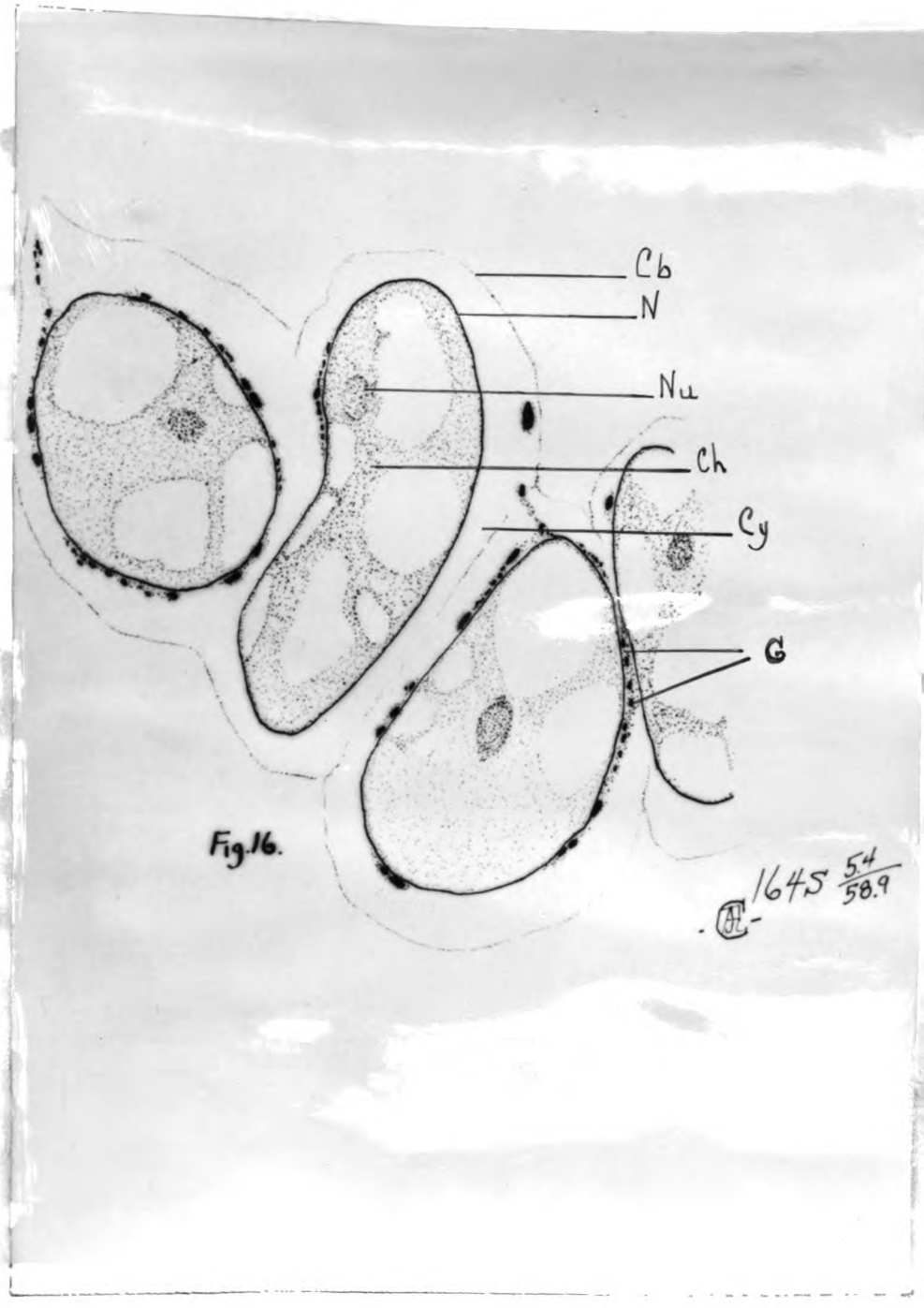

FIGURE 16

Light-bellied Agouti 1 day

Enlargement X 1500

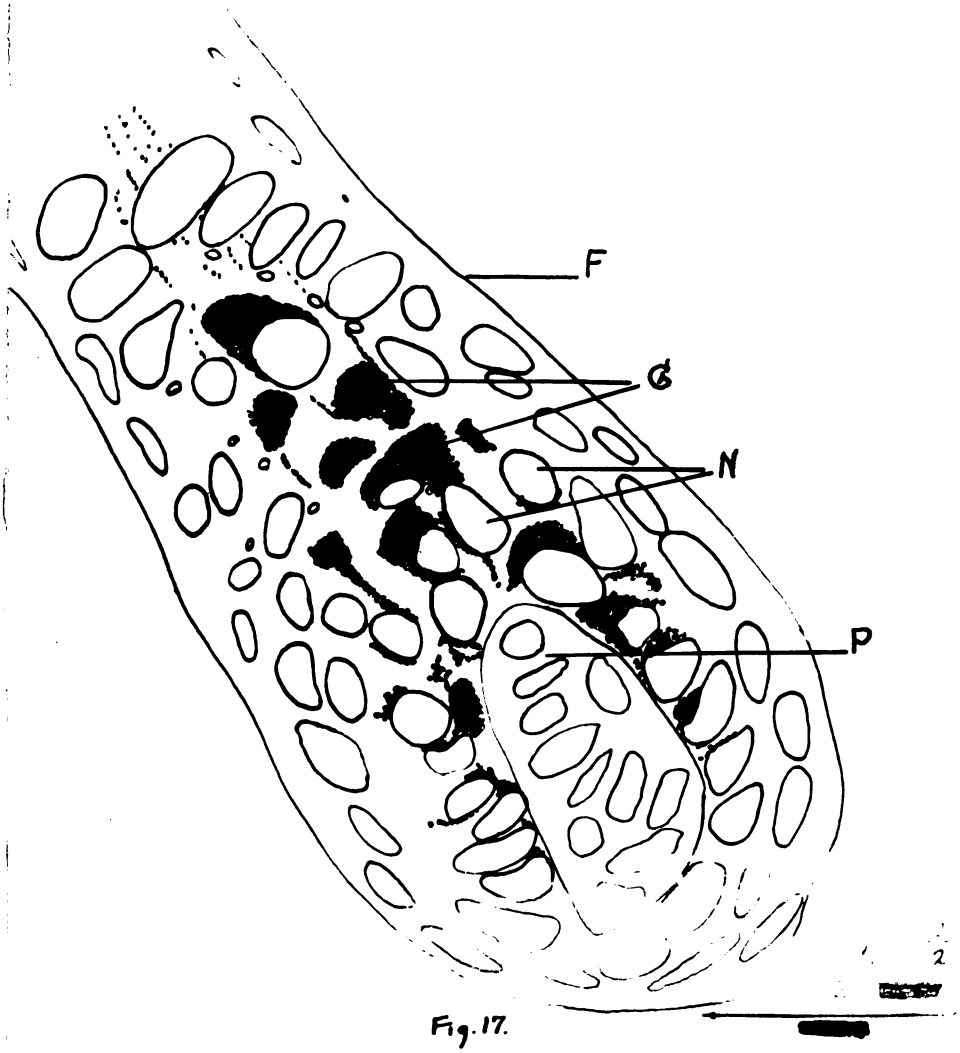

FIGURE 17

Light-bellied Agouti 2 days

General View X 600

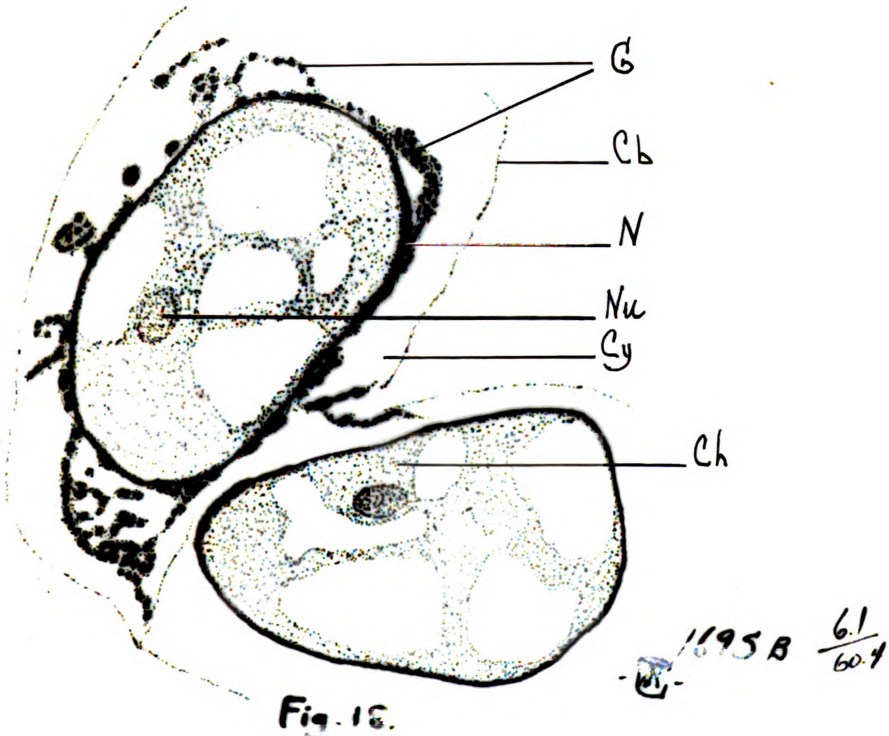

FIGURE 18

Light-bellied Agouti 2 days

Enlargement X 1500

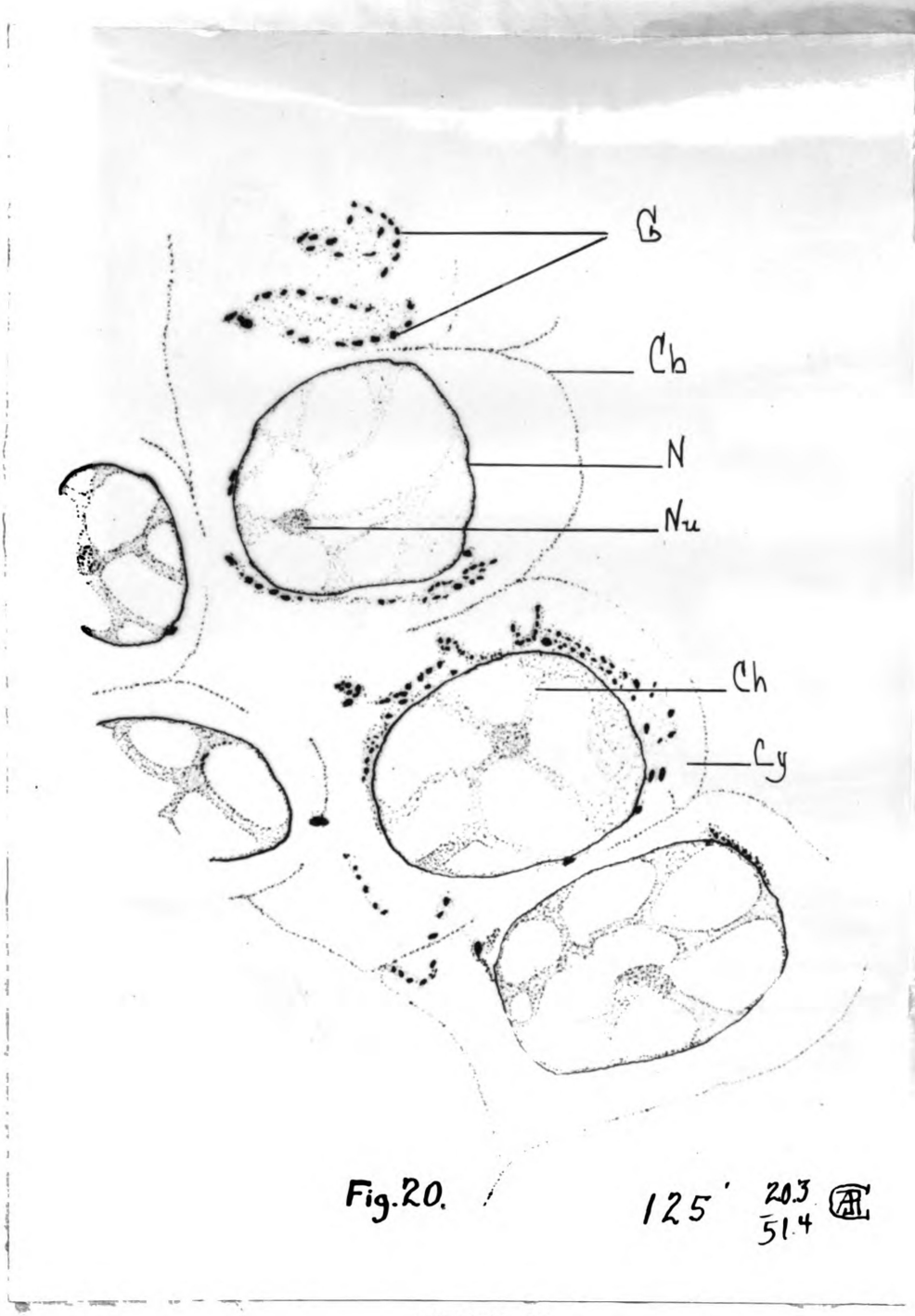

FIGURE 20

Dilute Brown Birth

Enlargement X 1500

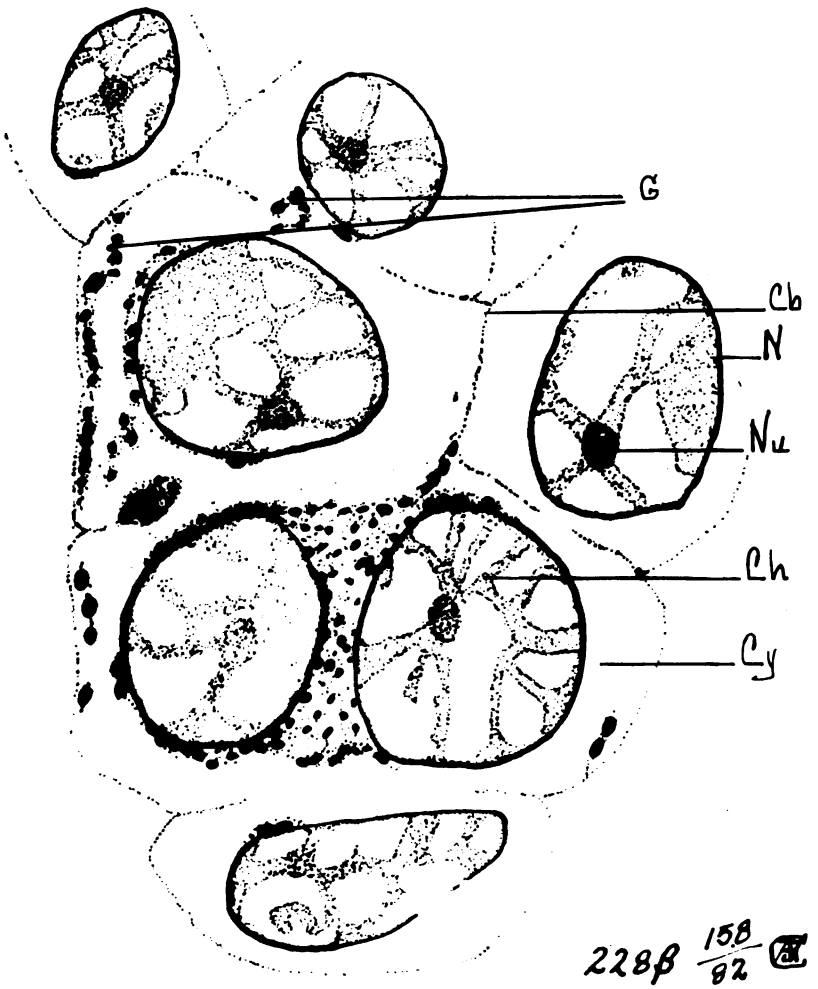

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Fig. 22.

FIGURE 22

Dilute Brown 1 day

Enlargement X 1500

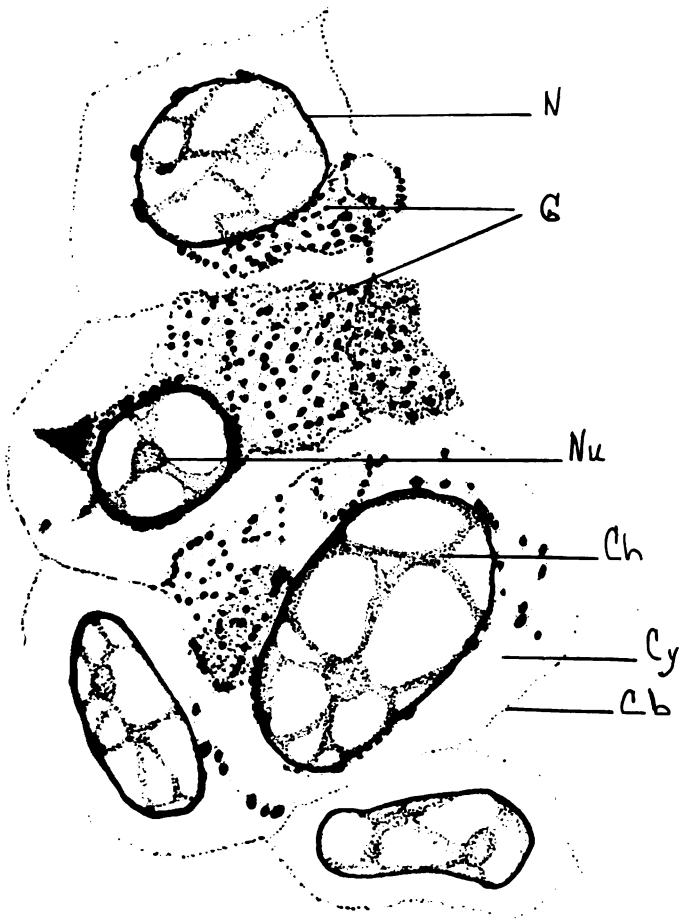

Fig. 24.

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FIGURE 24

Dilute Brown 2 days

Enlargement X 1500

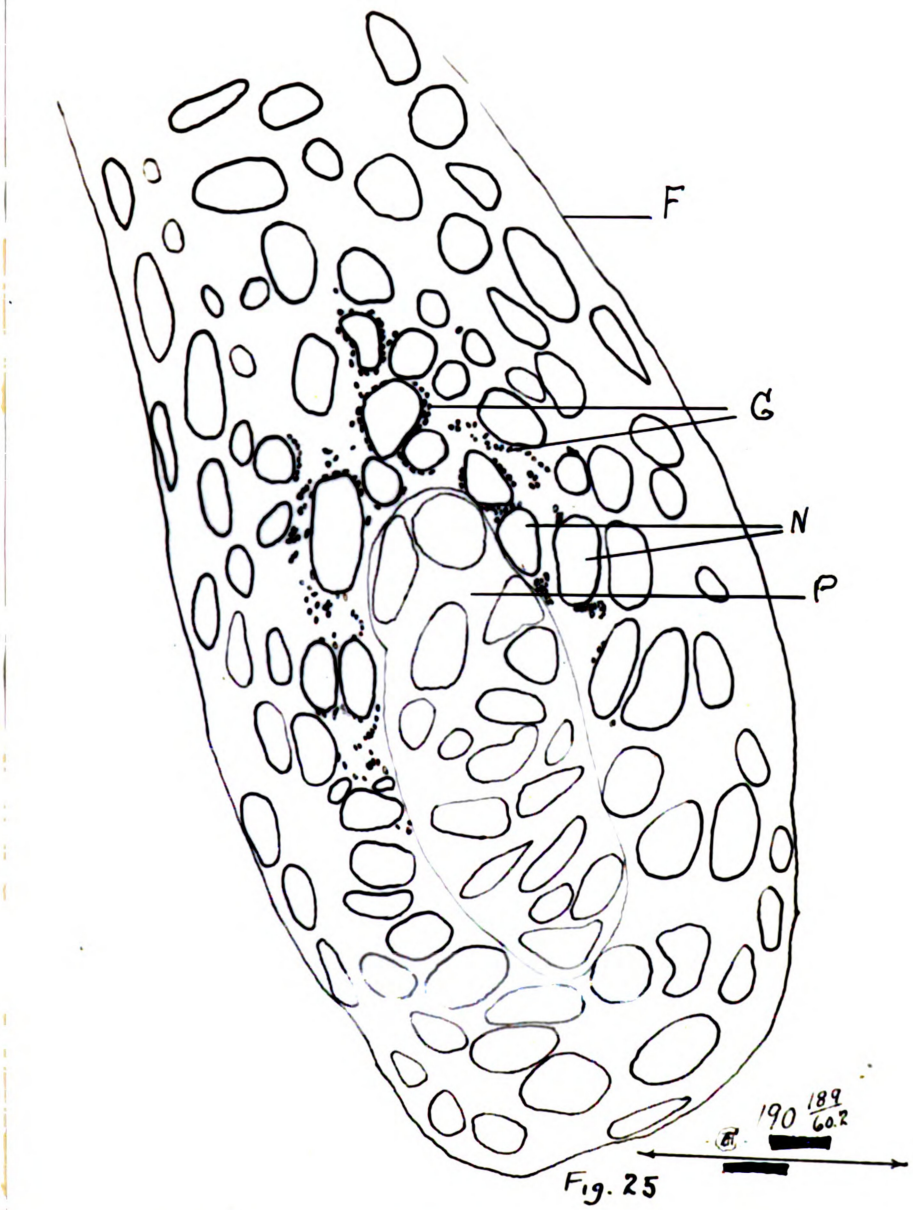

FIGURE 25

Chinchilla 1 day

General View X 600

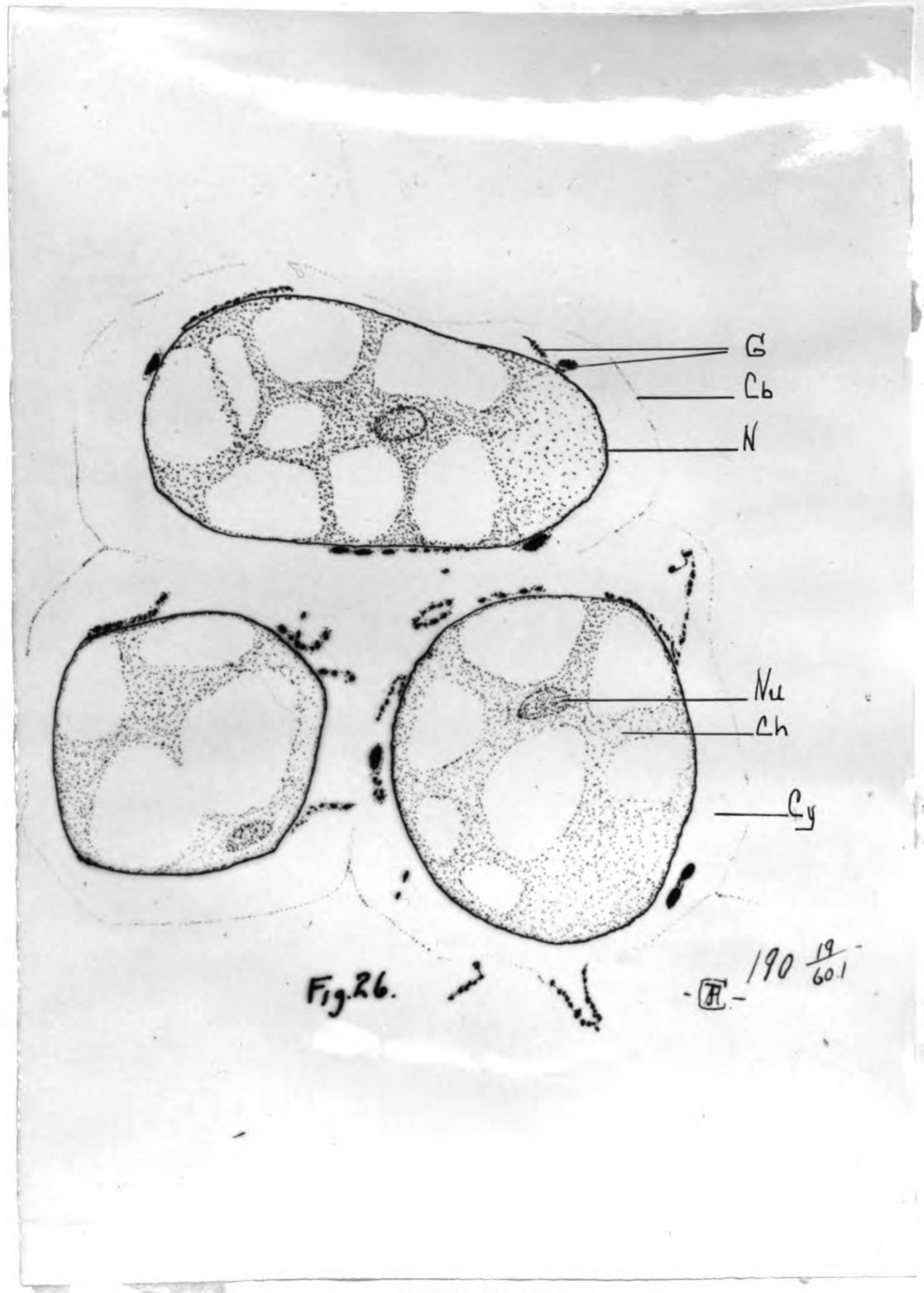

FIGURE 26

Chinchilla **1 day**

Enlargement **X 1500**

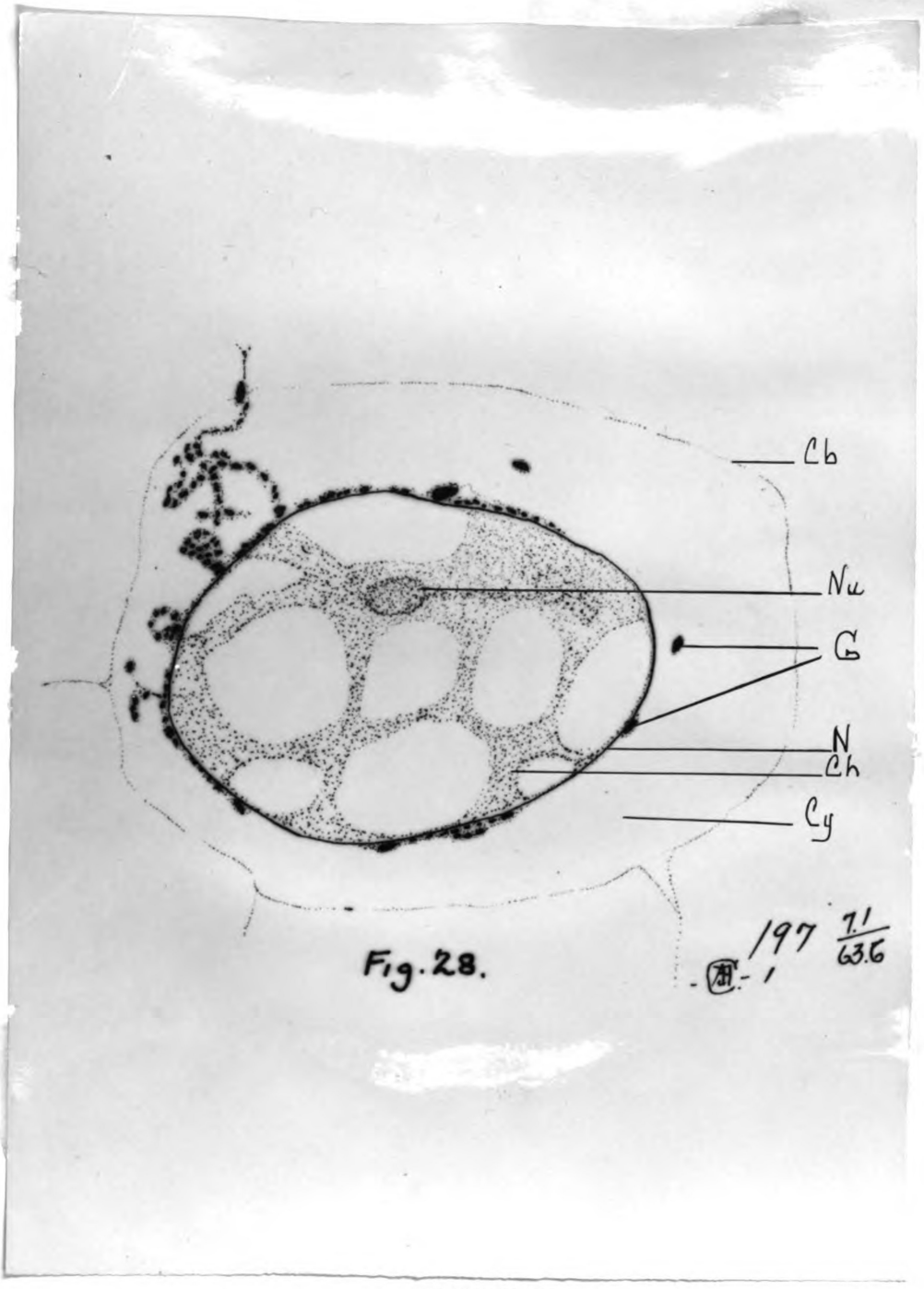

FIGURE 28

Chinchilla 2 days

Enlargement X 1500

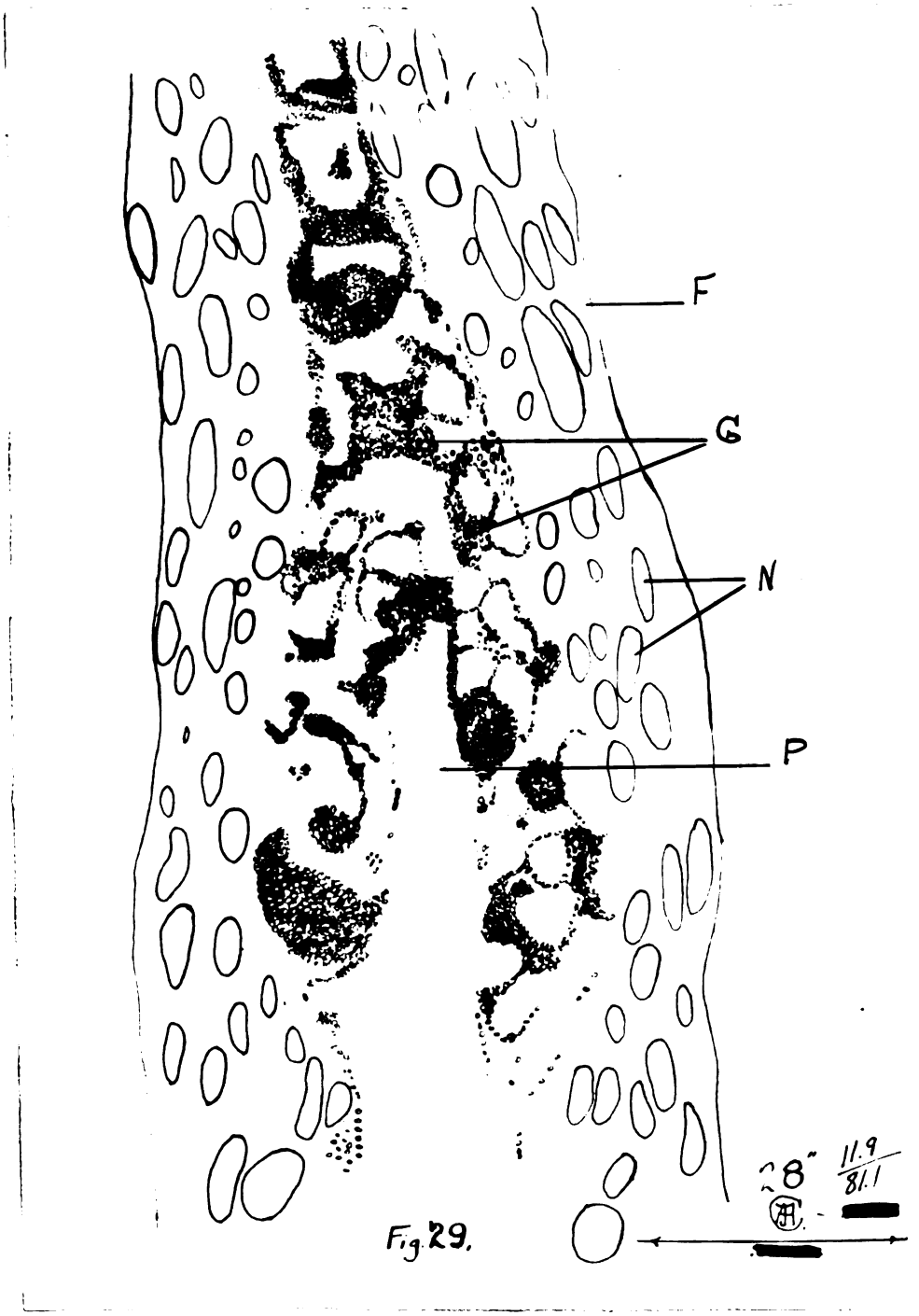

FIGURE 29

Chocolate 2 days

General View X 600

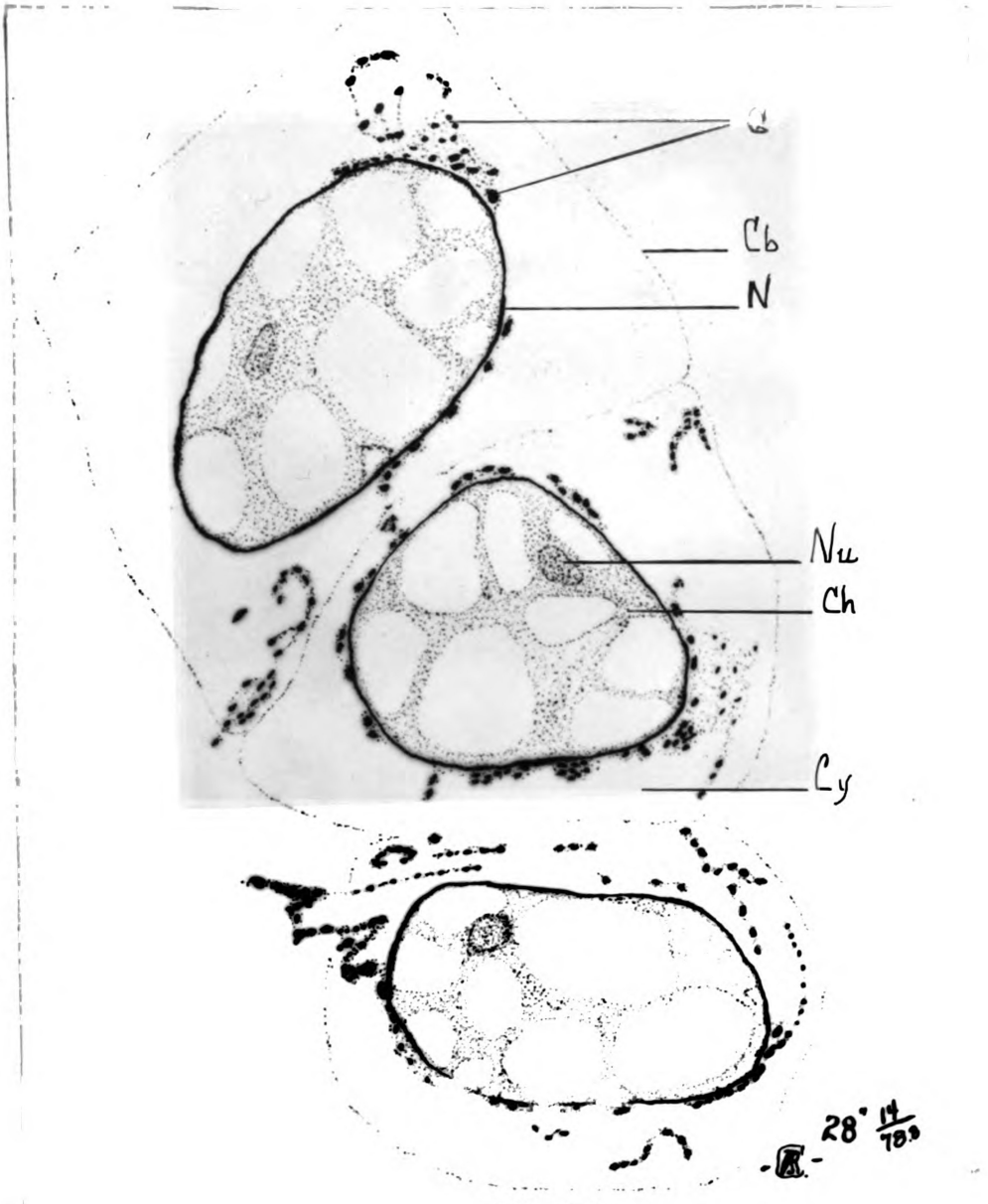

Fig. 30

FIGURE 30

Chocolate 2 days

Enlargement X 1500

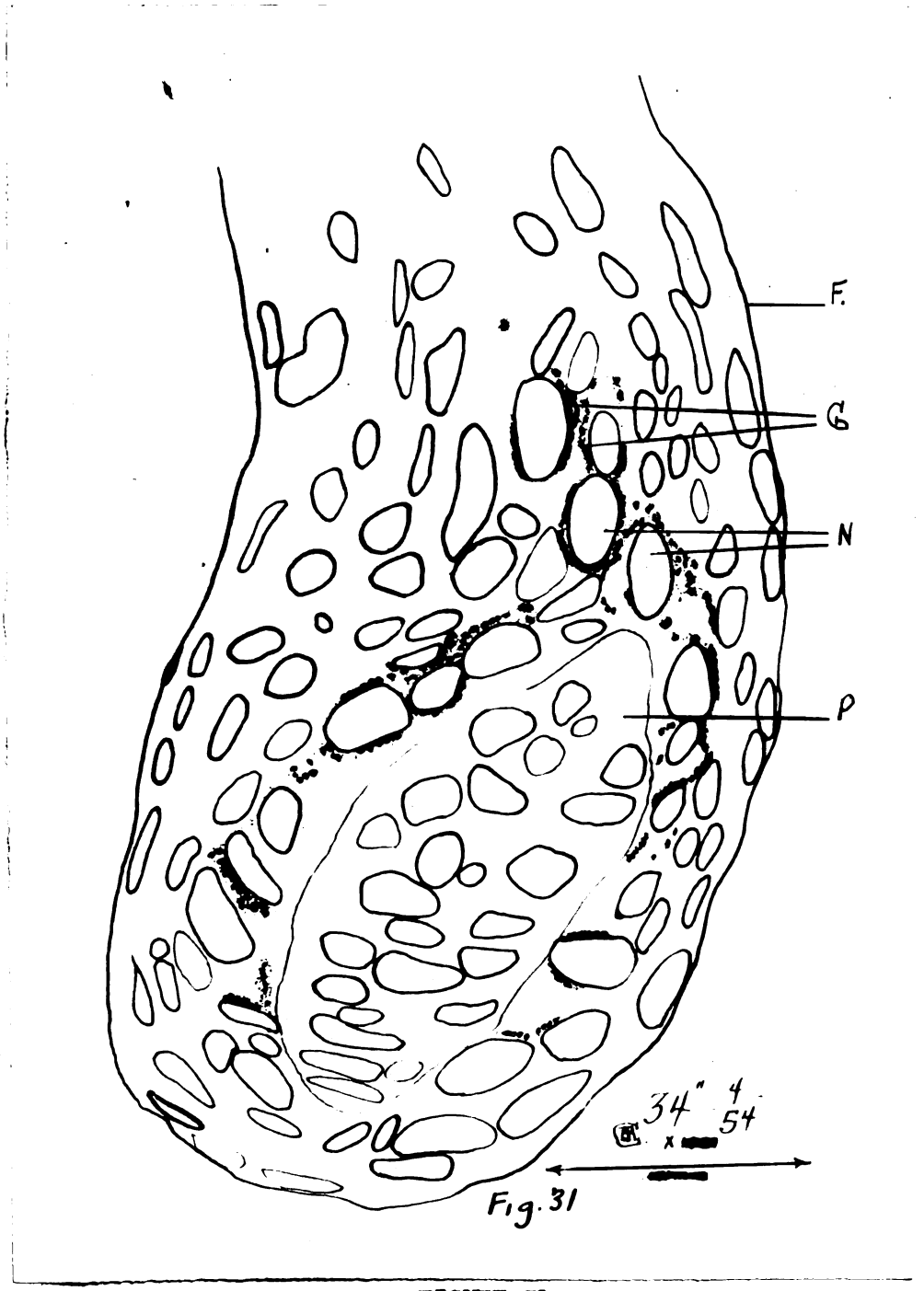

FIGURE 31

Chocolate 1 day

General View X 600

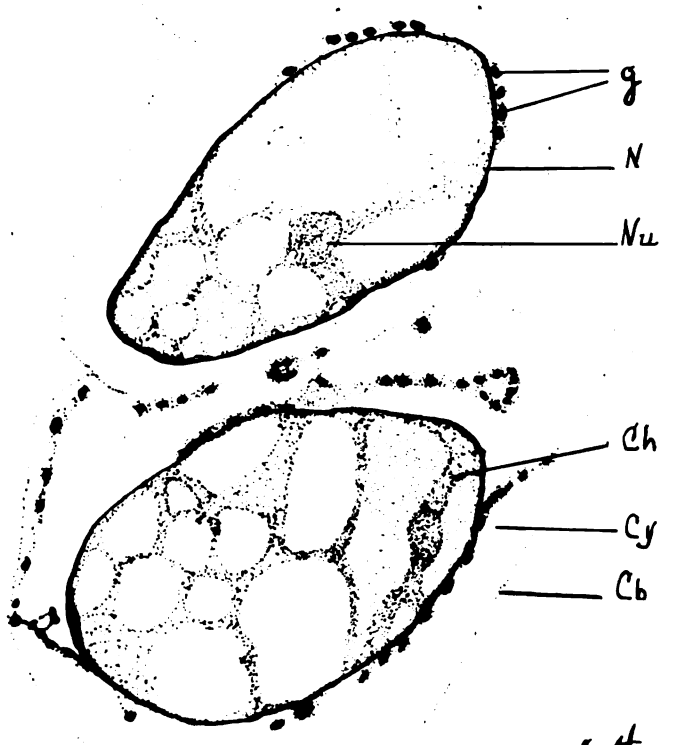

Fig. 32.

FIGURE 32

Chocolate 1 day

Enlargement X 1500