

## SEXUAL ABNORMALITIES IN *Bothrops insularis* (Amaral) 1921

(SERPENTES)

Alphonse Richard Hoge and Hélio Emerson Belluomini

(Laboratório de Ofiologia do Instituto Butantan, S. Paulo, Brasil)

Giorgio Schreiber

(Instituto de Biologia da Universidade de Minas Gerais, Belo Horizonte, Brasil)

Adolpho Martins Penha

(Instituto Biológico, Divisão de Defesa Animal, S. Paulo, Brasil)

### INDEX

#### 1 — INTRODUCTION

#### 2 — HABITAT, MATERIAL AND PROBLEMS

#### 3 — STATISTICAL RESEARCHES: ANALYSIS OF THE QUANTITATIVE DIFFERENCES OF CHARACTERS BETWEEN SEXES

##### A. — *Biometrical determination of the genetic sex.*

##### 1. — Analysis of the variability of length.

(Body length, head length, tail length).

##### 2. — Scale counts.

(Dorsals, ventrals, sub-caudals).

##### 3. — Regression: — body-tail.

##### 4. — Correlation: — ventral-subcaudal scales.

##### 5. — Application of the "discriminant function".

##### B. — *Variation of the sex-ratio in the population.*

#### 4 — EMBRYOLOGICAL RESEARCHES ON THE GONADS

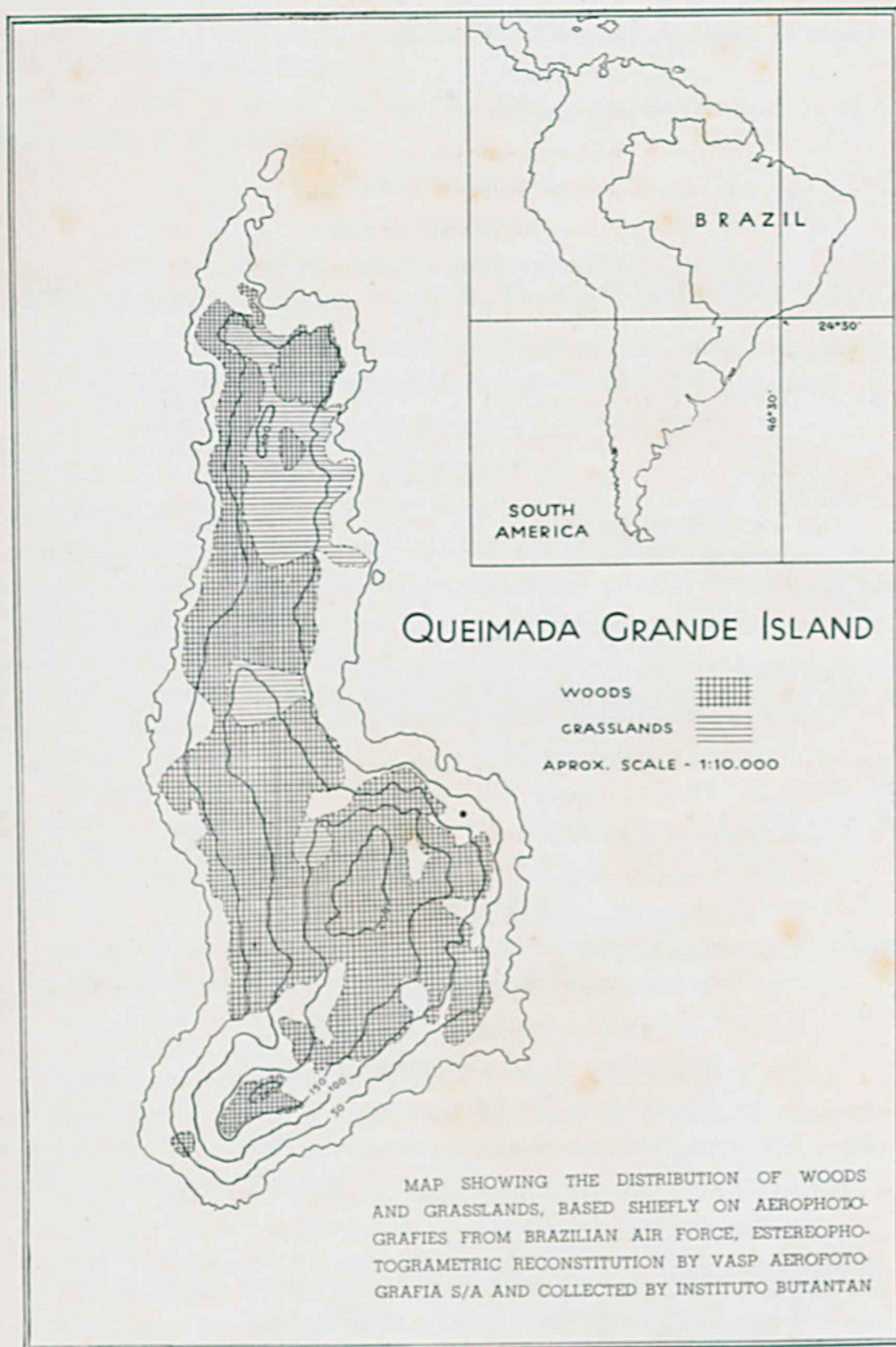
##### A. — *Introduction and techniques.*

##### B. — *Development of the gonads in Bothrops insularis and other species.*

##### 1. — *Bothrops insularis* embryos of 35-40 mm.

##### 2. — *Bothrops insularis* embryos of 60-70 mm.







3. — *Bothrops jararaca* embryos of 70 mm.
4. — *Xenodon merremii* embryos of 90 mm.
5. — *Bothrops insularis* embryos of 130 mm.
6. — *Bothrops insularis* embryos of 160 mm.
7. — *Xenodon merremii* embryos of 180 mm.
8. — *Crotalus durissus terrificus*, newborn.
9. — *Bothrops alternatus*, 24 hours old.

C. — *Discussion of the Embryological Researches:*

5. — A CASE OF HERMAPHRODITISM
6. — CARYOMETRIC RESEARCH FOR PLOIDY DISTURBANCES
7. — CLASSIFICATION OF THE SEXUAL ABNORMALITIES
8. — SUMMARY
9. — ACKNOWLEDGEMENTS
10. — LITERATURE
11. — EXPLANATION OF TABLES AND FIGURES.

1. — INTRODUCTION

At the 9 th International Congress of Genetica (1953) Hoge, Belluomini and Schreiber (38) presented the problem of the sexual abnormalities of *Bothrops insularis* (Amaral) 1921, a snake restricted to the "Queimada Grande" island at the coast of São Paulo, Brasil. (Plates 1 and 2). Detailed data are now added as a result of further captures and a deeper statistical and embryological analysis of the material.

*Bothrops insularis* (Amaral) 1921 (2 and 3) "jararaca ilhóa" (plate 3) (popular name), differs from all other snakes in having a certain number of female individuals with a more or less developed hemipenis (plate 4). This hemipenis can be present on one or on both sides. As in some of these individuals eggs with advanced embryos have been found, it was deemed interesting to study those embryos presenting a hemipenis at different stages of development.

In the population of the island three main groups of individuals can be distinguished: true males, females without hemipenis, and females having bilateral or unilateral hemipenis. For the present, we shall call this last group "intersexes". Later, the real meaning of the sexual abnormalities will be discussed in order to find out whether they represent a true intersexuality, a restricted type of gynandromorphism, or a new type of abnormality.



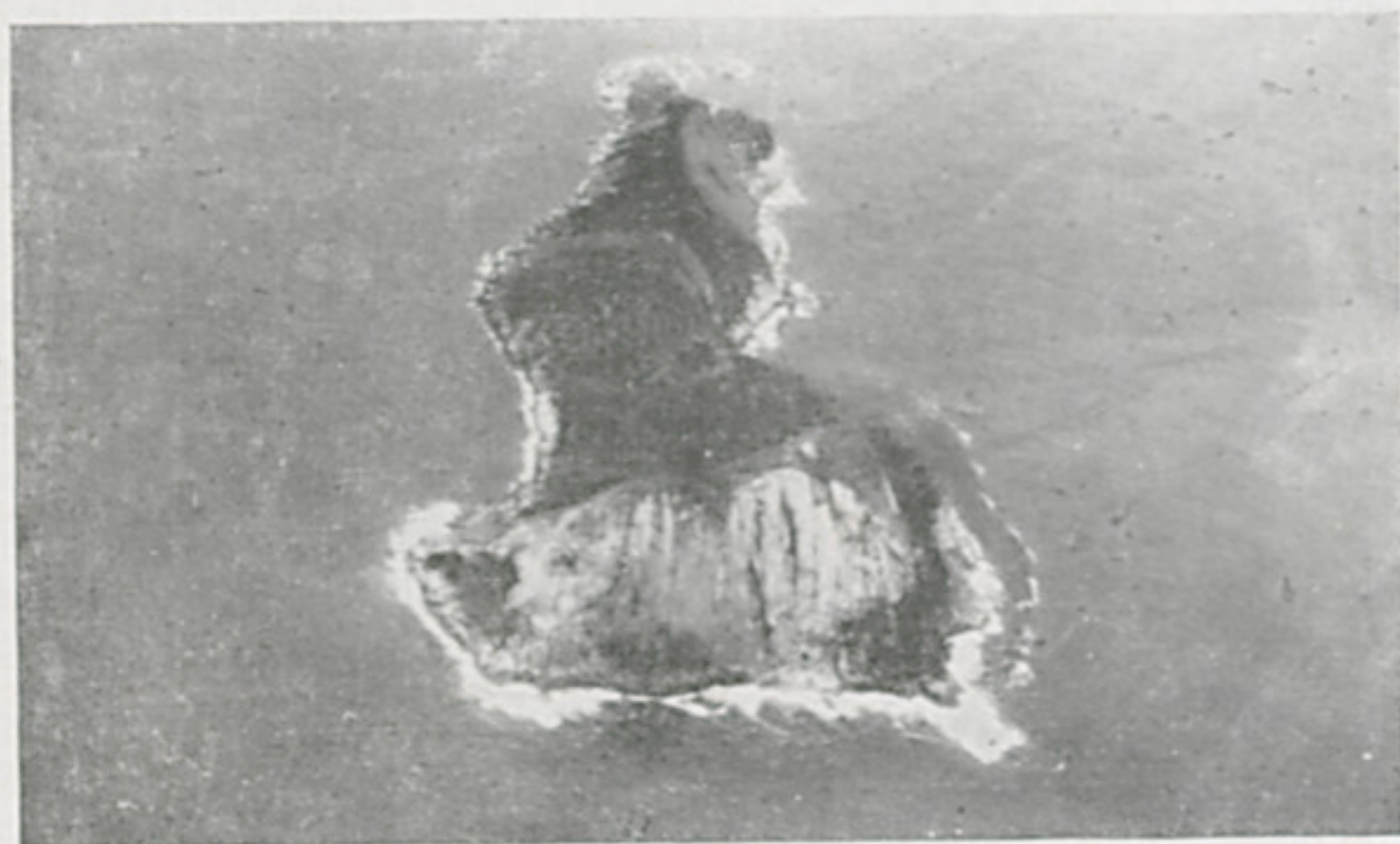


Plate 2 A and B. General views of the Island Queimada Grande, Brazil



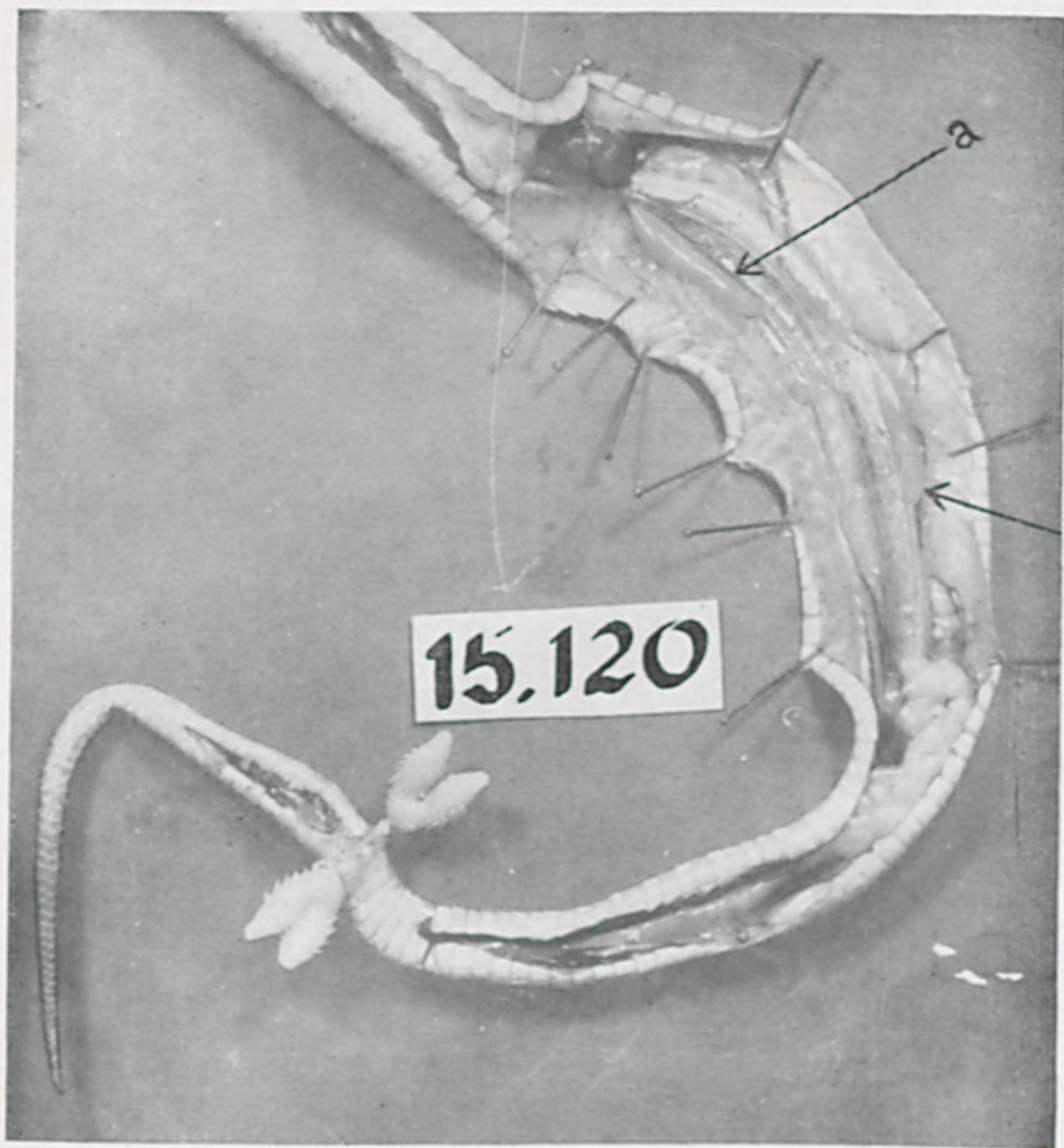


Plate 3. Dissected male specimen of *Bothrops insularis* showing gonads arrows, and hemipenis injected with paraffin.



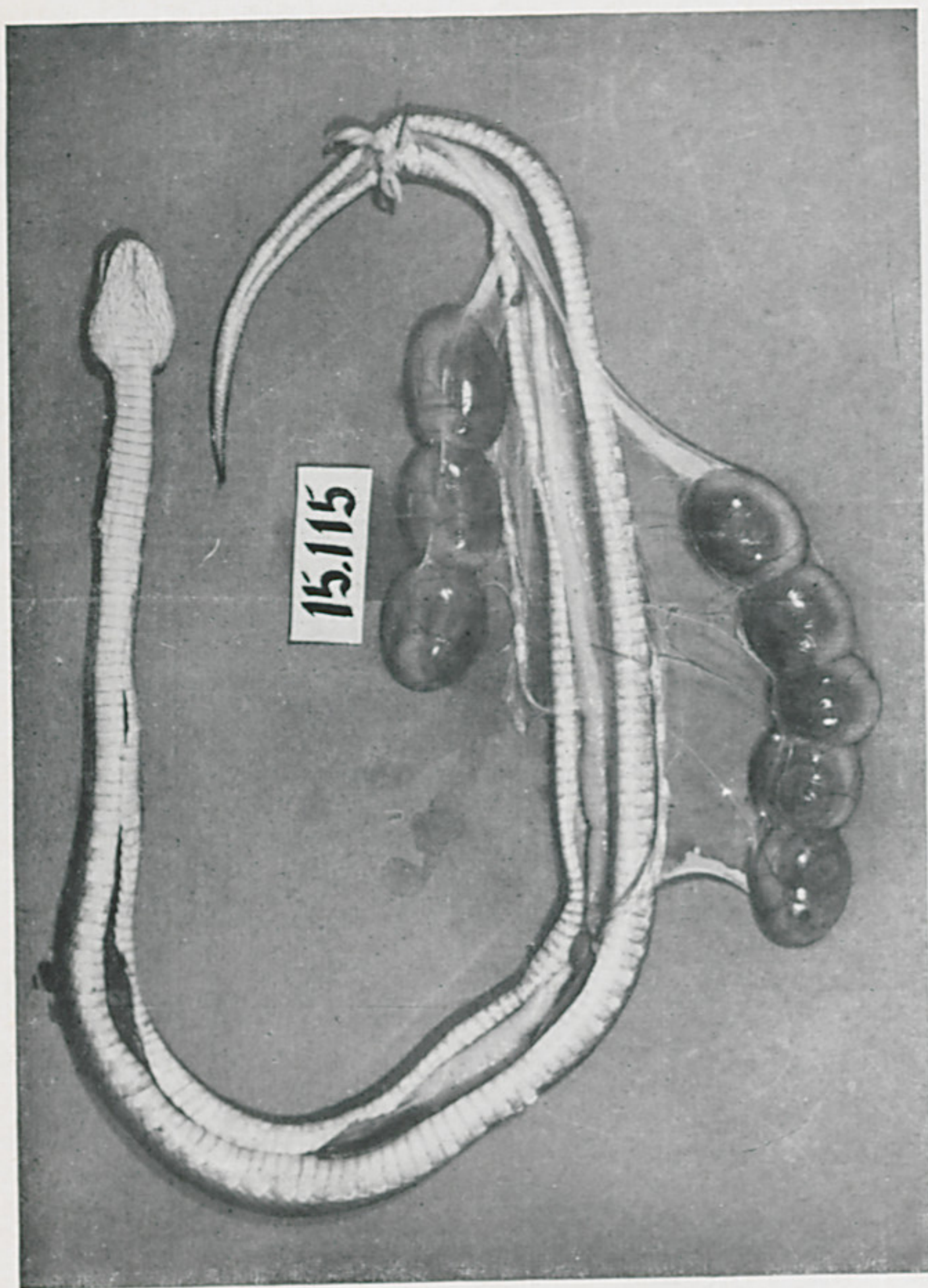


Plate 4. Dissected pregnant intersexes with embryonated eggs, oviducts, and hemipenis injected with paraffin (photograph *a*)



## 2. — HABITAT, MATERIALS AND PROBLEMS

The island "Queimada Grande" is situated in the South Atlantic at 24° 28' south and 46° 42' west Greenwich, approximately 40 miles off the harbour of Santos, and has about 430.000 m<sup>2</sup> with a maximum elevation of 200 meters above sea-level (Plate 5 a).

It is of continental formation, having the same geological formation as the coast, from which it is separated by a shallow sea. No climatological data exist, but the island belong to the Af. Zone (climate) of Koeppen (52), which means tropical humid. The countless visits done by one of the authors (Hoge) permitted the observation that, though there is no really dry season, the rains are relatively rare as compared to the continent, and the climate might not be Af. (perhaps Am).

The sea surrounding the island is extremely rich in fishes and for that reason the island is visited frequently by fishermen. However, they seldom go on shore, because they are afraid of the numerous snakes which abide the island and which have already caused some fatal accidents. Many tales go around among the sailors about shipwrecks and the island's snakes. The island is completely uninhabited. Its fauna is rich in sea birds (*Sula leucogaster* and *Fregata fregata*) and small terrestrial birds. The herpetological fauna consists in the *Bothrops insularis* (Amaral) 1921 (Serp. Crotal.), which is the dominating species and characteristic of the island, having never been captured elsewhere. Another snake, *Dipsas albifrons cavalheiroi* Hoge 1950 (Serp. Colubrin.), and the following lizards complete the land reptilian fauna of the island: *Leposternon microcephalum* Wagler 1824, *Amphisbaena darwinii* subsp., *Colobodactylus taunay* Amaral 1932, *Mabuya macrorhyncha* Hoge 1946, and *Hemidactylus mabouia* (Moreau de Jonnès 1818. Leão (54) related two kinds of *Anura*.

It is difficult to determine for how long the species *Bothrops insularis* has been isolated, since we have evidence that an island can be repopulated by snakes within a relatively short time, even very distant from the continent (Krakatoa after the eruption) Dammarmann (14). It is not an easy problem to ascertain the duration of the period required for the speciation. During the last glaciation (about 20.000 years ago) the island has probably been connected with the continent. As no specimen of *insularis* has been found on the continent, the problem of the specification of *Bothrops insularis* as differentiated from other similar island species must be considered in two ways. Either *B. insularis* existed previously on the continent as well as on the island and disappeared from the continent by negative selection, but persisted in the special habitat of the island, or the differentiation of *insularis*





Plate 5 a. Partial view taken from the Island Queimada Grande showing woods and grassland. (Photograph taken by Pirozelli).



Plate 5 b. *Bothrops insularis* in its habitat (Photograph taken by Pirozelli)



as a new species took place from a related species which reached "Queimada Grande" after its separation from the continent.

Amaral (2) described the species in 1921, based on 203 specimens, which were captured by the lightmen and sent to the Instituto Butantan between 1914 and 1920. Amaral visited the island and captured several snakes. Hoge (35 and 36) started his expeditions to the island in 1946 and brought back a collection of specimens. In 1952 Belluomini and Hoge observed that one of the specimens of *Bothrops insularis* (N.<sup>o</sup> 13992) possessed ovaries in the abdominal cavity, as well as follicles, oviducts and an atresic egg. This specimen, which had died in the terrarium of the Instituto Butantan and whose hemipenis was prepared and injected with paraffin, seemed to be a male. The absence of testicles was stated. Morphologically the snake appeared to be completely normal and did not show any external difference which might demonstrate such abnormality. Other snakes of the same species were investigated, specially since in his paper based on Amaral's data Klauber (50,51) showed no noticeable sexual dimorphism.

A large overlapping between males and females was stated in the specimens described by Klauber (50,51). We observed that the sexual abnormality was much more frequent than expected and after the examination of all the specimens of *Bothrops insularis* existing in the collection of the Instituto Butantan the intersexuality was demonstrated. Sex was determined by opening the abdominal cavity of the snakes to look for the presence of ovaries, testicles, eggs and oviducts. The tail was also opened to search for invaginated uni or bilateral hemipenis.

The present research aims a first contribution to the study of variation frequency in the population, by analysing the variation of the hemipenis character in the two groups of snakes during the period of 25 years.

In 1920 Amaral visited the island, and during his visit collected more snakes for the collection of the Institute. From 1946, when Hoge visited the island for the first time, until 1953 the island has been visited about 10 times in order to obtain more material. This material is divided in two groups: the first one consisting of snakes collected between 1914 and 1921, and the second one of those captured from 1946 to 1953. These two groups, each covering a period of about ten years and being separated by a gap of nearly 25 years, constitute a highly interesting material for the statistical study of the evolution of sex ratio in the population. The fact that this population is strictly localized and geographically isolated from any other similar species adds a new interest to the study of population genetics of this character, i.e. the presence of the male copulatory organ in many female individuals.



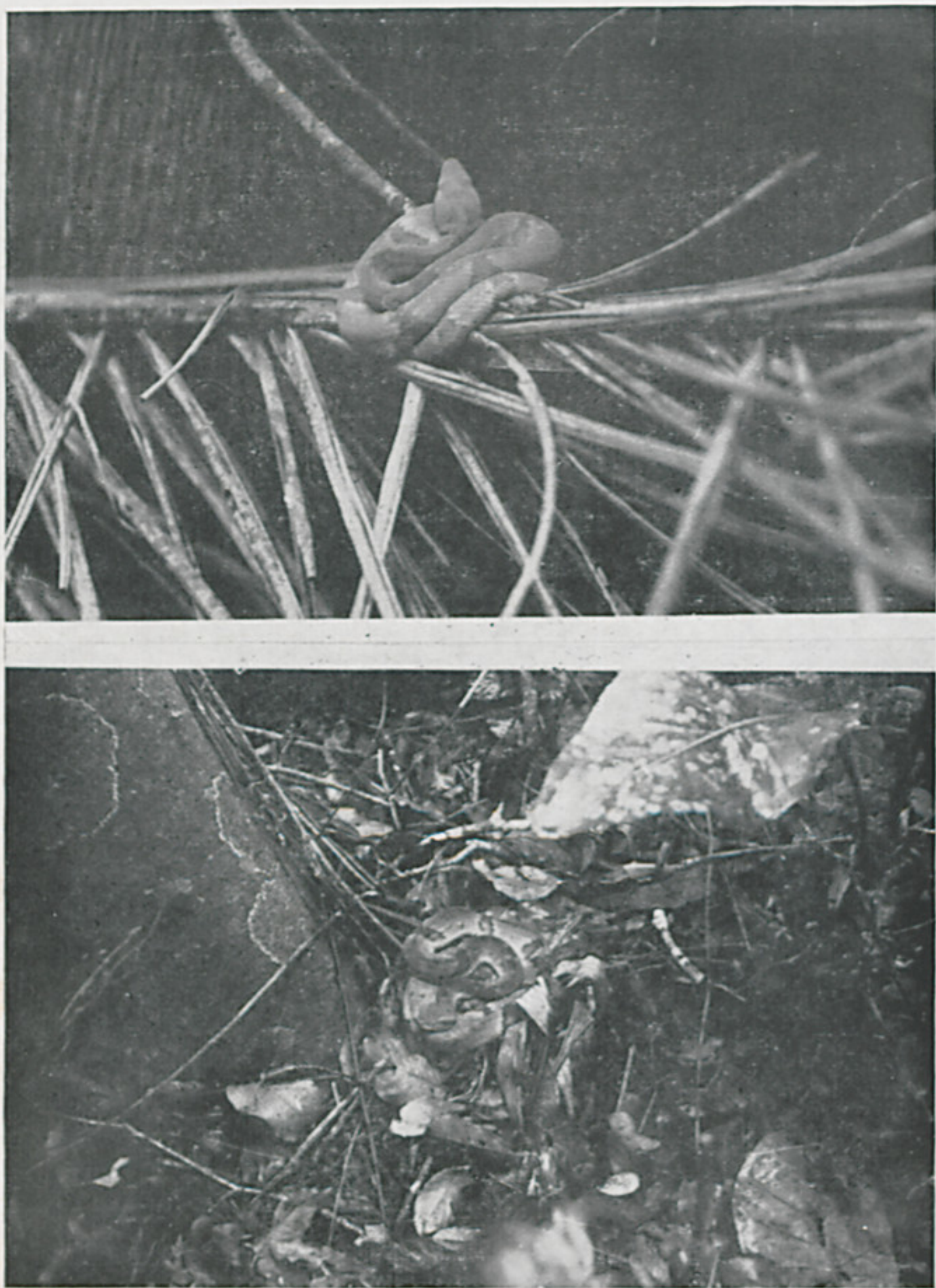


Plate 6 a and b. *Bothrops insularis* in its habitat (Photographs taken by Pirozelli)



*Bothrops insularis* can be found on trees or on the ground (plates 5 b and 6). Small birds constitute the normal food of the snakes; examination of the stomach content reveals occasional lizards (*Hemidactylus mabouia*) and in one case we found a *Dipsas albifrons cavalheiroi*. Frequently chilopods are found in the stomach of young specimens. The protective yellowish-brown coloration fits well the foliage on the ground and the rocks. The first description of *Bothrops insularis* reports it as "semi-arboreal", but further observations revealed the same habitat for the continental "jararaca" *Bothrops jararaca*. The arboreal habitat is perhaps more frequently used by *Bothrops insularis* because of the total absence of mammalian fauna on the island, so that birds are the chief food reserve of the snakes. Tables I and II give the indication of the whole material here studied, which will be analysed in chapters 3 (Statistic) and 4 (Embryology).

### 3. — Statistical Researches: ANALYSIS OF THE QUANTITATIVE DIFFERENCES OF CHARACTERS BETWEEN SEXES.

The statistical researches have been carried out along two main lines: 1 — the analysis of quantitative characters that could eventually differentiate both sexes and intersexes and 2 — the statistical treatment of the sex ratio of the two groups in order to investigate the trends of the genetic constitution of the population or other evolutionary facts.

#### A — Biometrical Determination of the Genetical Sex.

Some characters well suited to quantitative statistical analysis, for example number of scales (ventrals, dorsals and subcaudals) (46,51) are, generally speaking, a good discriminating criterion for sex. We studied in *Bothrops insularis*, in both the first and second group, the statistical distribution of these pholidetic characters in individuals classified as males, females and intersexes. The sexual classification has been based on the presence of testicles and hemipenis in males, the presence of ovaries and oviducts and absence of hemipenis in the females, and ovary, oviducts and hemipenis for the intersexes (See tables I and II).

We must state here that Klauber in 1943 (50), in a paper dealing with sex dimorphism in *Bothrops insularis*, concluded that in this species the statistical analysis of the pholides's does not clearly define the sex. The data analysed by Klauber are those published by Amaral before the discovery of the sexual abnormality of this species, and thus the conclusions of this author cannot actually be taken into consideration. The first attempt to analyse statistically the sex differences in *Bothrops insularis* has been made by comparing head, body and tail length in the three groups of males, females and intersexes. The two samples constituted by the two groups of snakes captured



TABLE I

*Sex distribution of the individuals analysed*

Numbers of the Collection from the Institute Butantan

*Males:*

666	1854	1885	1938	1962	1977	2031	2087	14083	14481	15114	15543
667	1855	1886	1939	1963	1980	2042	3091	14186	14482	15119	15694
668	1864	1887	1941	1964	1983	2047	14000	14187	14487	15120	15740
670	1865	1895	1942	1965	1984	2048	14001	14188	14490	15123	15742
674	1867	1899	1943	1966	1991	2051	14012	14189	14491	15126	15743
675	1870	1905	1944	1967	1992	2052	14036	14190	14494	15127	15804
676	1873	1907	1945	1969	1994	2054	14037	14229	14495	15128	15006
679	1875	1911	1951	1970	1995	2055	14059	14231	14497	15136	15815
682	1876	1918	1952	1971	2006	2057	14060	14233	14500	15140	15852
684	1877	1928	1953	1972	2008	2069	14062	14295	14507	15146	15854
685	1880	1932	1956	1974	2017	2070	14064	14313	14510	15150	Total: 154
1254	1882	1933	1960	1975	2020	2071	14068	14478	14515	15175	
1731	1884	1937	1961	1976	2021	2074	14082	14480	15004	15183	

*Intersexes:*

Hemipenis present in the right side:

669	1896	1934	1962	2043	13014	14072	14261	14512	15155	15805
671	1897	1940	1993	2044	13926	14075	14294	14577	15137	15807
673	1900	1946	1996	2045	13998	14077	14321	15111	15138	15811
678	1908	1947	1999	2050	14011	14979	14334	15112	15142	15860
688	1909	1949	2001	2056	14016	14084	14350	15113	15144	Total:
1737	1913	1954	2007	2058	14025	14086	14483	15116	15451	105
1739	1914	1955	2019	2076	14038	14213	14492	15117	15695	
1881	1925	1957	2025	2162	14063	14228	14496	15121	15739	
1883	1929	1959	2040	3086	14065	14230	14499	15129	15801	
1889	1930	1978	2041	3089	14071	14260	14503	15132	15802	

Hemipenis present on the left side: — Only the number 2027

Hemipenis present on both sides (left and right)

665	1871	1893	1998	14010	14070	14224	14485	15115	15160
672	1874	1901	2233	14013	14074	14236	14486	15118	15171
683	1878	1916	2156	14017	14076	14281	14489	15122	15174
686	1888	1930	13908	14044	14078	14293	14493	15125	15223
1857	1890	1927	13996	14049	14080	14476	14505	15130	15696
1866	1891	1936	14002	14058	14087	14477	14509	15133	15861
1871	1892	1979	14008	14069	14088	14484	14511	15145	15867
Total: 70									

*Females:*

617 (19004)	1952	2033	3669	14073	14508
680	1935	2046	14003	14085	15619
681	1950	2053	14006	14089	Total: 37
1869	1958	2068	14007	14232	
1872	1968	2075	14040	14296	
1904	2013	2077	14066	14351	
1922	2032	2078	14067	14498	

Hermaphrodite n.º 15843

The italic numbers correspond to the paratypes of the first sample whose sexes were re-checked. The number 1996 correspond to the Type of *Bothrops insularis*.



TABLE II

Intersex individuals included in table I which were found to be pregnant.  
Both samples presented a 10% occurrence of pregnancy.

First sample		
Hemipenis present on the right side Col. I. B. n.º	Right oviduct	Left oviduct
1737	7 embryonated eggs	1 embryonated eggs
1914	2 " "	5 " "
2341	5 " "	5 " "
Hemipenis present on both sides: Col. I. B. n.º		
665	3 " "	2 " "
686	3 " "	3 " "
1878	4 " "	6 " "
1888	6 " "	4 " "
1998	3 " "	4 " "
Second sample		
Hemipenis present on the right side Col. I. B. n.º	Right oviduct	Left oviduct
15111*	3 embryonated eggs	2 embryonated eggs
15801	6 " "	4 " "
15802	2 atresic eggs.	2 " "
	1 embryonated egg	—
15803	7 embryonated eggs.	—
15860	2 atresic eggs.	1 embryonated eggs.
	4 embryonated eggs.	—
Hemipenis present on the both sides: Col. I. B. n.º		
15115*	5 embryonated eggs.	3 embryonated eggs
15122	2 " "	—
	2 atresic eggs.	1 atresic egg
15130	3 embryonated eggs	—
	3 atresic eggs	—
15171 Birth of 5 off-springs. 3 died posteriorly		
14485 Eggs at the initial phasis of development		

\* These were studied embryologically.



respectively in 1914-1921 and 1946-1953, have been treated separately and in the histograms are represented by different lines. In all calculations these two groups have been kept separate in order to see if there have been variations in this lapse of time. In view of the fact that the size of the animal, or parts of it, can be influenced by ecological and by ageing factors, a successive analysis has been carried out in the same way as the preceding one, the scale numbers being used as the statistical variable factors. \*

The statistical analysis of both length measurements and scale numbers leads approximately to the same conclusions, *i.e.* the scale counts show sharper differences between the sexes. The relation between scale number vertebrae and ribs in snakes are well established by Gadow (30). The increase in number of vertebrae in Ophidia is a phylogenetic problem largely discussed by Sewertzoff (70) and reviewed from the modern evolutionary point of view by Goldschmidt (33-34).

The genetic influence on the metamerization of the vertebral column is discussed by Kuhne (53) and Fischer (22). All these facts give the scale number a relevant position as a criterion for the determination of the genetical sex, because the segmentation of the mesoderm into somites is an embryological step that occurs far before the appearance of the gonadic ridge and the sexual differentiation of the gonads. It must therefore depend only on the genetic individual constitution XX or XY, being a true somatosexual trait, like all the sexual characters of insects, and not influenced by the hormones.

The biometrical analysis for the genetical sex has been carried out by the following methods:

1 — Analysis of the variability of body length (head, body and tail) of males, females and intersexes, considering the two groups.

2 — Scale countings (dorsal, subcaudal and ventral) in the same individuals as "1".

3 — Regression line between body and tail length.

4 — Scatter diagram correlating to ventral and sub-caudal scales.

5 — Study of the "discriminating function" between two groups of three characters; first body, tail and head length and second, dorsal, ventral and subcaudal scales.

The methods indicated at 1 and 2 consider each character independently from this variability; the methods indicated at 3 and 4 analyse the relationships

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\* This number is a quantitative character fixed during the early period of development and is far more exact than a differential criterion, both for sexes and as taxonomic character.



between two characters related to sex differentiation, and finally the method indicated at 5 considers jointly three characters for the evolution of the sex differences.

# 1 — Analysis of the Variability of Length

Figs. 1, 2, 3 show the following facts: the head of females is slightly longer than that of males. The intersexes appear to be definitively females. An interesting increase in the head length in intersexes of the second group of captures is clearly evidenced. Statistical significance is given by the gra-

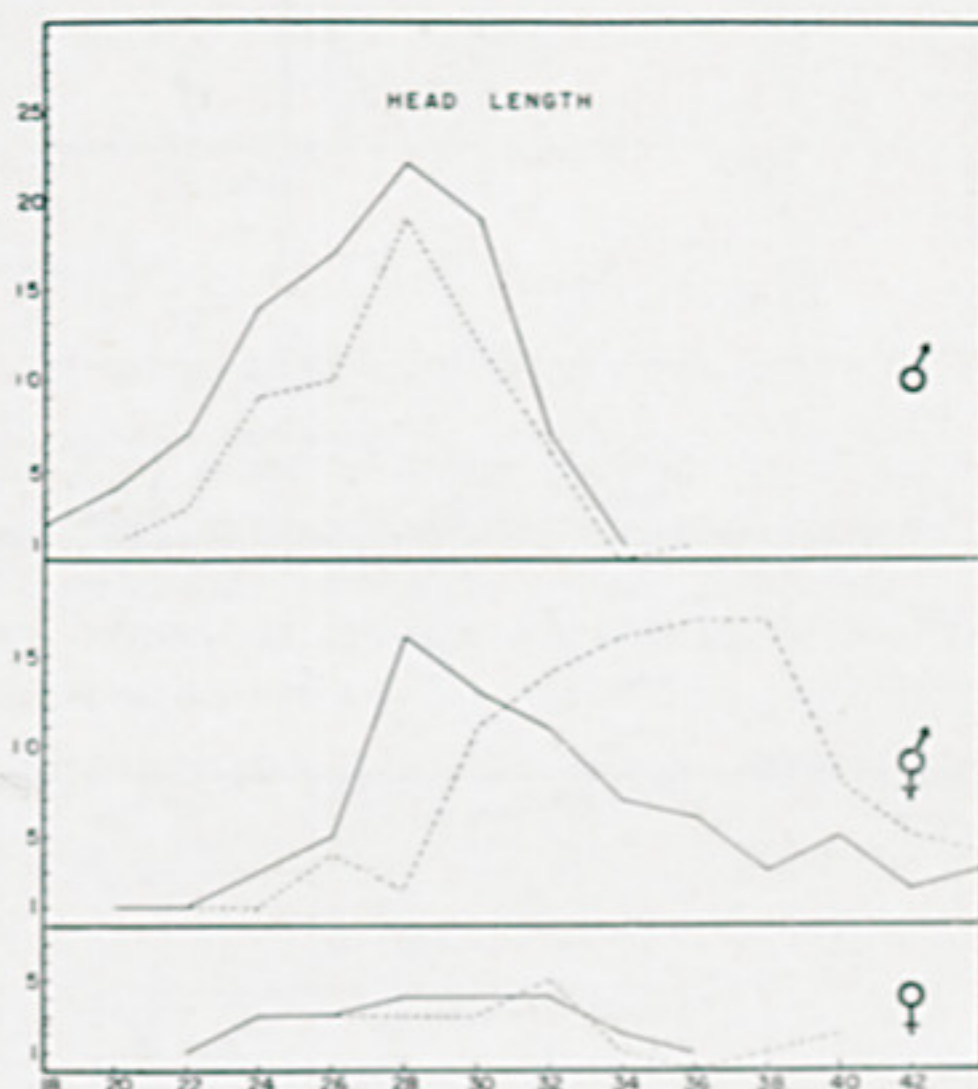


Fig. 1. Frequency distribution of the head length of males (♂), intersexes (♂) and females (♀) of snakes collected between 1914-1920 (full lines) and of snakes collected 1946-1953 (broken lines). Ordinates: number of snakes. Abscissae: head length (mm).

phical method (see fig. 4). Body length is also larger in females than in males, and the intersexes behave also in their character as females. The increased body length in females and intersexes is also clearly indicated for the second group of captures (fig 2). The tail length of the snakes is generally larger in males than in females. These facts are shown in Fig. 3. The intersexes of the first group of captures behave as females, but in the second group there is a clear increase in the length, the second group of intersexes being were more masculine than the first one (Fig. 3).



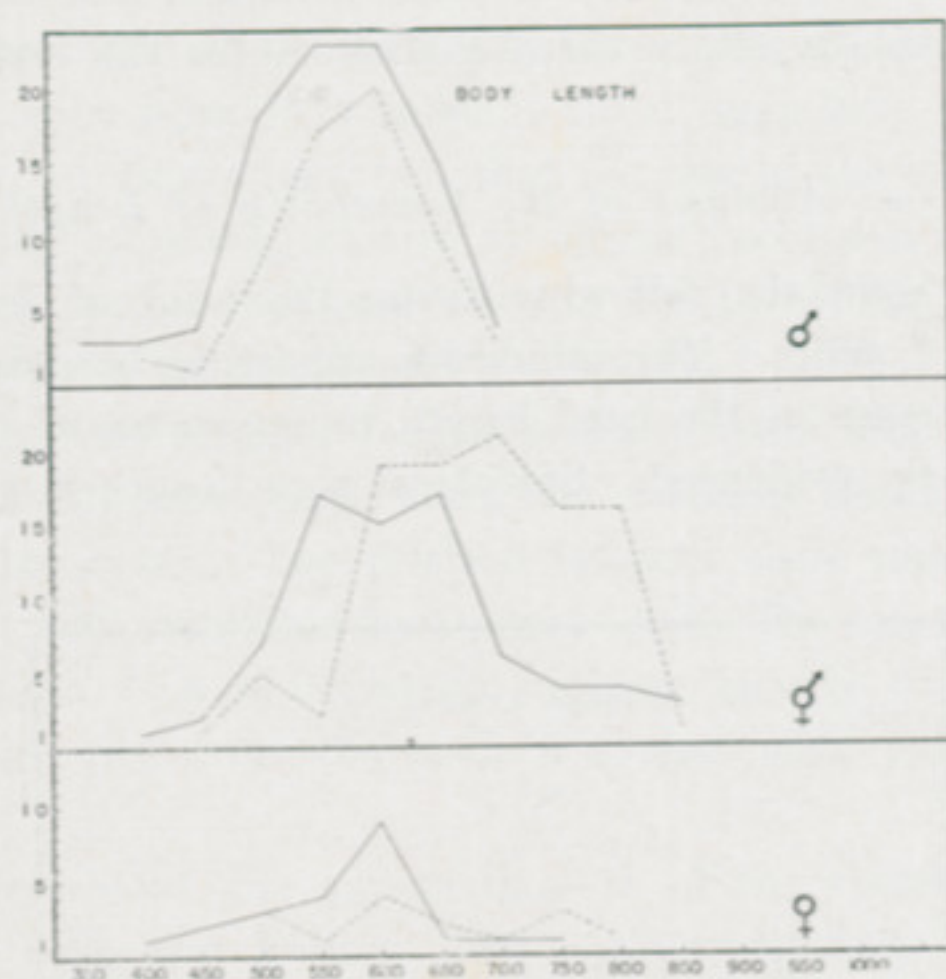


Fig. 2. Frequency distribution of the body length of males (♂), intersexes (♂♀) and females (♀) of snakes collected between 1914-1920 (full lines) and 1946-1953 (broken lines). Ordinates: number of snakes. Abscissae: body length (mm). The open circle corresponds to a hermaphrodite individual.

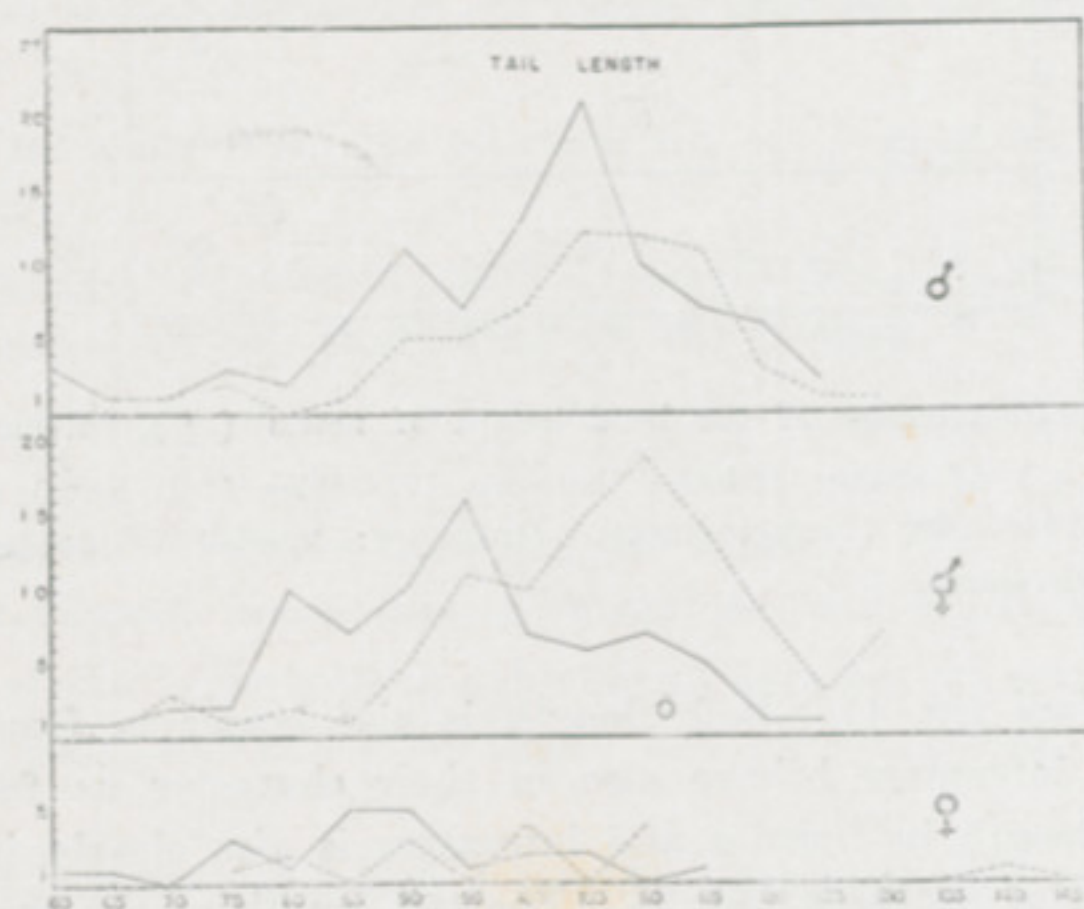


Fig. 3. Tail length of males (♂), intersexes (♂♀) and females (♀) of snakes collected between 1914-1920 (full lines) and between 1946-1953 (broken lines). Ordinates: number of snakes. Abscissae: tail length (mm). The open circle corresponds to a hermaphrodite individual.



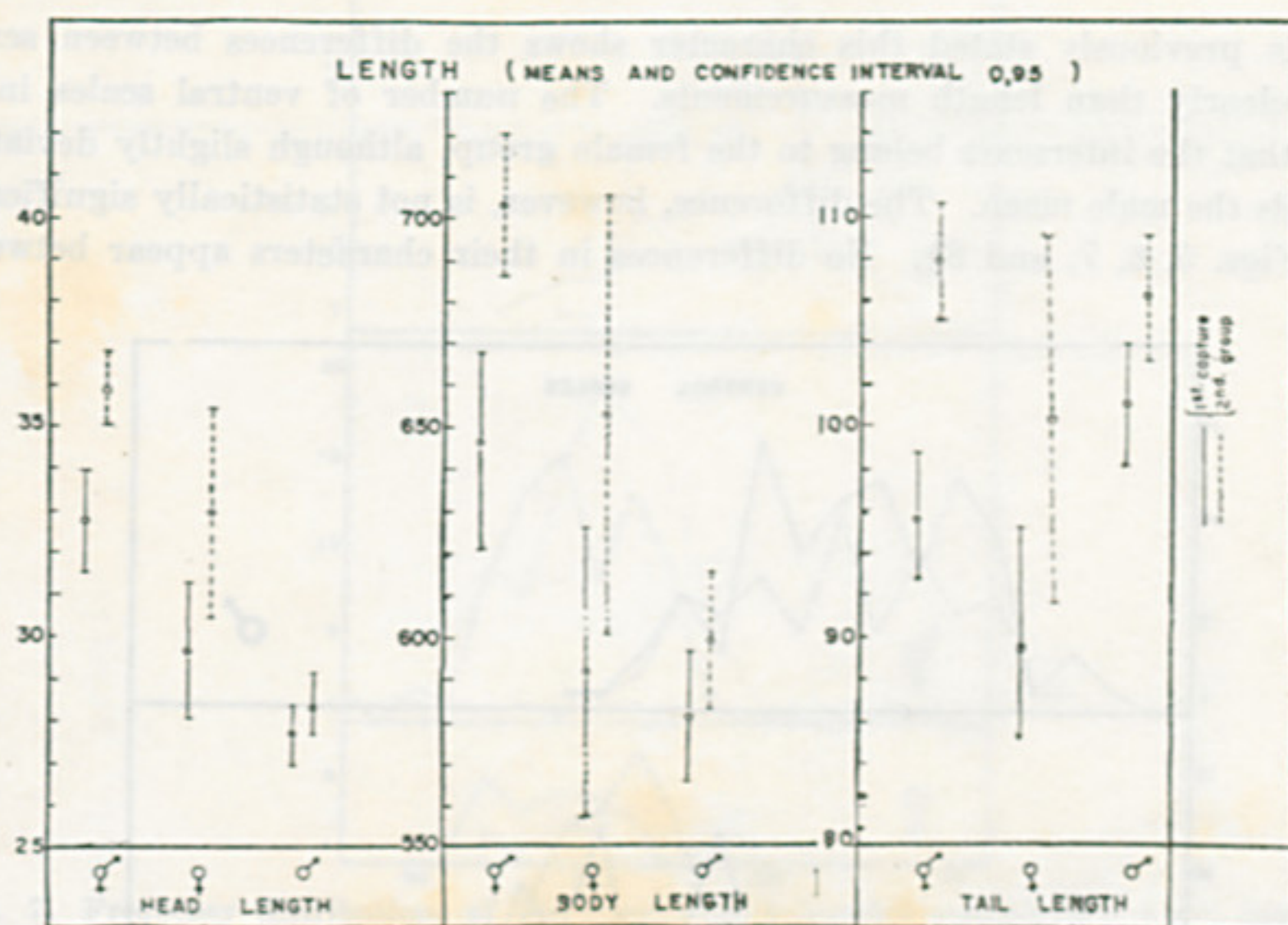


Fig. 4. Confidence intervals at the level of 0,95 probability of the head-body-and tail length length of intersexes (♂), females (♀) and males (♂) of snakes collected between 1914-1920 (full lines) and between 1946-1953 (broken lines).

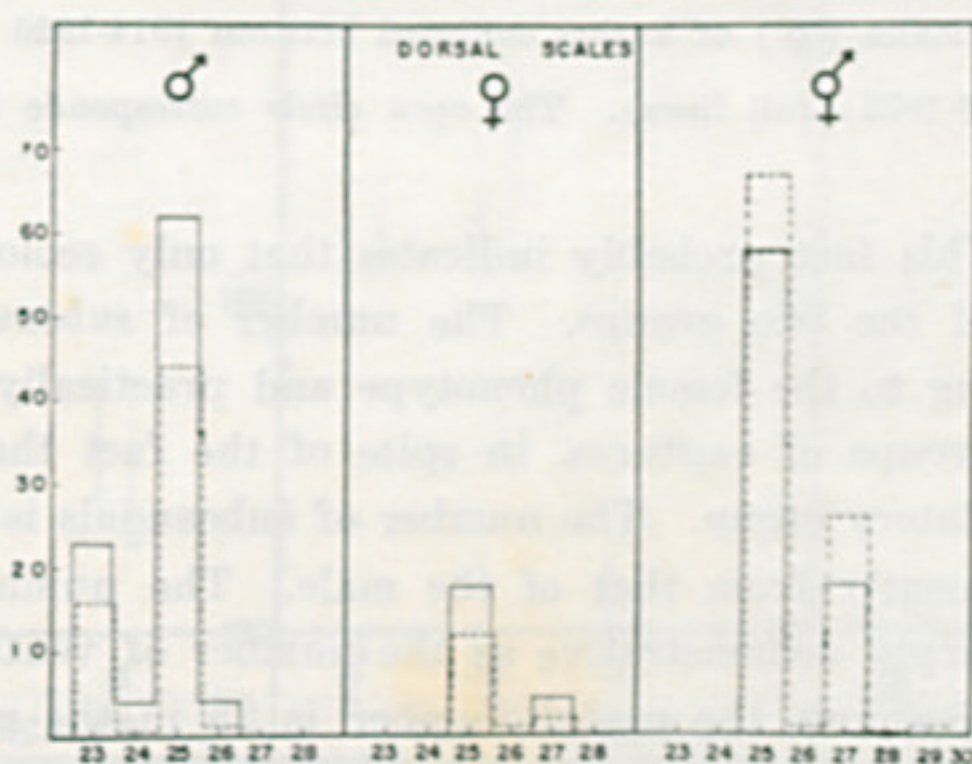


Fig. 5. Frequency distributions of the number of dorsal scales of males (♂), females (♀) and intersexes (♂) of snakes collected between 1914-1920 (broken lines) and between 1946-1953 (full lines).



## 2 — Scale Number

As previously stated this character shows the differences between sexes more clearly than length measurements. The number of ventral scales indicates that the intersexes belong to the female group, although slightly deviated towards the male mean. The difference, however, is not statistically significant (see Figs. 5, 6, 7, and 8). No differences in their characters appear between

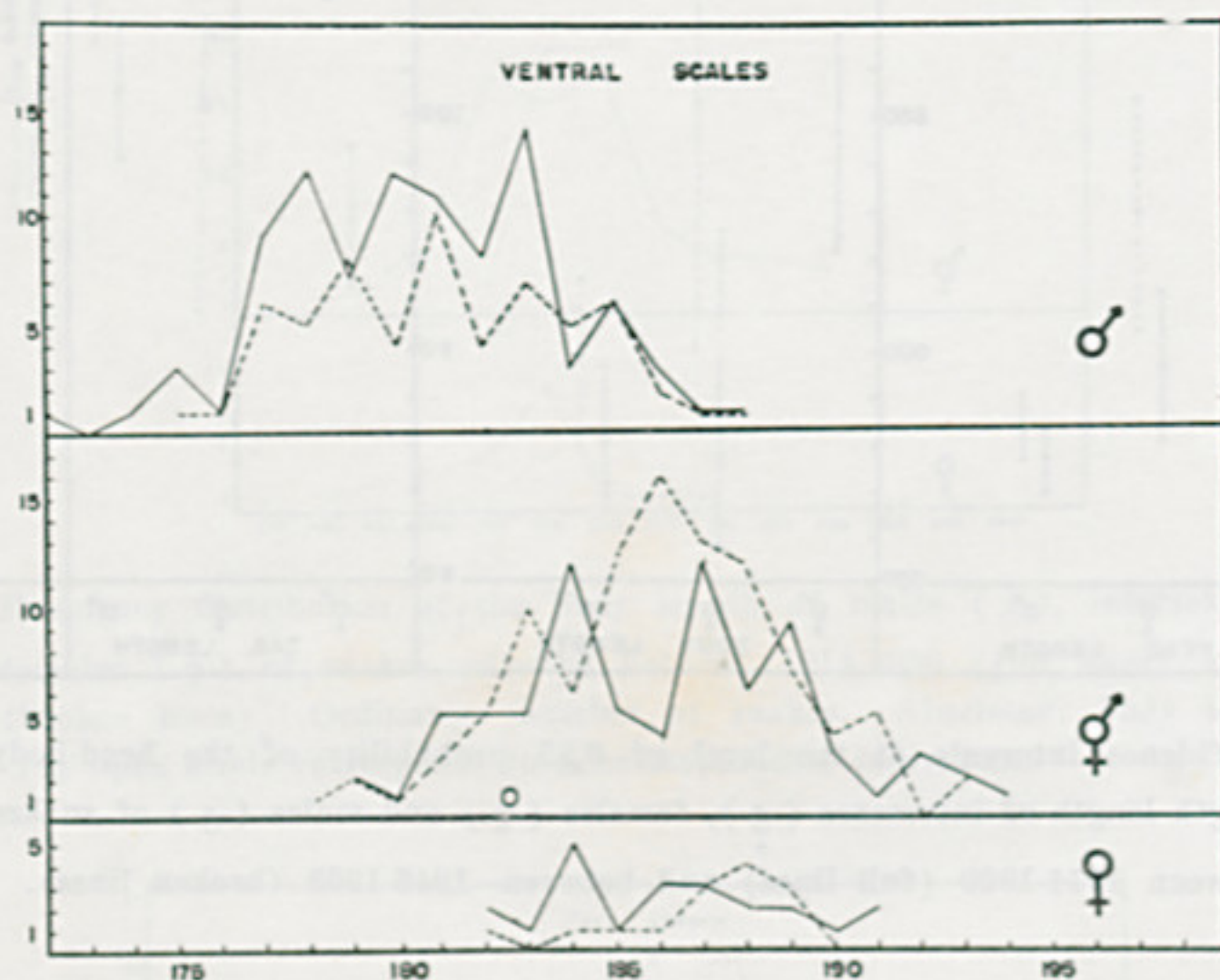


Fig. 6. Frequency distributions of the number of ventral scales of males (♂), intersexes (♂) and females (♀) of snakes collected between 1914-1920 (broken lines) and between 1946-1953 (full lines). The open circle corresponds to a hermaphrodite individual.

the two groups. This fact probably indicates that only ecological factors act on the body size of the two groups. The number of *subcaudals* shows that the intersexes belong to the female phenotype and practically does not differ between the two groups of captures, in spite of the fact that the intersexes bear the male copulatory organ. The number of subcaudals is typically female and differs significantly from that of the male. The number of the *dorsal* scales is not so sharply demonstrative as the number of ventrals and subcaudals, but as Fig., 5 shows, the modal number is 25 in the males with 23 as minor mode. Females show the same main mode at 25, a second one being also evident in 27 and there is an indication for a deviation towards a mode at 27, but not at 23. The female type of scaling here is true higher than in the true females.



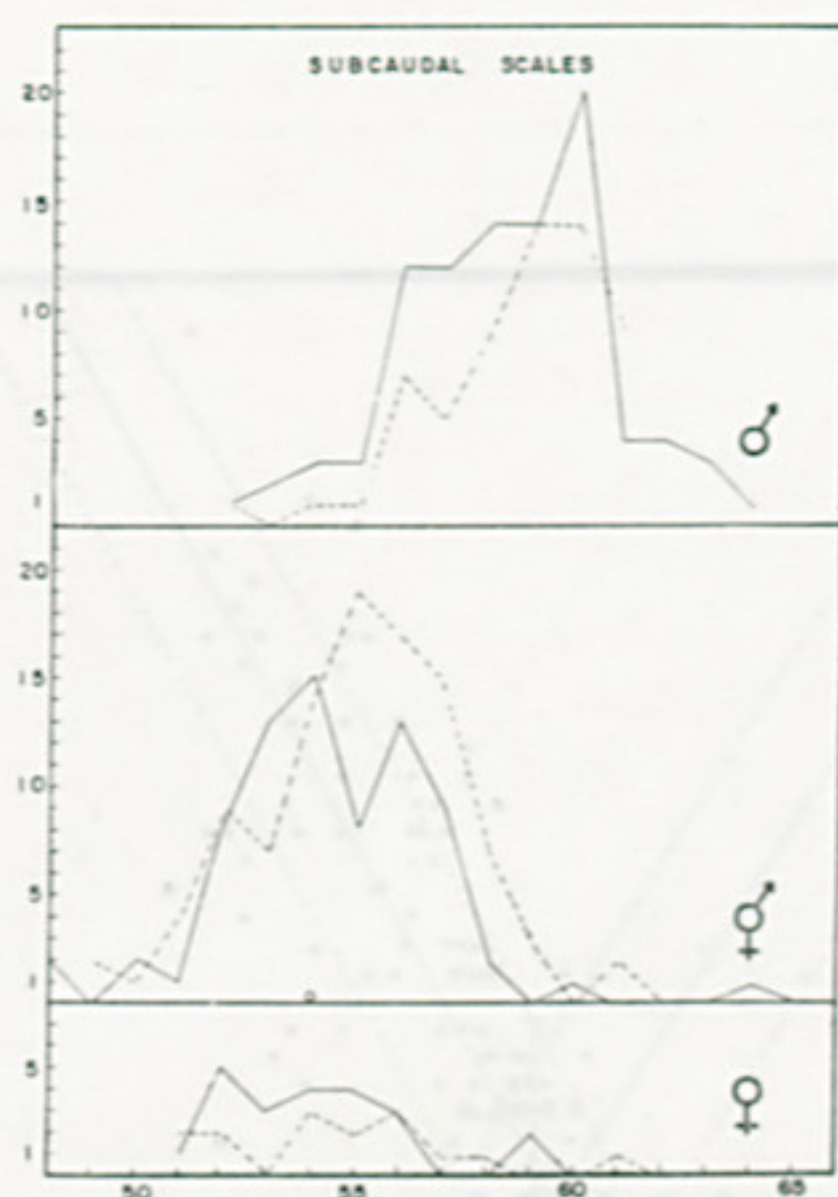


Fig. 7. Frequency distributions of the number of subcaudal scales males ( $\sigma$ ), intersexes ( $\sigma$ ) and females ( $\phi$ ) of snakes collected between 1914-1920 (broken lines) and between 1946-1953 (full lines). The open circle corresponds to a hermaphrodite individual.

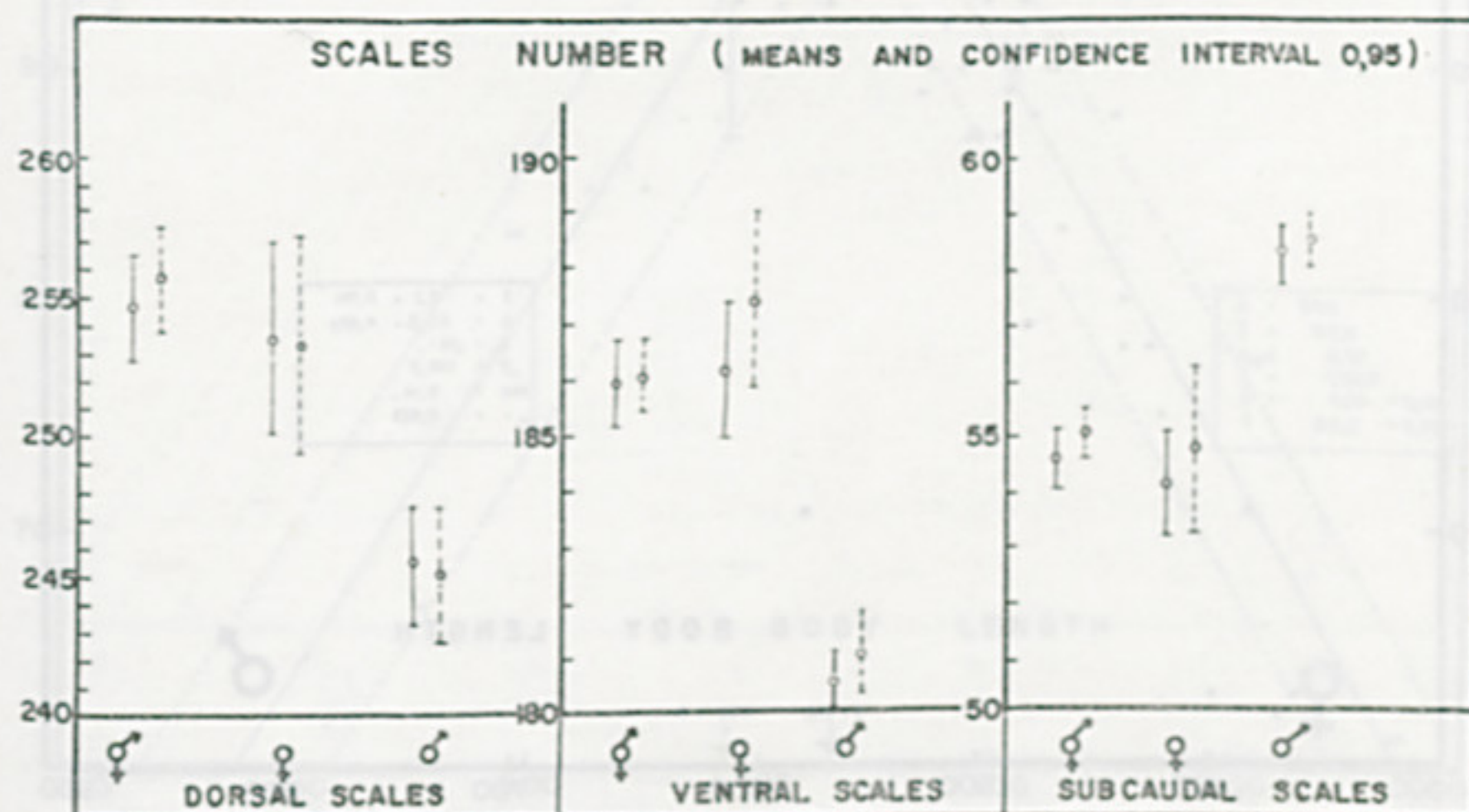


Fig. 8. Confidence intervals, at the level of probability of 0,95 of the averages of numbers of dorsal, ventral and subcaudal scales of intersexes ( $\sigma$ ), females ( $\phi$ ) and males ( $\sigma$ ) of snakes collected between 1914-1920 (full lines) and between 1946-1953 (broken lines).



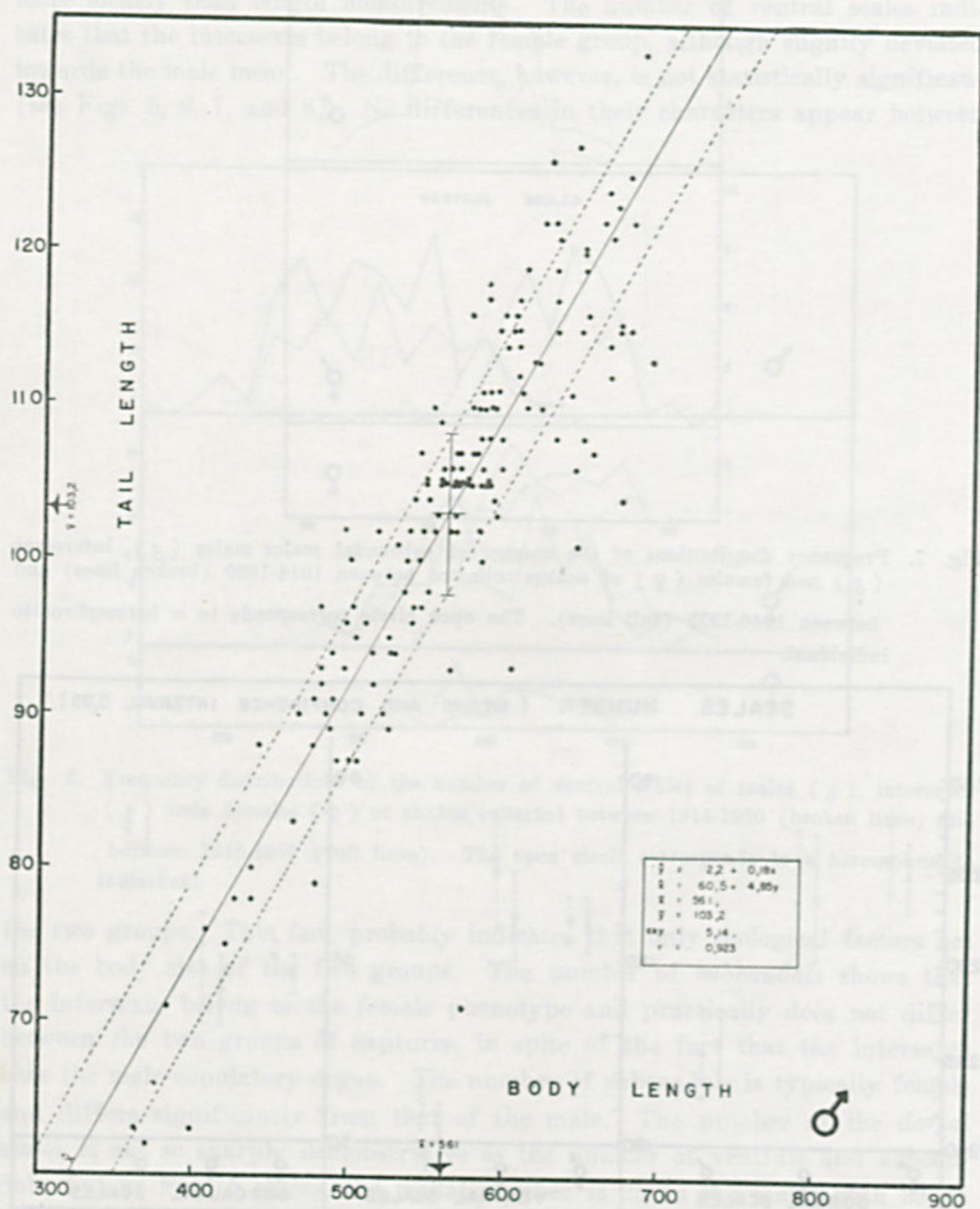


Fig. 9. Tail-body length regression line for all males snakes included in table I.



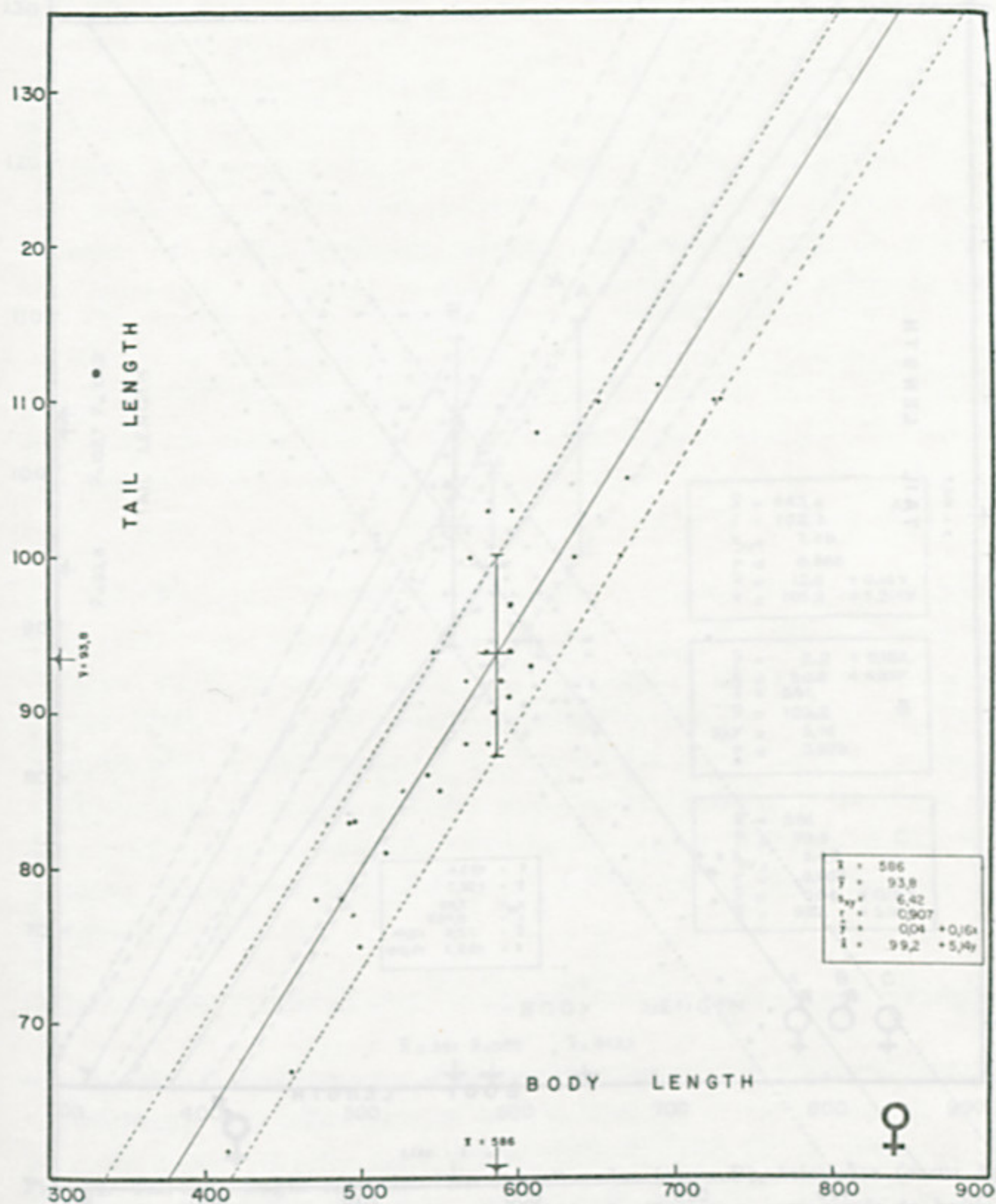


Fig. 10. Tail-body length regression line for all females snakes included in table I.



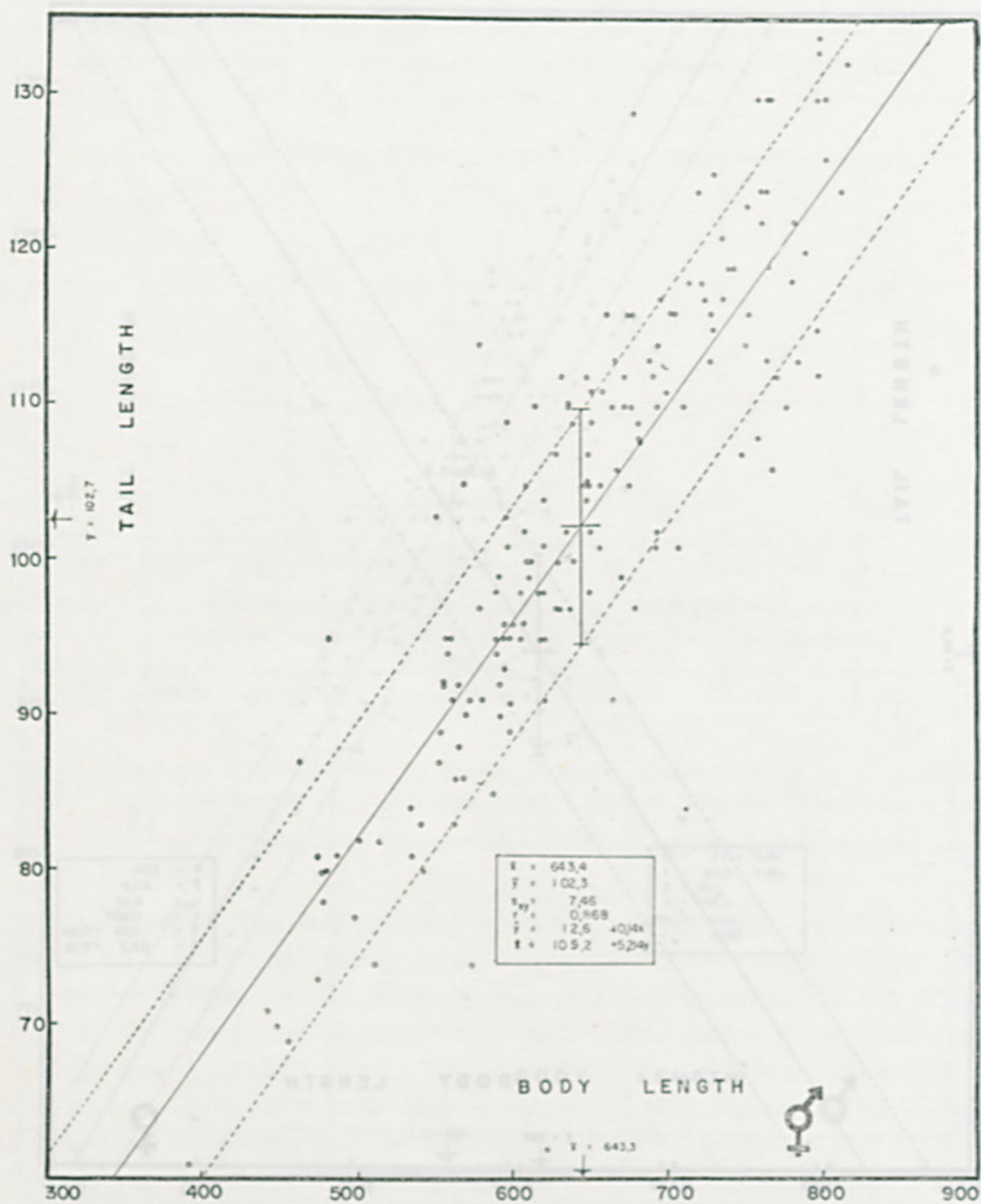


Fig. 11. Tail-body length regression line for all intersexes included in table I.



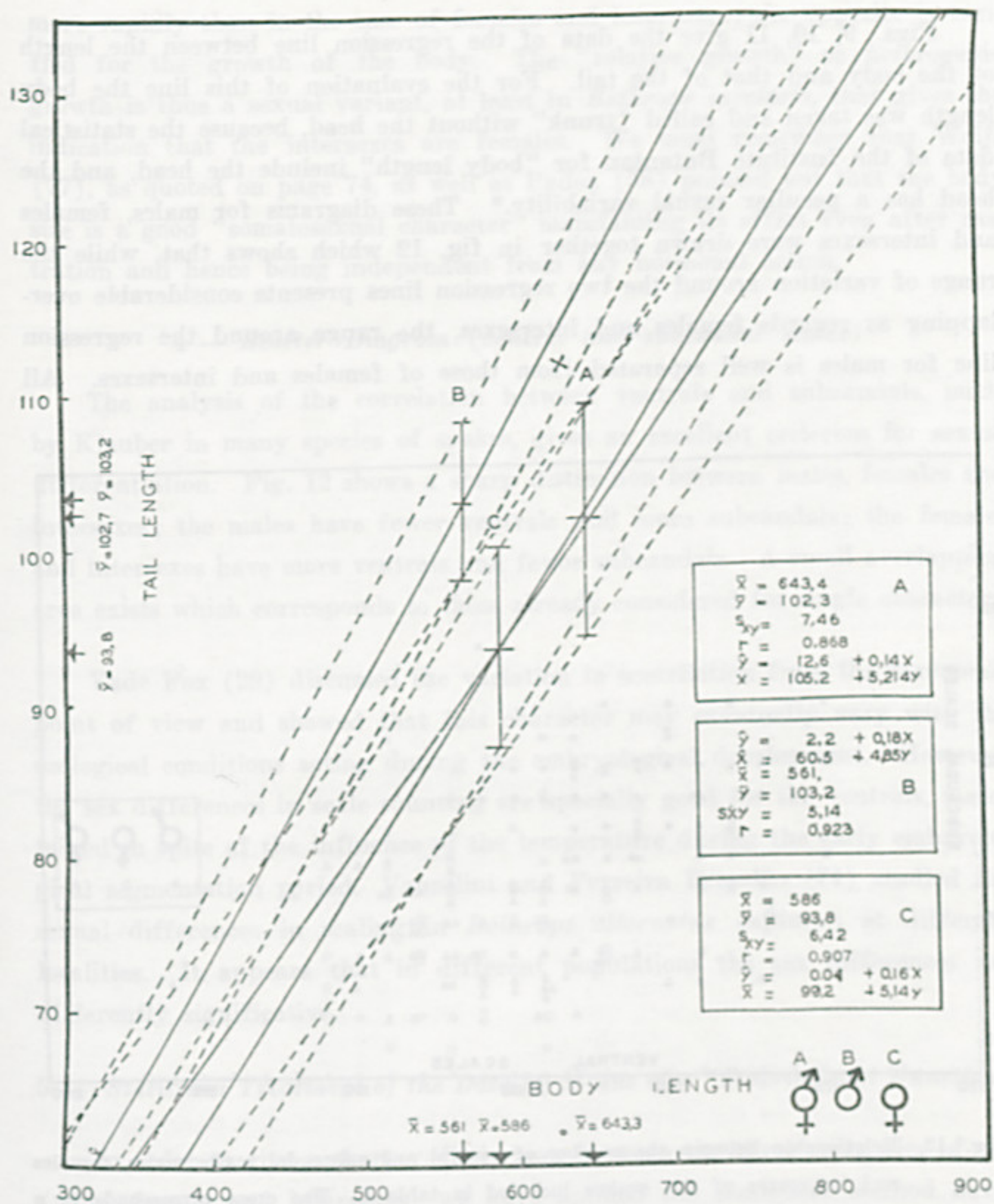


Fig. 12. Tail-body length regression lines for all males (curve B), intersexes (curve A) and females (Curve C) included in table I. The cross corresponds to hermaphrodite individual (snake 15843).



## 3 — Regression: Body-Tail Length

Figs. 9, 10, 11 give the data of the regression line between the length of the body and that of the tail. For the evaluation of this line the body length was taken and called "trunk" without the head, because the statistical data of the Instituto Butantan for "body length" include the head, and the head has a peculiar sexual variability.\* These diagrams for males, females and intersexes were drawn together in fig. 12 which shows that, while the range of variation around the two regression lines presents considerable overlapping as regards females and intersexes, the range around the regression line for males is well separated from those of females and intersexes. All

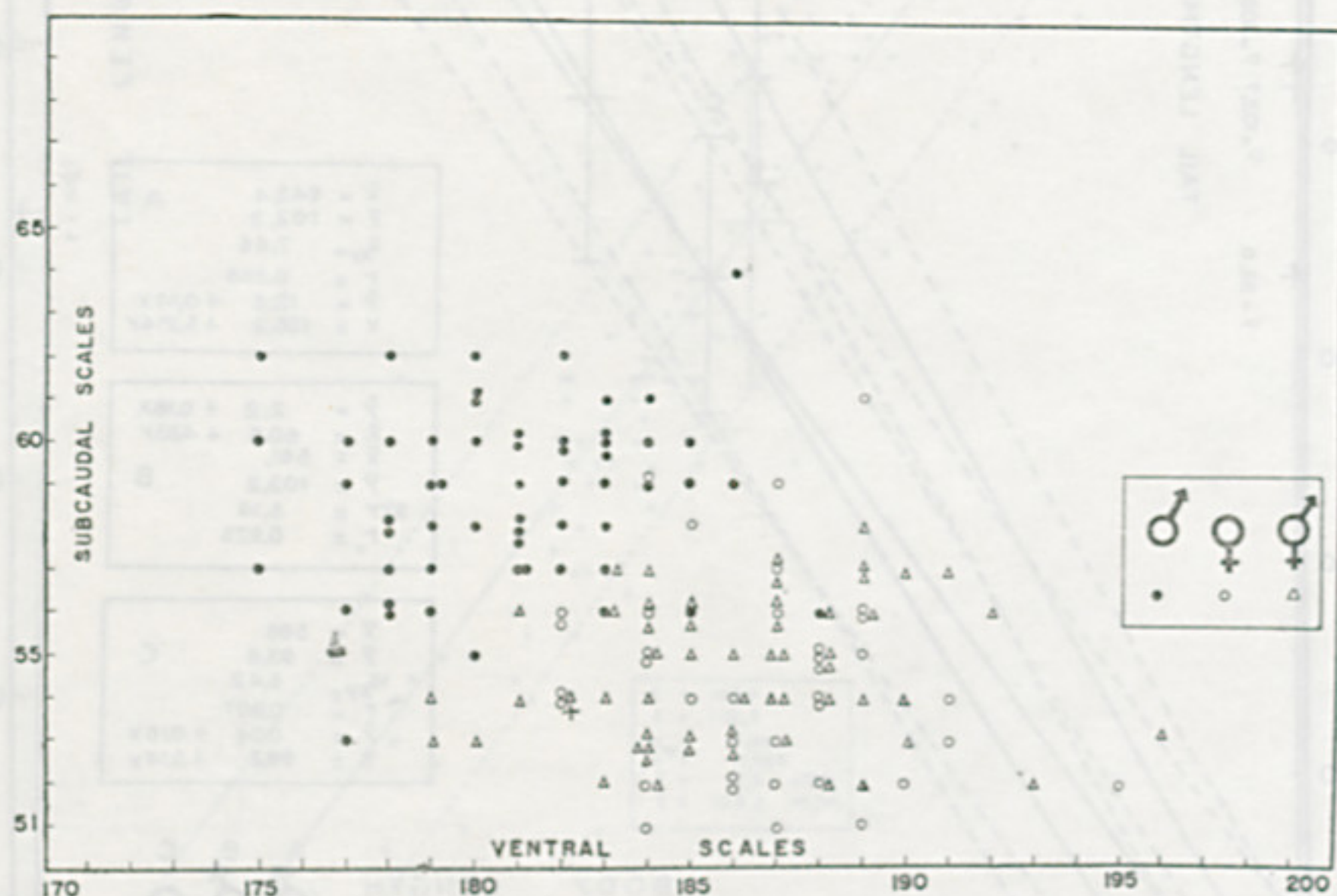


Fig. 12. Relationship between the number of ventral and subcaudal scales males, females and intersexes of the snakes included in table I. The cross corresponds to a hermaphrodite individual (snake 15843).

\* The correlation coefficient "r" has been calculated for all sexes and intersexes, and was found to be close to 1. The standard error of estimate gives an indication for the variability of the regression.



these facts indicate that during the development the tail of the males grows more rapidly than in the case of females and intersexes; the opposite is verified for the growth of the body. The "relative growth" or heterogenic growth is thus a sexual variant, at least in *Bothrops insularis*, that gives the indication that the intersexes are females. We must remember that Wolff (77), as quoted on page 74, as well as Padoa (58) pointed out that the body size is a good "somatosexual character" maintaining its effect even after castration and hence being independent from any hormonal action.

#### 4 — Scatter Diagram (ventral and subcaudal scales)

The analysis of the correlation between ventrals and subcaudals, made by Klauber in many species of snakes, gives an excellent criterion for sexual differentiation. Fig. 12 shows a sharp distinction between males, females and intersexes; the males have fewer ventrals and more subcaudals; the females and intersexes have more ventrals and fewer subcaudals. A small overlapping area exists which corresponds to those already considered for single characters.

Vade Fox (29) discussed the variation in scutellation from the taxonomic point of view and showed that this character may eventually vary with the ecological conditions acting during the embryological development. However, the sex differences in scale counting are specially good for the ventrals, maintained in spite of the influence of the temperature during the early embryological segmentation period. Vanzolini and Ferreira Brandão (74) studied the sexual differences in scaling in *Bothrops alternatus* captured at different localities. It appears that in different populations the sex differences are differently significative.

#### 5 — Statistical Treatment of the Data by Means of a "Discriminant Function"

In order to give a sharper distinction between the three categories of sex (male, female and intersexes) we have applied the statistical method developed by Fisher (23) for the taxonomic distinction of groups differentiated by several quantitative characters, each of which contributes a certain amount of weight to the differentiation of the taxonomic groups. For this purpose, a linear function of the measurements, called discriminant function, was thought of, based on the principles that it would minimize the ratio of the



differences between the mean values of the groups and the standard deviations within the groups. This condition enables one to compute the value of each unknown coefficient which enters into the discriminant function, and so to solve the algebraic problem connected with the complete specification of the investigated function.

In our case, we have captured in each expedition three different sexual groups of snakes: the typical male and female groups, and the so called intersexes group. The problem was to decide whether the intersex group was a typical male, as it seemed at first sight by the presence of a male external copulatory organ, or a real female, as it later proved to be by the finding of embryonated eggs inside the body. As pointed out by Fisher (23) in his original paper, this sex problem is one that can be properly solved by the discriminant function, two different groups of characters being available for its computation in our case: the length of different parts of the body of each specimen, and the number of scales. To simplify the purposes, the number of independent variable factors was limited to three: head, body and tail for lengths; dorsals, ventrals and subcaudals for scale numbers, the discriminant function then taking for both the form of

$$x = l_1x_1 + l_2x_2 + l_3x_3 \quad (1)$$

where  $x_1$ ,  $x_2$ ,  $x_3$  are the values of each of three independent variables and  $l_1$ ,  $l_2$ ,  $l_3$  parametric constants to be determined in each case by the available data of the typical male and female groups. The necessary data for the computation of the parameters are summarized in table IV for lengths and in table V for scale numbers, the general disposition of elements following Johnson's (40) text book for both tables.

As explained in the above mentioned Fisher's paper, the computation of the parameters  $l_1$ ,  $l_2$ ,  $l_3$  depends on the solution of three simultaneous linear equations with coefficients whose matrix is found in table VI for lengths and in table VII for scale numbers, the second members of the linear equations being the differences  $d$  of the tables IV and V respectively. The value itself of each computed parameter is found in table VIII, as well as each of its standard error calculated according to Kendall (45). The last column of this same table shows that the least discriminative variable factor is the length of body



for one of the discriminant functions, and the dorsal scale numbers for the second discriminant function.

Table III gives the values of each discriminant function computed with the averages of male, female and intersex groups, which can be found in tables IV and V. The figures show that intersexes belong definitely to the female group and in the case of discriminant function relative to length, its

TABLE III  
*Mean values for discriminant functions (See equation 1)*

Discriminant function	Independent variables		
	Male averages	Female averages	Intersex averages
Relative to length.....	0,019	0,066	0,076
Relative to scale numbers.....	0,553	0,598	0,594

TABLE IV  
*Data for the computation of a discriminant function relative to head lenght (X<sub>1</sub>), body lenght (X<sub>2</sub>) and tail lenght (X<sub>3</sub>).*

	MALE	FEMALE	INTERSEX
N	154	37	176
SX <sub>1</sub>	4310,2	1149,0	6081,0
$\bar{X}_1$	27,9883	31,0540	34,5511
SX <sub>2</sub>	90704	22830	119313
$\bar{X}_2$	588,9870	617,0270	677,9148
SX <sub>3</sub>	15884	3472	18073
$\bar{X}_3$	103,1428	93,8378	102,6875
SX <sub>1</sub> <sup>2</sup>	122285,40	36360,48	
SX <sub>2</sub> <sup>2</sup>	54221104	14375700	
SX <sub>3</sub> <sup>2</sup>	1665820	333856	
SX <sub>1</sub> X <sub>2</sub>	2571688,8	721756,3	
SX <sub>1</sub> X <sub>3</sub>	450300,3	109876,6	
SX <sub>2</sub> X <sub>3</sub>	9488511	2186285	
d <sub>1</sub>		3,0657	
d <sub>2</sub>		28,0400	
d <sub>3</sub>		-9,3050	

N = number of specimens.  
S = summation sign for whole sample.  
d = differences between female and male averages.



TABLE V

Data for the computation of a discriminant function relative to number of dorsal scales ( $X_1$ ), ventral scales ( $X_2$ ), and subcaudal scales ( $X_3$ ).

	MALE	FEMALE	INTERSEX
N	154	37	176
$SX_1$	3778	938	4493
$\bar{X}_1$	24,5325	25,3513	25,5284
$SX_2$	27844	6909	32739
$\bar{X}_2$	180,8052	186,7297	186,0170
$SX_3$	8999	2014	9653
$\bar{X}_3$	58,4351	54,4324	54,8466
$SX_1^2$	92830	23800	
$SX_2^2$	5035782	1290401	
$SX_3^2$	526599	109832	
$SX_1X_2$	683044	175151	
$SX_1X_3$	220742	51056	
$SX_2X_3$	1627213	376036	
$d_1$		0,8189	
$d_2$		5,9245	
$d_3$		-4,0026	

(Notation similar to that of table IV)

TABLE VI

Sums of squares and products of three measurements, table IV, within male and female sexes, for length

	Head ( $X_1$ )	Body ( $X_2$ )	Tail ( $X_3$ )
Head ( $X_1$ ).....	2329,5509	45829,223	7790,8816
Body ( $X_2$ ).....	—	1086598,96	177008,453
Tail ( $X_3$ ).....	—	—	35549,886

TABLE VII

Sums of squares and products of three measurements, table V, within male and female sexes, for scale number

	Dorsal side ( $X_1$ )	Ventral side ( $X_2$ )	Subcaudal side ( $X_3$ )
Dorsal side ( $X_1$ ).....	166,7702	-39,511	-27,2969
Ventral side ( $X_2$ ).....	—	1727,454	109,377
Subcaudal side ( $X_3$ )..	—	—	946,9318



value is even beyond that for females. As pointed out also by Fisher, to each discriminant function an analysis of variance can be applied which shows the degree of reliability attached to its discriminative power. This is shown in table IX where the value of the probability corresponding to the  $F$ -test of each discriminant function specified in the first column is expressed in the last column. For comparison, the same table contains besides the discriminant function for length and scale numbers, each with three independent variables, the  $F$ -test for six other discriminant functions with only two or even one variable alone, obtained by similar processes with the appropriate data of table IV. Judging by the  $F$ -test, the least discriminative functions is that based on length of body alone, a conclusion which is in agreement with the information given in the last column of table VIII; on the other hand the

TABLE VIII

*Discriminant functions with three independent variables*

Discriminant function	Independent variable	Parameter ( $\times 10^6$ )	Standard error ( $\times 10^6$ )	Ratio parameter standard error
Relative to length	Head ( $X_1$ )	6708 ( $l_1$ )	808	8,3
	Body ( $X_2$ )	132 ( $l_2$ )	44	3,0
	Tail ( $X_3$ )	-2391 ( $l_3$ )	196	12,2
Relative to scale number	Dorsal side ( $X_1$ )	5078 ( $l_1$ )	1206	4,2
	Ventral side ( $X_2$ )	3832 ( $l_2$ )	375	10,2
	Subcaudal side ( $X_3$ )	-4523 ( $l_3$ )	507	8,9

The variable for body length ( $X_2$ ) actually consists of head length ( $X_1$ ) plus body length in narrow sense ( $X_2 - X_1$ ).

TABLE IX

*Significance tests for discriminant functions*

Component independent variables	d. f.	Error variance	F	Probability level
Head length.....	1:189	21,35	22,743	< 0,01
Body length.....	1:189	3,82	4,083	> 0,05
Tail length.....	1:189	12,89	13,724	< 0,01
Head $\times$ Body (Length).....	2:188	51,40	27,106	< 0,01
Body $\times$ Tail (Length).....	2:188	156,30	82,398	< 0,01
Head $\times$ Tail (Length).....	2:188	235,76	124,292	< 0,01
Head $\times$ Body $\times$ Tail (Length).....	3:187	248,79	86,515	< 0,01
Dorsal $\times$ Ventral $\times$ Subcaudal (Scales)...	3:187	240,46	83,617	< 0,01

d. f. = degrees of freedom



best one seems to be the dual discriminant function based on length of head and tail together, instead of three variable discriminant functions used at first; the *U*-statistic test of Rao (62) showed, however, no significant difference between both functions, which means again that the third variable body length, does not contribute significantly to the differentiation of snake sexes. (It can be proved that the *F*-test with one variable alone here used is equivalent to a *t*-test between male and female mean values of the involved variables).

TABLE X

*Mean and standard deviation of each variable according to sex and capture sample, for length*

Sex	Capture sample	Number of specimens	Head		Body		Tail		Discriminant function(*)
			Mean	S. D.	Mean	S. D.	Mean	S. D.	
Intersex	I	76	32.78	5.29	644.06	96.96	95.79	13.27	0.076
	II	100	35.90	4.42	703.64	84.97	107.93	14.06	0.076
Female	I	22	29.73	3.80	592.04	77.72	89.36	13.08	0.064
	II	15	32.99	4.49	653.67	95.71	100.40	15.61	0.068
Male	I	93	27.71	3.41	581.76	75.05	101.05	14.13	0.021
	II	61	28.41	3.08	600.00	66.03	106.33	11.62	0.016

(\*) Discriminant function with three independent variables: head, body and tail length. Values computed with more decimals than those given for the mean sample variables.

TABLE XI

*Mean and standard deviation of each variable according to sex and capture sample, for scale number*

Sex	Capture sample	Number of specimens	Dorsal scales		Ventral scales		Subcaudal scales		Discriminant function(*)
			Mean	S. D.	Mean	S. D.	Mean	S. D.	
Intersex	I	76	25.47	0.85	185.98	3.46	54.59	2.37	0.595
	II	100	25.57	0.94	186.04	3.09	55.04	2.34	0.594
Female	I	22	25.36	0.79	186.23	2.72	54.18	2.15	0.597
	II	15	25.33	0.72	187.47	2.87	54.80	2.72	0.599
Male	I	93	24.55	1.00	180.57	3.09	58.32	2.37	0.553
	II	61	24.51	0.94	181.16	3.00	58.61	1.92	0.554

(\*) Discriminant function with three independent variables: dorsal scales, ventral scales, and subcaudal scales. Values computed with more decimals than those given for the mean sample variables.

Table X gives the averages and standard deviations of head, body and tail lengths for intersex, male and female groups in each of the captures I or II, along with the value of the discriminant function with three variables for length; similar data are presented in table XI for scale numbers. Con-



firming previous statements, both tables show that intersexes and females seem to form a homogeneous group with values of their discriminant functions almost independent from the capture samples, but quite distinct from the corresponding values for males. The values for the respective discriminant functions for lengths and scale numbers were plotted in fig. 13.

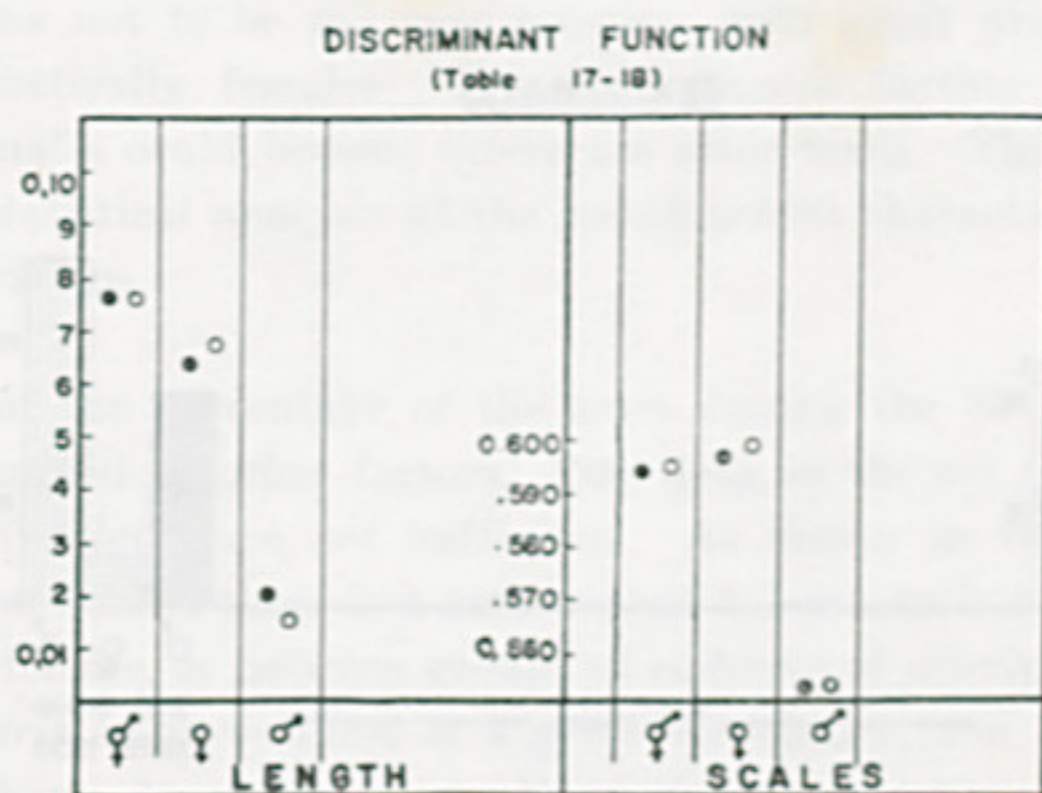


Fig. 13. Discriminant function for length and scale numbers for snakes collected between 1914-1920 (full circles) and between 1946-1953 (empty circles).

#### B — Variation of the sex ratio in the population

As stated in our introduction chapter, the specimens of *insularis* her studied have been captured in successive expeditions to "Queimada Grande" island and have been grouped in two distinct periods, separated by a gap of time of about 25 years. From the collected snakes 367 well prepared specimens have been selected for the statistical analysis. The snakes of the first group have been captured between 1914 and 1920, and those of the second group between 1946 and 1953. A third group of 81 individuals captured during 1954 and 1957 is not included in the data here published and will be the subject of a future paper. In the last captures special emphasis has been paid to the ecological distribution of the snakes in the different parts of the island, taking in account the frequency of the sexes in the different environments, but as far as we can infer from these captures no selective ecological difference exists between sexes or intersexes.

Figs. 14 and 15 show the percentage of sexes in the three groups captured. A decrease in the frequency of males and an increase of intersexes is evident. The frequency of the females appears almost invariable. The diffe-



rence between the males of the groups, as well as those of the intersexes, are statistically significant. The difference between the two groups of females is not significant. These facts show that a variation in the composition of the

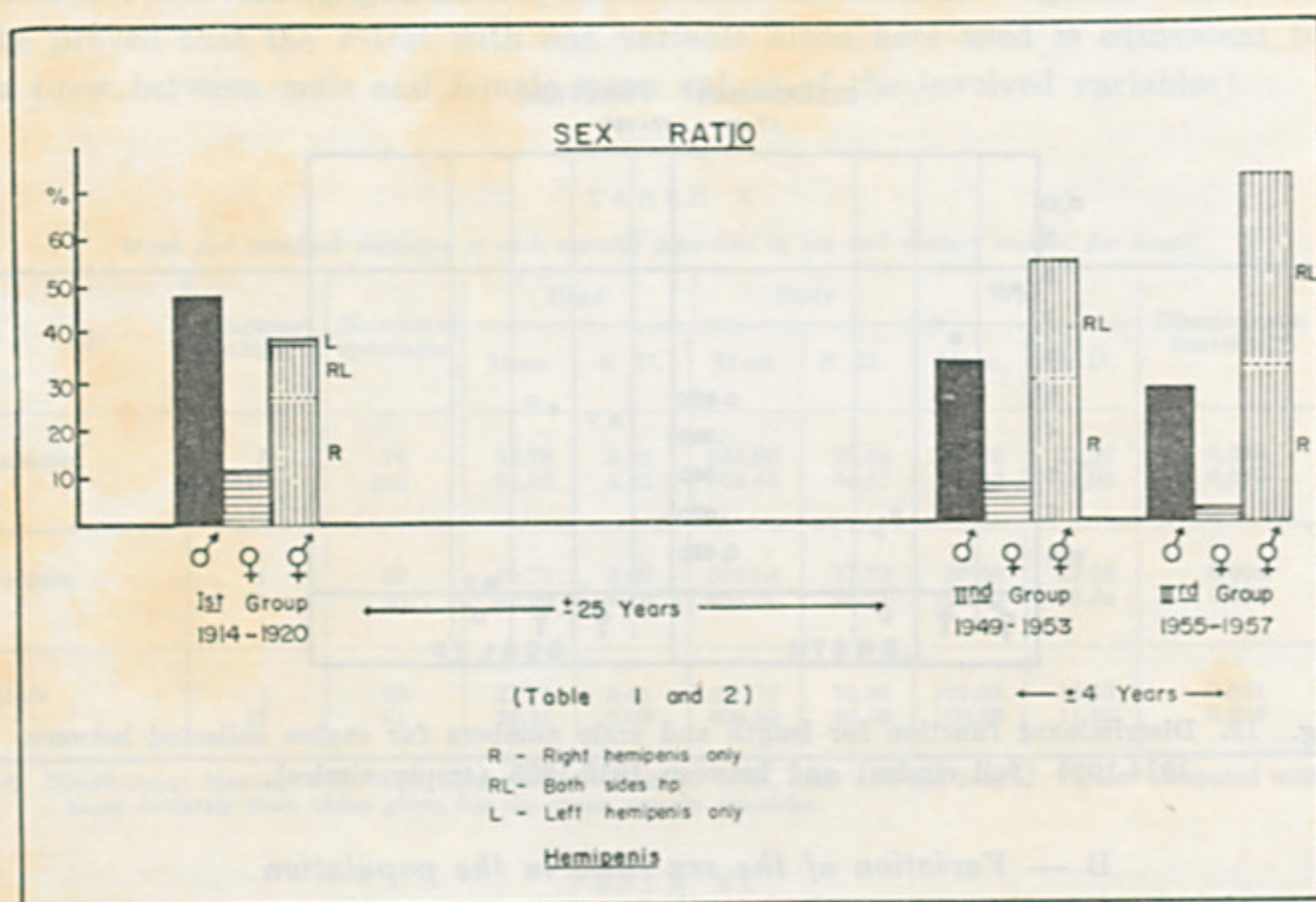


Fig. 14. Sex ratio in *Bothrops insularis* at different periods. The data of the third sample will be discussed in a future paper.

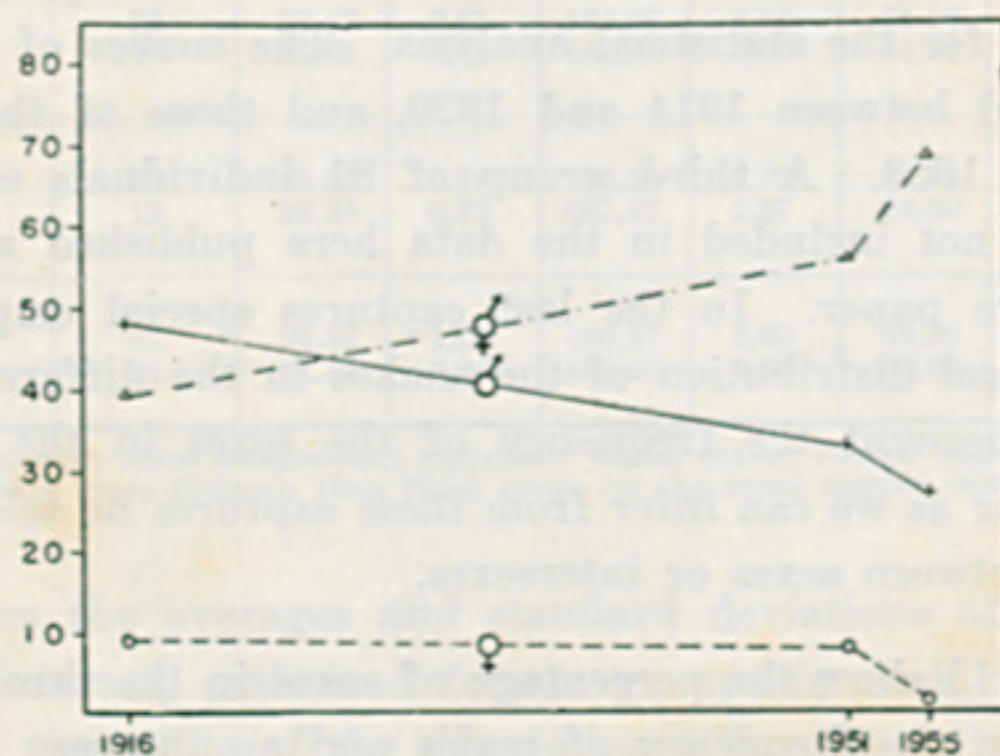


Fig. 15. Sex ratio in *Bothrops insularis* at different periods.



population from the sexual point of view is in action, and although far from being easy to interpret, are here considered very important.

If the intersexes were genetically males, their increased frequency and the corresponding decrease of males would be interpreted as an increase of the factors influencing intersexuality in the population during the 30 years. But this seems not to be the case because, with great probability, the intersexes are genetically females. We shall discuss further on the possibility, that some females could become intersexes after birth. This would be possible by both the statistical analysis of the somatosexual characters and the embryological observations.

A shift of the percentage of the sexes during the 30 years period would thus be determined by other factors. Our data on the sex ratio of the embryos of *Bothrops insularis* are not sufficient. As shown in the next chapter, in other species of snakes there is a rather good 1:1 sex ratio between the embryos borne by one female, or between groups of embryos of ovoviparous species. But, for the *insularis* embryos there is a great variability, and a good deal of fertilized eggs shows degenerescence. Even if we could have more data on this fact in the second and third groups of captures, these data would not have great interest for our problem because of the lack of information in this respect for the first group of captures. This group had been collected before the discovery of the abnormality, and no embryo was examined by the collector at that time. From the first group of captures we can infer that, if really the intersexes were genetically females, the sex ratio would be 1:1, that is the approximate ratio between the number of males and the sum of both true females and intersexes (Fig. 15). This is not true for the second group. If the first group really had the sex ratio as assumed above, we could present the facts as follows: some of the individuals genetically females have a factor that determine the appearance of the hemipenis. This factor does not interfere with the breeding capacity of these females, although perhaps there is a diminution in fertility, in comparison, for instance, with the *Bothrops jararaca*, common "jararaca", and with great probability there is some factor that inhibits the fertility in true females (those without the factors for hemipenis).

During the last 25 years the approximate ratio of 1:1 between the males and the sum of females and intersexes showed a decrease from the initial value of 1:1 in favour of the intersexes. It is possible that lethal factors eliminate a certain number of males during the first period of life. The increase in the



percentage of intersexes may perhaps be ascribed to the selective pressure of the ecological environment, to which the intersexes are better fitted. Secondly the increase in the percentage of intersexes may be the result of the high grade of inbreeding in the isolated population that results in the appearance of a higher number of recessive homozygotic phenotypes, and finally a third factor can be supposed: the increase of the rate of the eventual mutation that determines the intersexuality in the the population. For the present the problem has to be left at this stage. A further attempt to interpret these facts, in terms of population genetics based on some working hypothesis on the genetical factors of the abnormality, will be published in the future.

### EMBRYOLOGICAL OBSERVATIONS IN THE GONADS

#### A — Introduction and Technique

Having captured some pregnant *Bothrops insularis*, it has been possible to study the development of the gonads of the embryos in order to inquire whether some disturbances on the relation between the ambisexual parts of histological constitution of the embryonal gonad are related to the presence of the hemipenis. In all Ophidia it is possible to recognize easily the sex of the embryos by the hemipenis everted during the whole period of development. In other species of snakes (*Bothrops jararaca*, *Crotalus*, *Xenodon*, etc...), as we have already stated, the sex ratio is always 1:1, one half of the embryos with, and the other half without, hemipenis. The histological constitution of the gonads always confirms the sex as determined by the hemipenis. In *Bothrops insularis*, however, the presence of hemipenis is insufficient to indicate the sex of the animal examined. In fact, of all the embryos studied, only one was completely deprived of this organ and all the others presented a more or less developed hemipenis on both sides or on the right side only (Figs. 16, 17 and 18). The degree of development of the hemipenis was conventionally indicated by three stages (+++) (++) and (+) as shown in Table XII and in Fig. 18. We shall return to this argument further on.

All pregnant mothers were intersexes and, as we have already stated, no true females have ever been encountered in a pregnant state. The development of the gonads and their anomalies have been studied specially by Forbes (24-28) in alligators and Risley (63-66) in Chelonia. In Chelonia there exists in the testicles of the embryos a transitory cortex, in alligators the transitory cortex persists in the testicles and a medullary zone persists longer in the juvenile ovaries. The only case of intersexuality in adult Reptilians has been



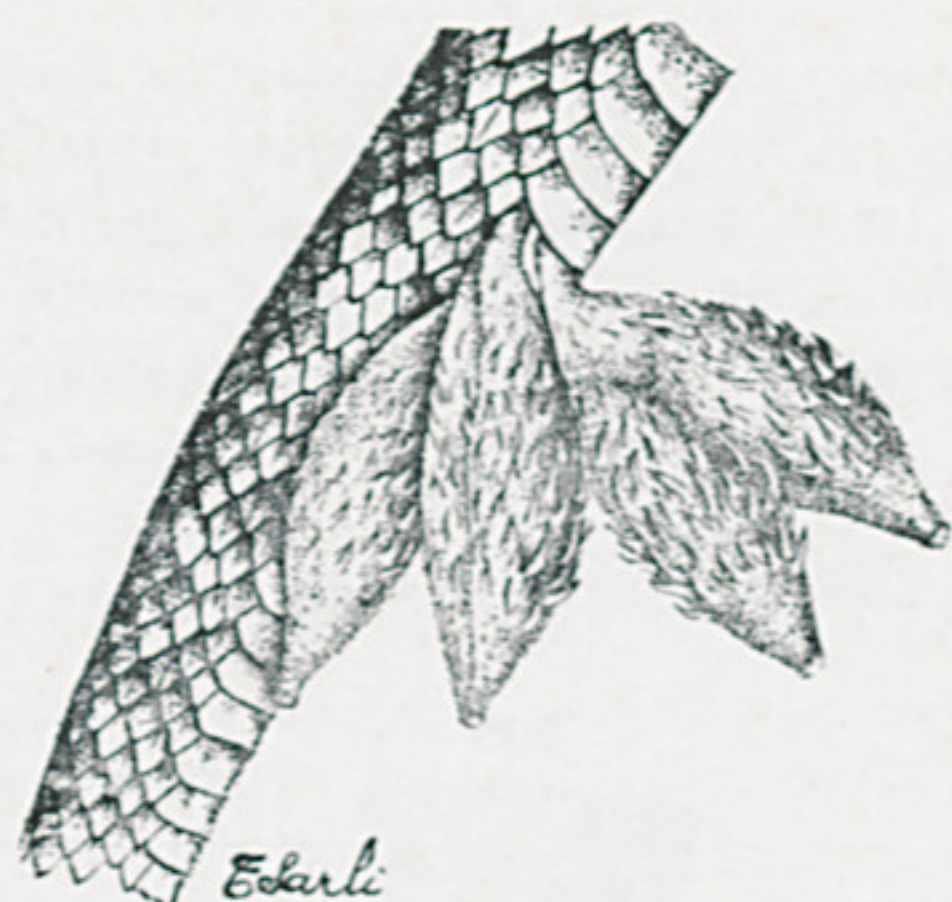


Fig. 16 — Male *Bothrops insularis* with the hemipenis injected with paraffin.

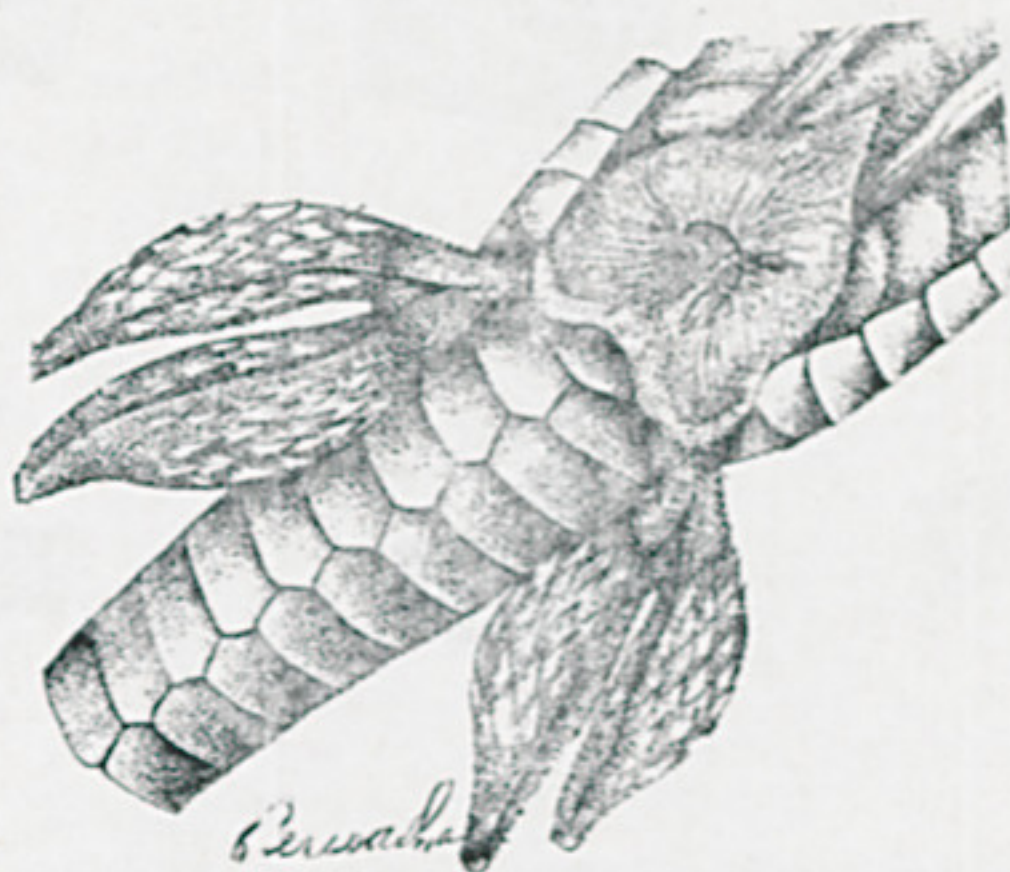
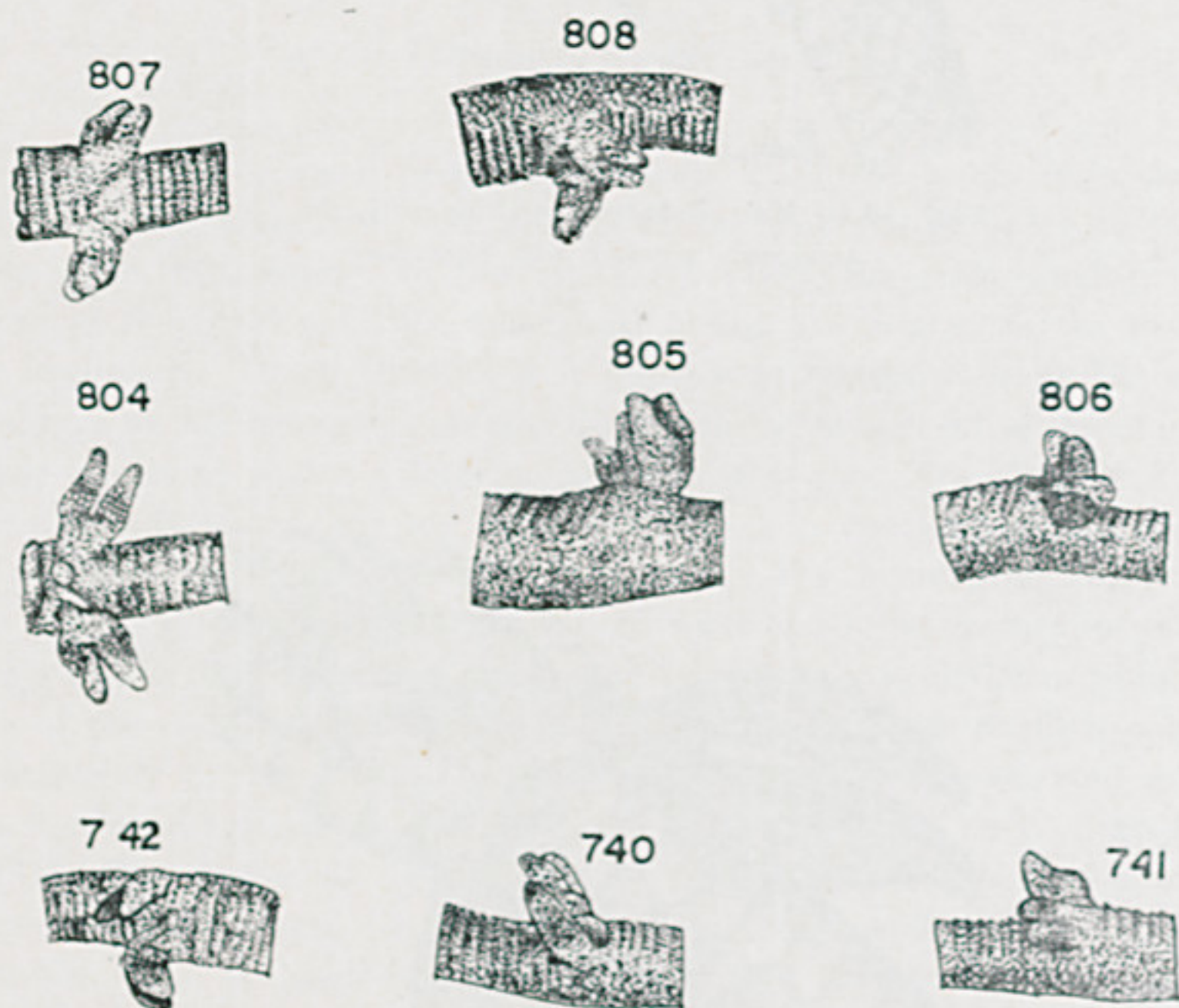


Fig. 17 — Ventral view of a dissected *Bothrops insularis* showing oviduct, cloaca and hemipenis at both sides.



described by Matthey (57) in the turtle *Emys europea*, in which an ovary existed on one side and an ovotestis on the other.

Experimental intersexuality has been obtained by Dantchakoff (15-18) in *Lacerta*, by Forbes (24-28) in Alligator, by Kehl (41-44) in various lacer-  
tids, and by Risley (63-66) in turtles. By treating the embryos in a later period of development in which the gonads were sexually determined, the last author obtained only the stimulation of the copulatory organs towards



*A. dir/s.*

Fig. 18 — Different types of hemipenis presented by embryos of *Bothrops insularis*. The figures correspond to the embryos included in table XII



the male sex, although the gonads do not respond to the hormonal stimulation towards an inversion to the originary sex. Nothing is known in this respect about Ophidia.

Summing up, it is evident that in reptile embryos, the gonads retain their bipotentiality through a period rather advanced of their development, and the ovaries retain the medulla longer than the testicles retain cortex.

B — *Description of the Embryonal Gonads in Bothrops insularis and other Snakes.*

The following embryos have been studied: (Tables XIII and XIV).

TABLE XII

Fully developed embryos of *Bothrops insularis* studied histologically

Mothers	Embryos	Sex			Observations
		Sex		Sex	
	Slide number	Embryo identification	Sex*	Degree of hemipeni ** development	
Intersex n.º 15115 IB Embryos of 130mm	740	a	male	++	Cortex traces in testicles  Total absence of hemipenis
	741	b	male	+	
	742	c	male	+	
	733	d	female	—	
6 males — 1 female	806	e	male	+	Histological lost material
	808	f	male	++	
	807	g	male	++	
	732	h	—	++	
Intersex n.º 15111 IB 3 males — 2 intersexes	727	a	male	+++	Only right hemipenis present
	731	b	female	+	
	804	c	male	+++	Only left hemipenis present
	805	d	male	++	
	728	e	female	+	

\* Sex — as determined histologically.

\*\* See text.



TABLE XIII

Early embryos of *Bothrops insularis* studied histologically

Species	N.º I. B. mother	Embryos n.º mm.	Prep. n.º	Observations
<i>B. insularis</i> ...	15.860	35-40 35-40 35-40	763 769 770	Germinative epithelium Without gonocytes
<i>B. insularis</i> ...	15.868	60-70 60-70	773 872	Gonocytes Both in cortex and marrow
<i>B. jararaca</i> ...	15.862	70	774	Gonocytes

TABLE XIV

Embryos from other species and new-born snakes Studied histologically

Species	mm.	Sex histological N.º preparation	Observations
<i>Xenodon merremii</i> .....	99	386 male	Cortex traces in testicles - Gonocytes in cortex and marrow
<i>Xenodon merremii</i> .....	180 180	730 male 729	Testicles well differentiated Ovocytes in leptotene and pachytene
<i>Crotalus d. terrificus</i> ...	newborn	832 male 837 female	Gonads sexually well differentiated.
<i>Bothrops alternatus</i> .....	24 hours after the birth	848 male 854 female 859 female	Gonads sexually well differentiated Ovocytes in leptotene and pachytene

*Bothrops insularis* embryos of 35-40 mm.*Bothrops insularis*: embryos of 60-70 mm.*Bothrops insularis*: embryos of 130 mm.*Bothrops insularis*: embryos of 160 mm.*Bothrops jararaca*: embryos of 70 mm.*Bothrops alternatus*: embryos of 24 hours (newborn)



*Crotalus durissus terrificus*: embryos of 24 hours (newborn).

*Xenodon merremii*: embryos of 99 mm.

*Xenodon merremii*: embryos of 180 mm. (almost at hatching).

All the embryos have been transversally sectioned in four segments of equal length, indicated as A, B, C, starting from the cloacal region in caudo-cranial direction. The embryos of 35 to 70 mm were arranged with all consecutive segments paralleling each other in the same paraffin block and sectioned together. This method facilitates the reconstruction and the immediate identification of the gonads. More advanced embryos and newborns have been dissected and the gonads and kidney fixed separately. All embryos have been fixed in Bouin fluid, stained with Harris or Haidenhain hematoxylin and eosin. Some sections have been stained with trichromic Mallory or with Feulgen.

In the younger embryos (35 mm) the gonadal ridge is very long and in the transverse section it appears on both sides. In older embryos there is an asymmetry as in the adults, and the right gonad is more cranial than the left one. For this reason in the older *insularis* embryos of 130-170 mm the gonad is present only at one side of a section.

We shall briefly describe here the situation of the gonads of the embryos in an age sequence.

1 — Embryos of *Bothrops insularis* of 35-40 mm from the pregnant intersex n.º 15.860. The embryonated eggs have been opened and the embryos fixed as soon as the amniotic cavity was open. The embryos have been stretched on a Petri dish, measured and fixed. They were then sectioned in the A, B, C and D segments as previously described.

Plate 7 A (embryo 763 and 769) (35-40 mm) shows a transversal section of this stage. The genital ridge on both sides of the mesentery is constituted by one layer of epithelium with some rare primary gonocytes distinguished by their larger size. The interrenal medullar blastem is clearly distinguished also by bearing some rare gonocytes.\* The gonads in this stage are thus completely undifferentiated. The mullerian duct is clearly visible as a little groove on the external and dorsal side of the coelomatic epithelium.

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\* Dorsally, this medullar blastem of the interrenals is clearly visible and in some section it is in direct continuity with the medulla of the gonads. These interrenal blastems are well defined and completely independent from the mesonephric tissue.

These observations show a histogenetic identity of the gonadic medulla and the interrenal blastem as shown by Vannini (72-73) in Amphibians, Birds and Mammals and by Chieffi in Selachians (9). We shall emphasize here these observations that complete the picture of the identity of origin of the two tissues (interrenal and medullar of the gonads) in the Ophidia.



## Plate 7



Fig. A



Fig. B

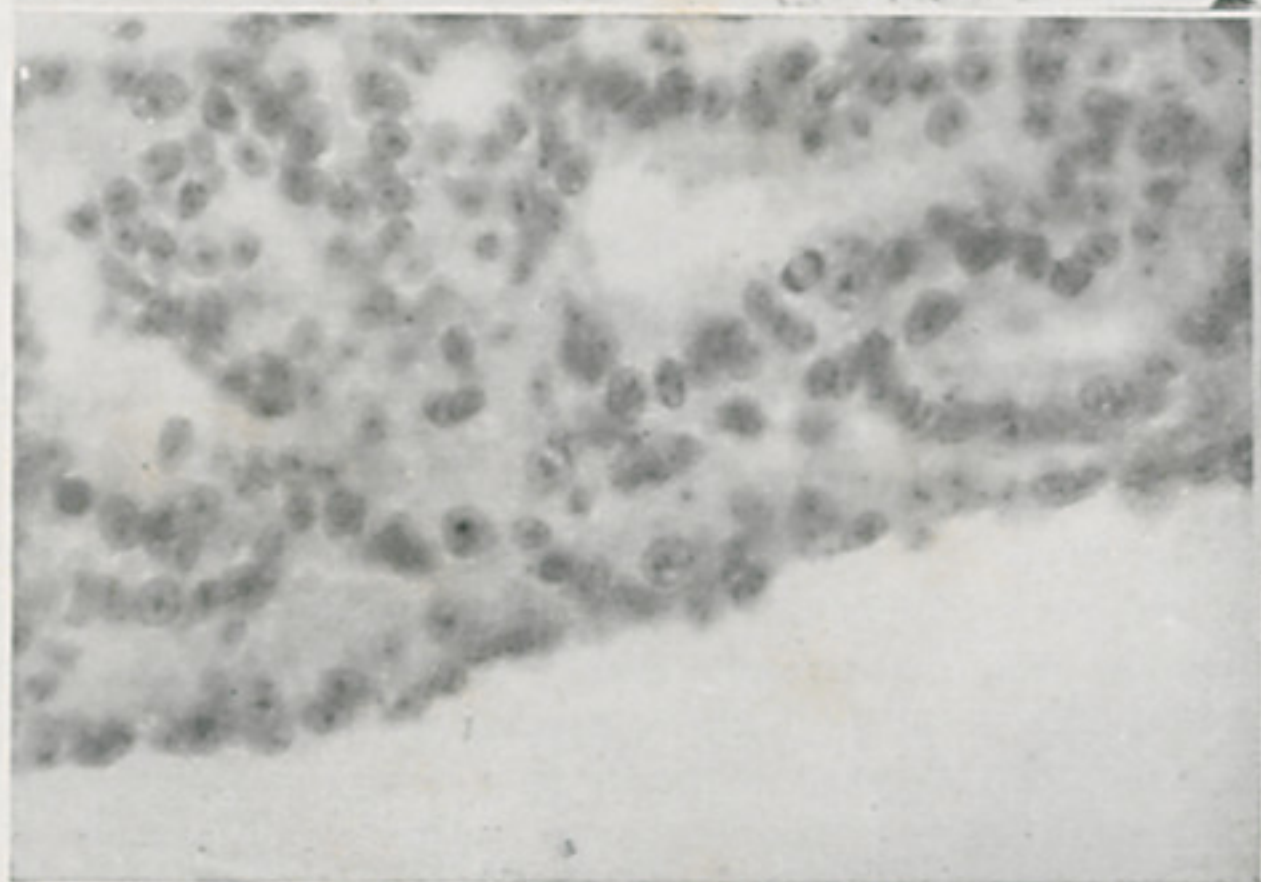


Fig. C



Plate 8

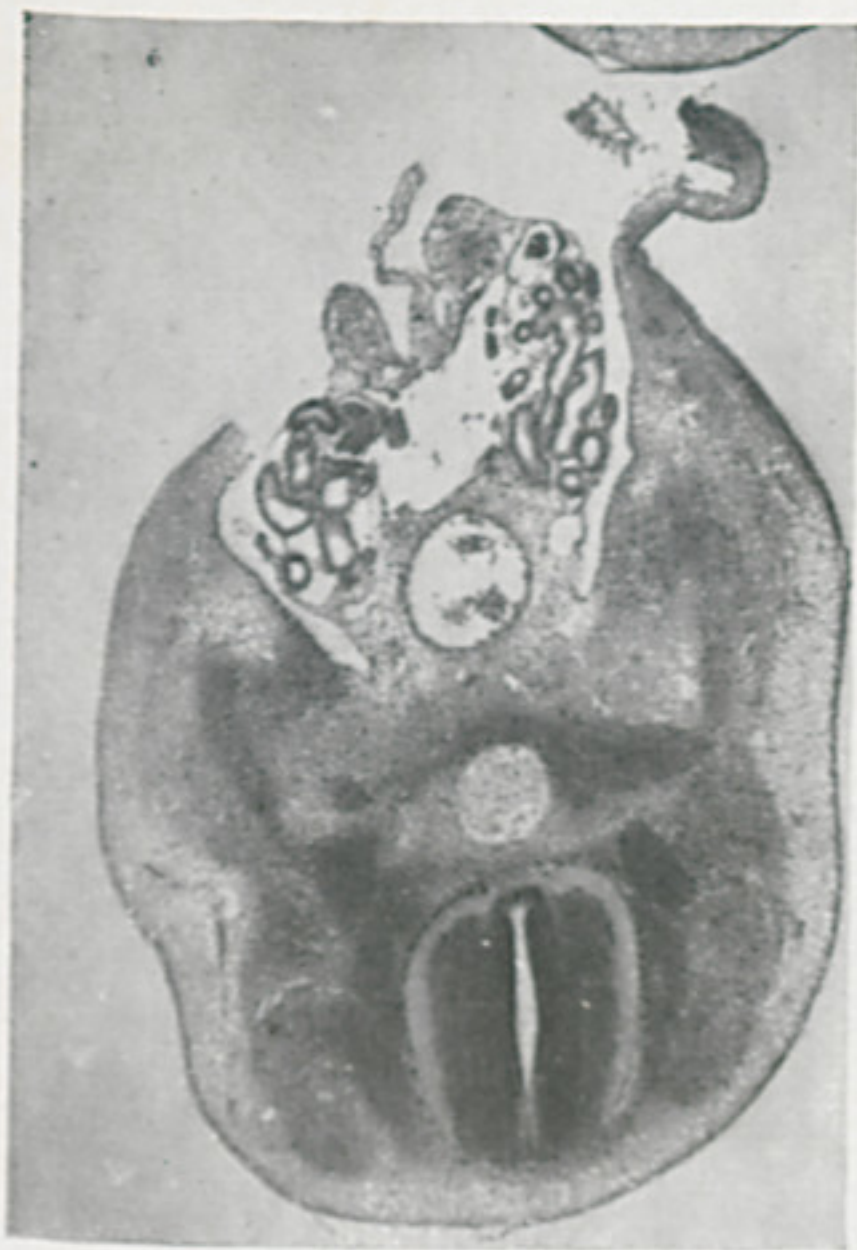


Fig. D

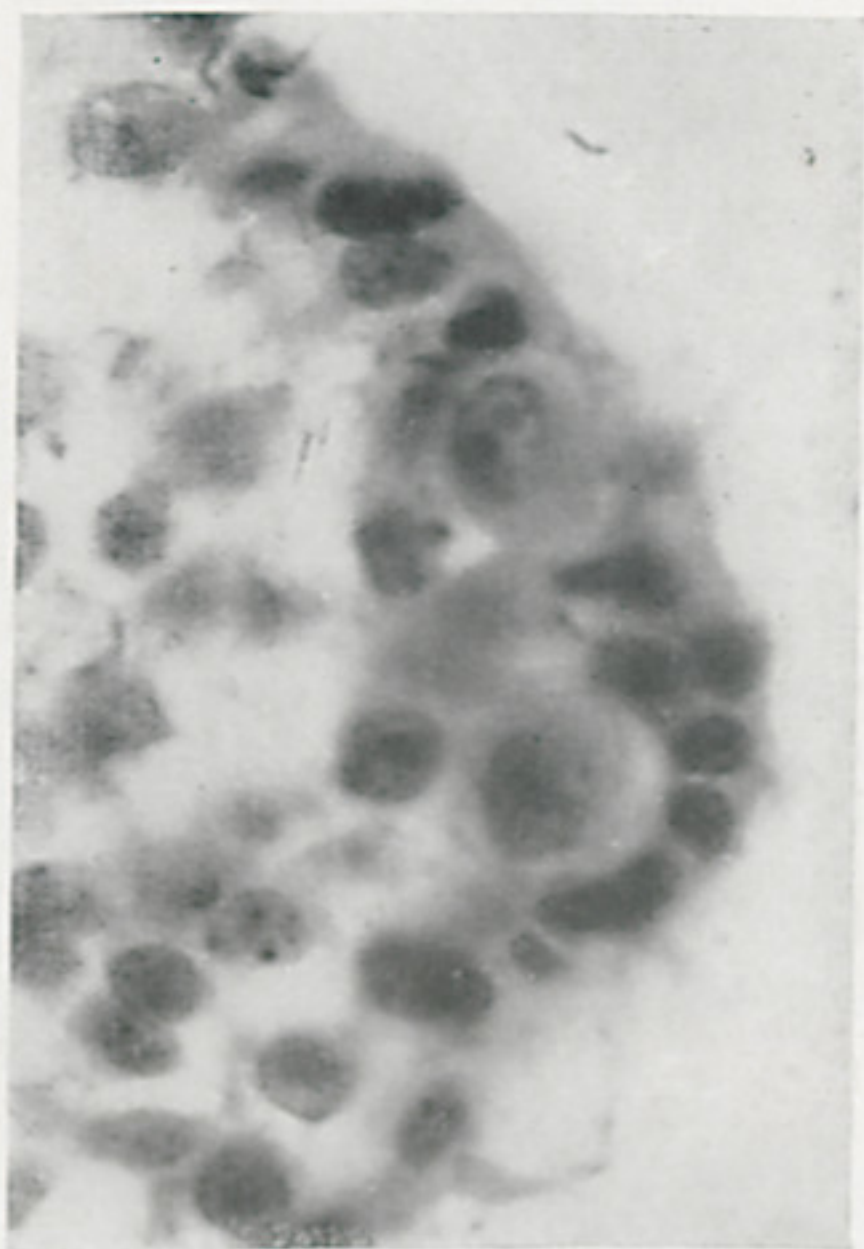


Fig. F

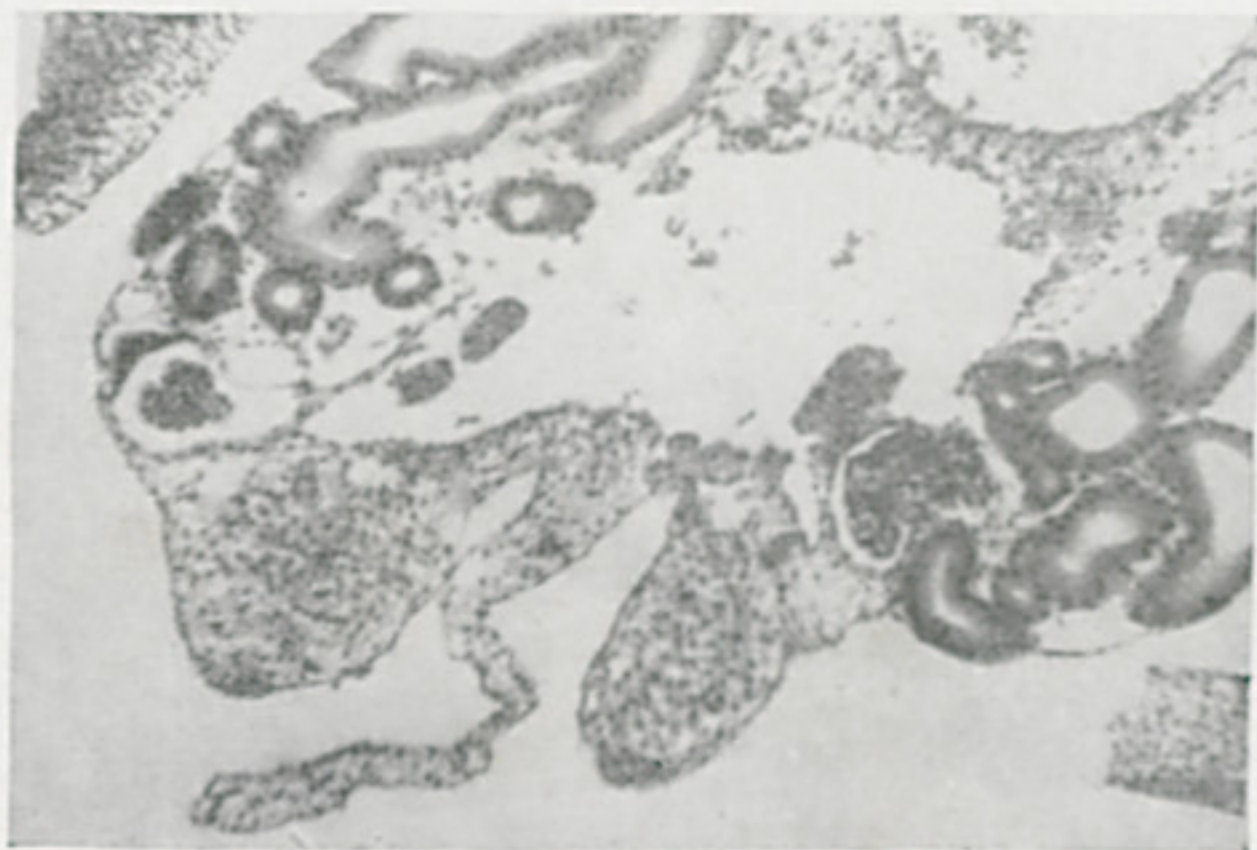


Fig. E



Plate 9

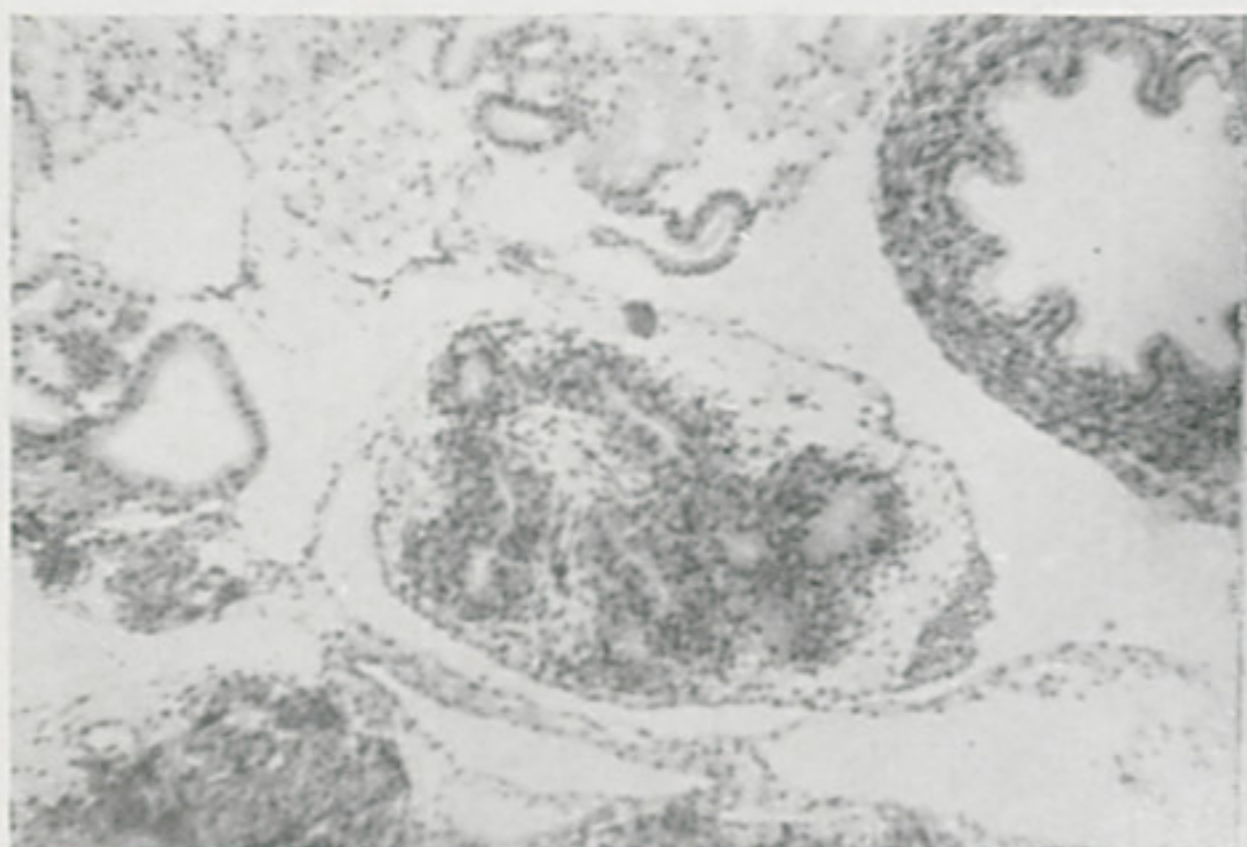


Fig. G

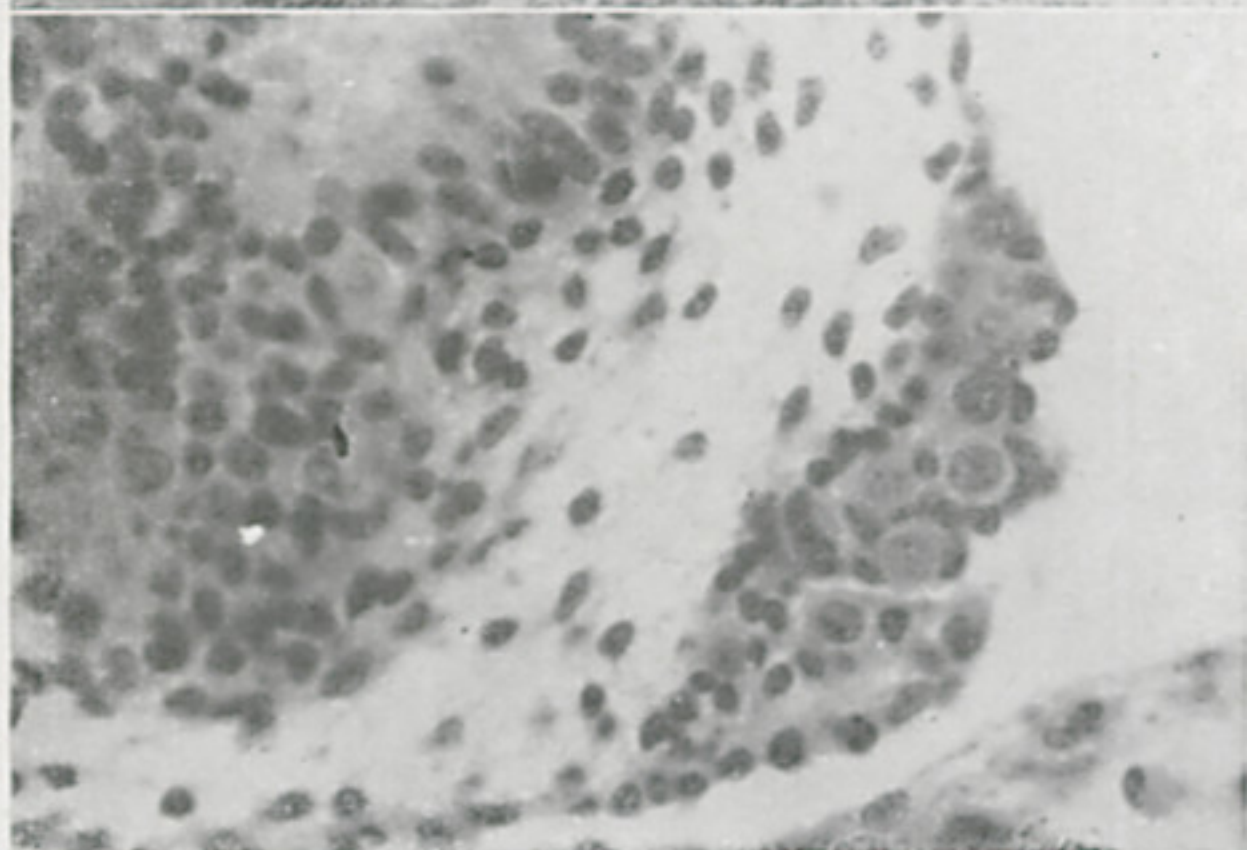


Fig. H



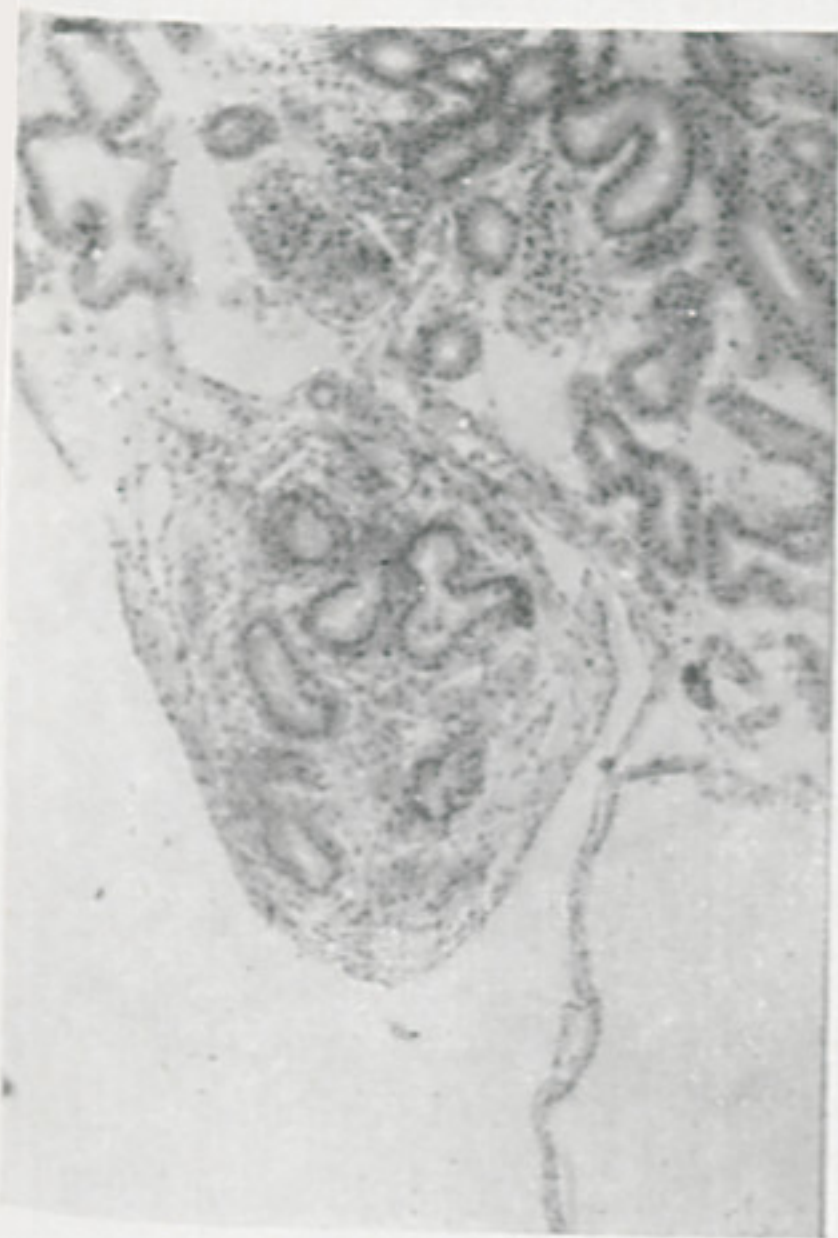


Fig. I



Fig. J

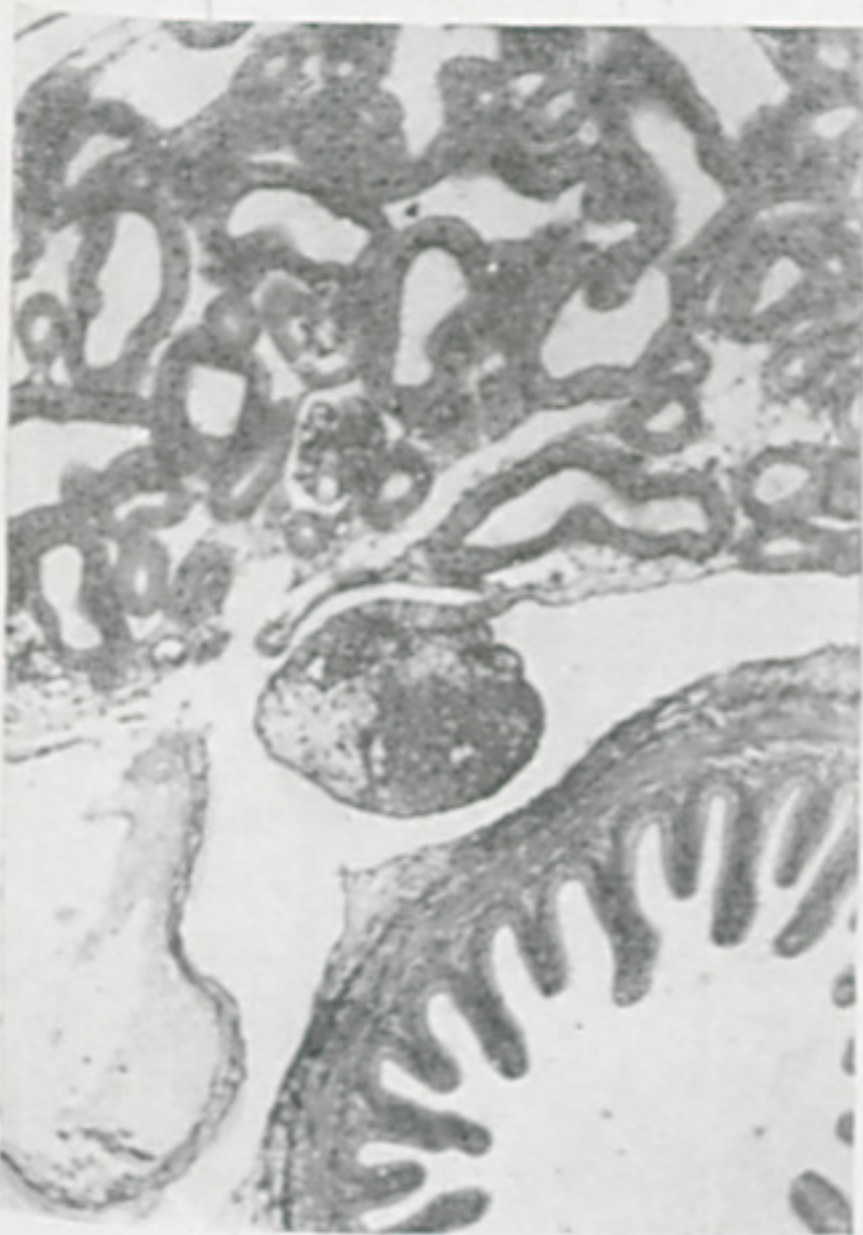


Fig. L

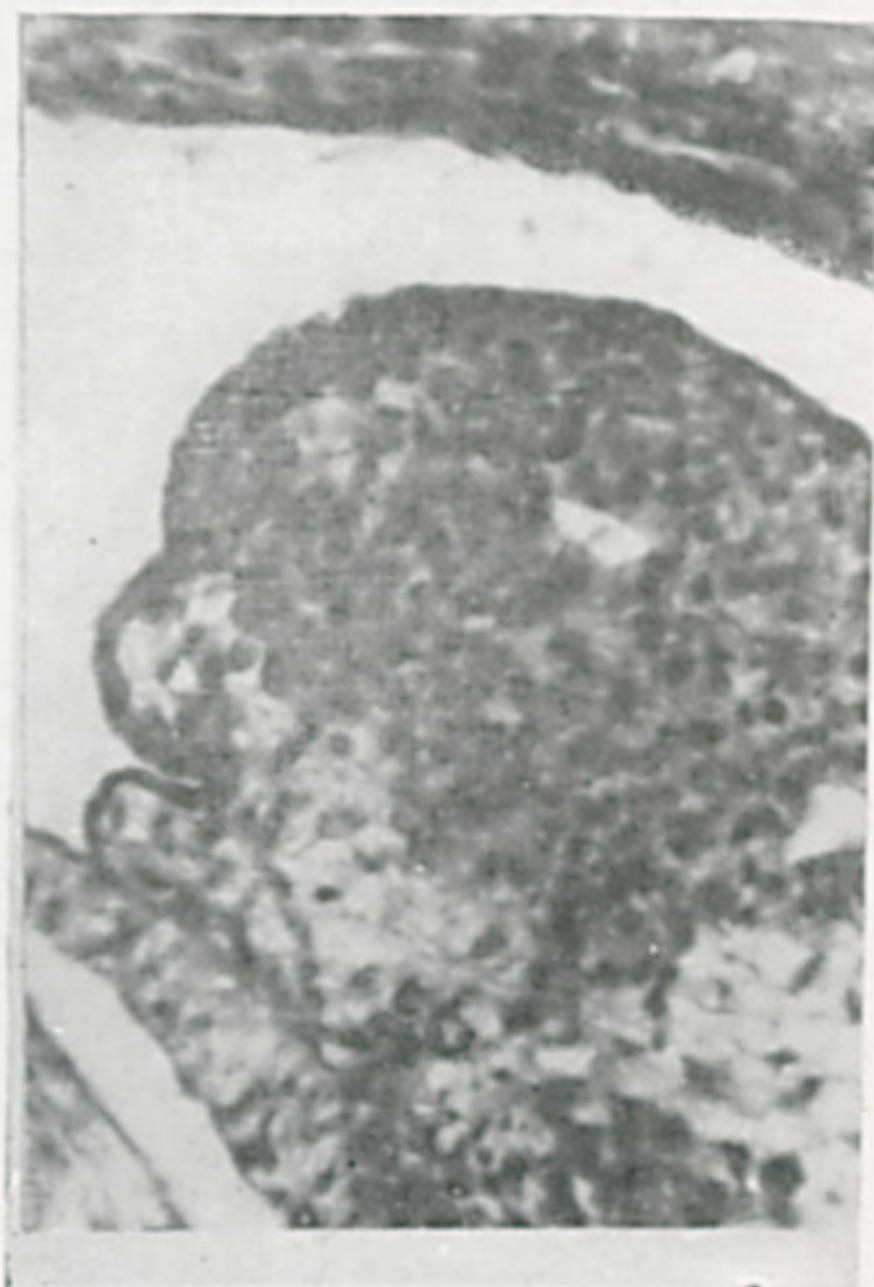


Fig. M



The same situation is shown in another embryo (n.<sup>o</sup> 769) (35-40 mm) in which some mitotic figures are shown by the gonocytes. The embryos n.<sup>o</sup> 770, (plate 7B and C) of the same age (35-40 mm) as the preceding ones, was sectioned frontally longitudinally and shows the gonadic area in which the medullar is disposed in cords, and rare gonocytes are equally distributed in both cortex and medulla.

2 — *Bothrops insularis* of 60-70 mm., n.<sup>o</sup> 773, plate 8 D, E, F from the intersexual mother n.<sup>o</sup> 15.868. The gonadic area is more prominent into the coelomic cavity. There are more gonocytes, the cortex is more developed than the medulla, and pluristratified, and it seems richer in gonocytes than the medullar region. The embryo n.<sup>o</sup> 872 of the same age (60-70 mm) seems to have more gonocytes in the medulla than in the cortex. The müllerian ducts are clearly visible on both sides.

3 — *Bothrops jararaca* embryo of 70 mm. This is at the same stage of development of the gonads as the embryos n.<sup>o</sup> 773 and 872 of *insularis*.

4 — *Xenodon merremii* Embryo of 90 mm. This embryo has been fixed from an egg when more or less one month old, having been layed on moist sand by a female; this happened at the "Ezequiel Dias Institute", Belo Horizonte, Brazil. The section at the region "D", that is rather cranial, presents two gonads with a distinct testicular structure. Medulla with tubular structure, with many gonocytes. At the cranial region of the testicle the interrenal blastem is visible caudally. This testicle presents a rather prominent region of cortex bearing some gonocytes. (Plate 9 G-H). At this stage, therefore in *Xenodon*, the gonad is well differentiated, although with a residual feminine cortex, as encountered in other Reptiles mentioned before. This embryo must have been at approximately one third of its developmental period.

5 — *Bothrops insularis* 130 mm. These embryos are at a rather advanced (Plate 10 I-J-L-M) stage of pregnancy. Although it is not possible to determine the date, we think it must have been close to eclosion. The two pregnant intersexes n.<sup>o</sup> 15.115 and 15.111 were captured by the same expedition and killed at the same time. The embryos of n.<sup>o</sup> 15.115 were at a less advanced stage than those of n.<sup>o</sup> 15.111. The hemipenis is perfectly recognizable, and Fig. 19 shows the post-cloacal segment in each different stage of development of the copulatory organ.

All embryos of this lot have been prepared according to the scheme indicated before and the gonads were generally situated at segment D, a few partly at segments C or E.



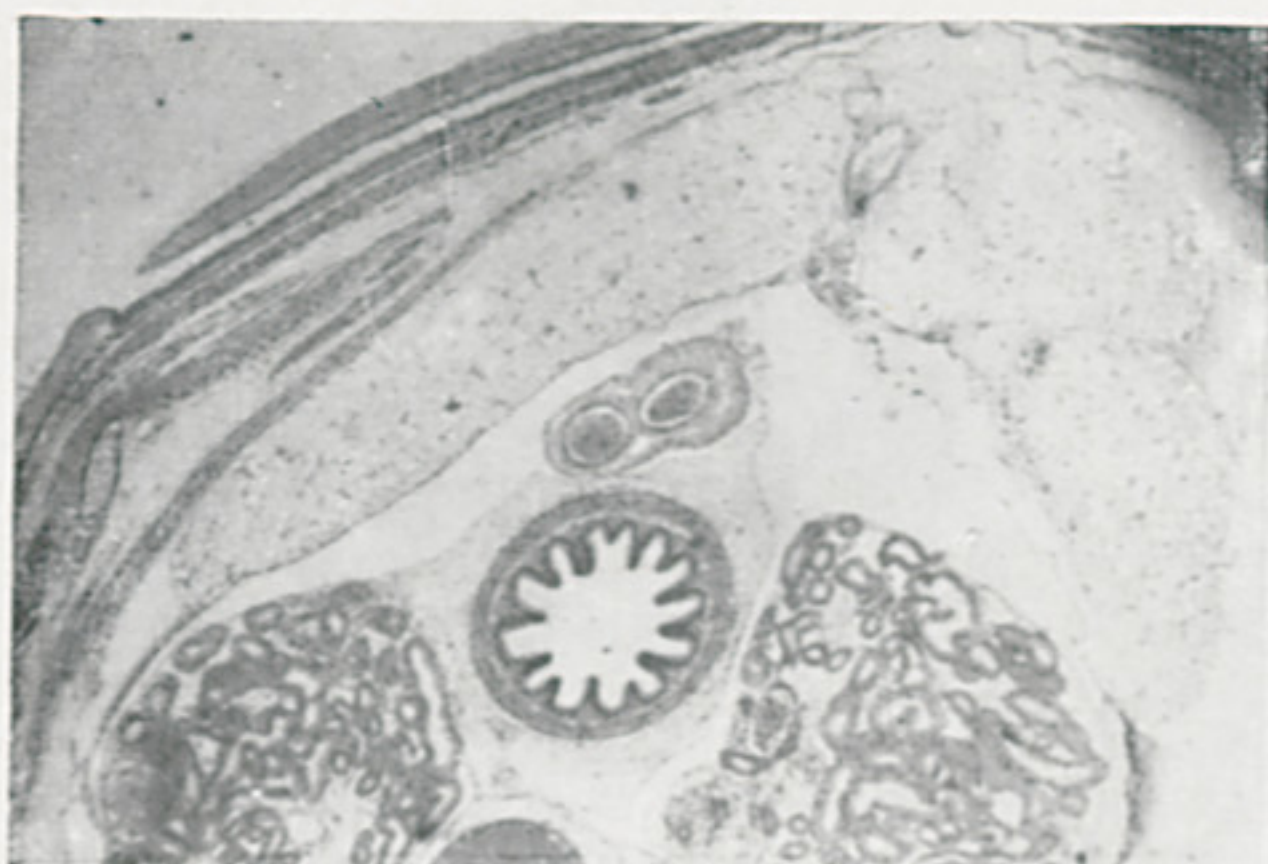


Fig. N

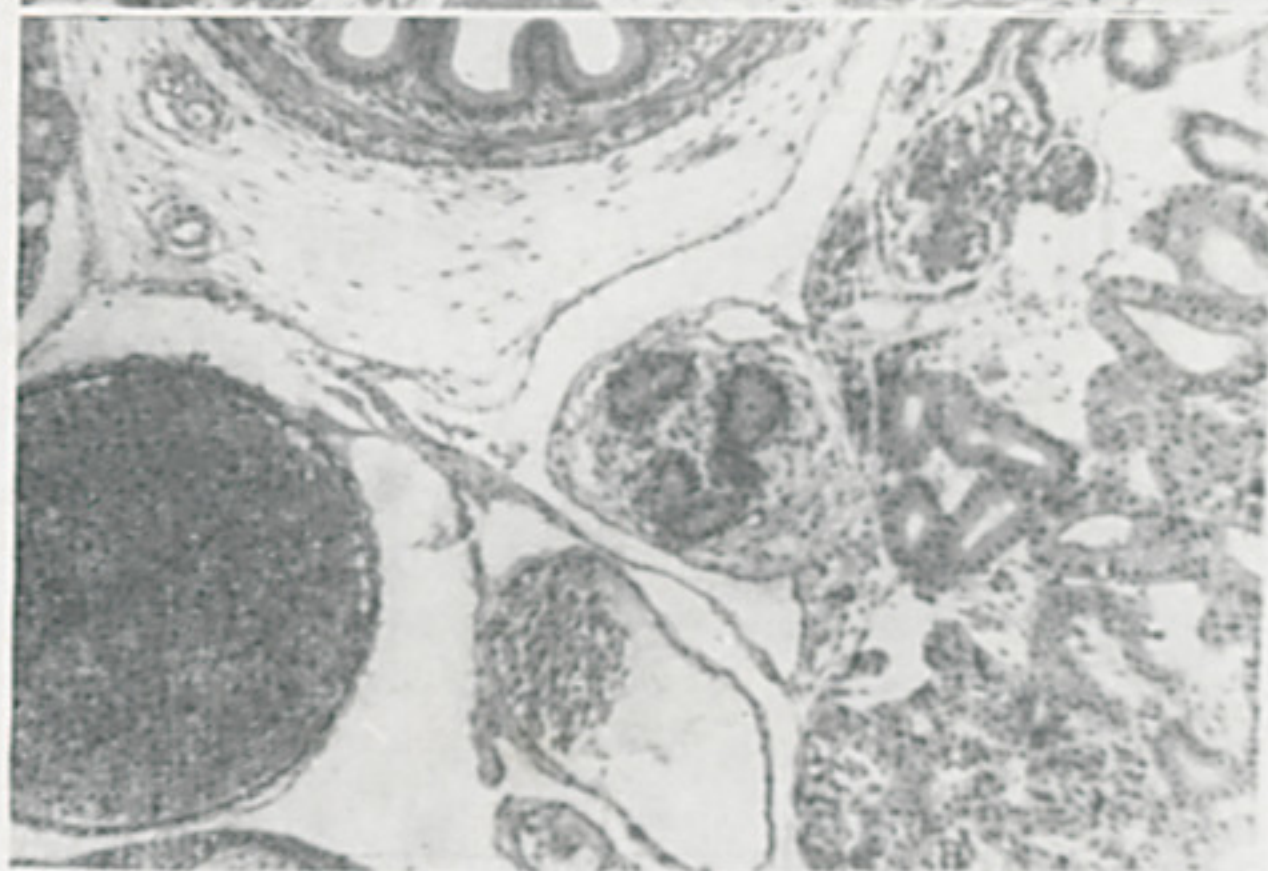


Fig. O

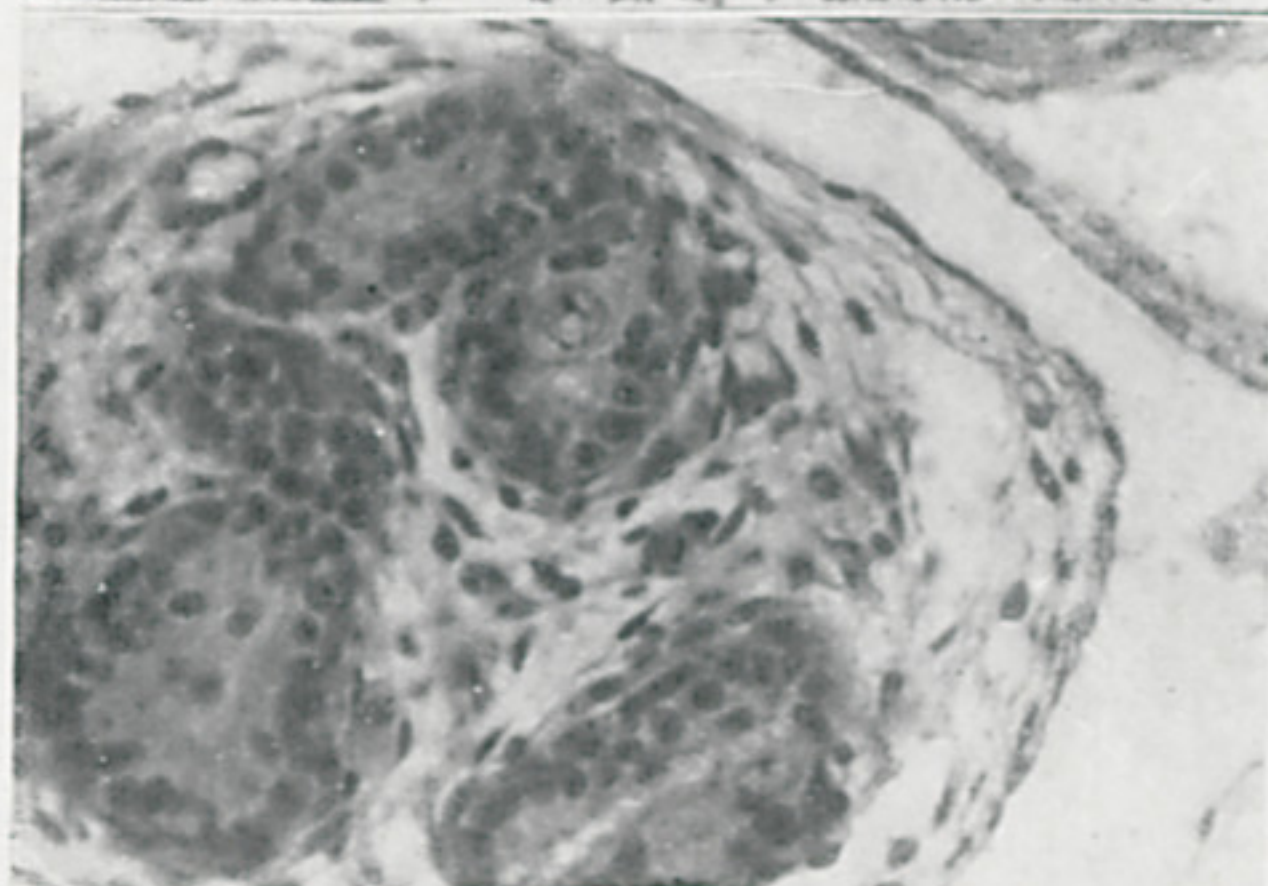


Fig. P



## Plate 12

Fig. Q

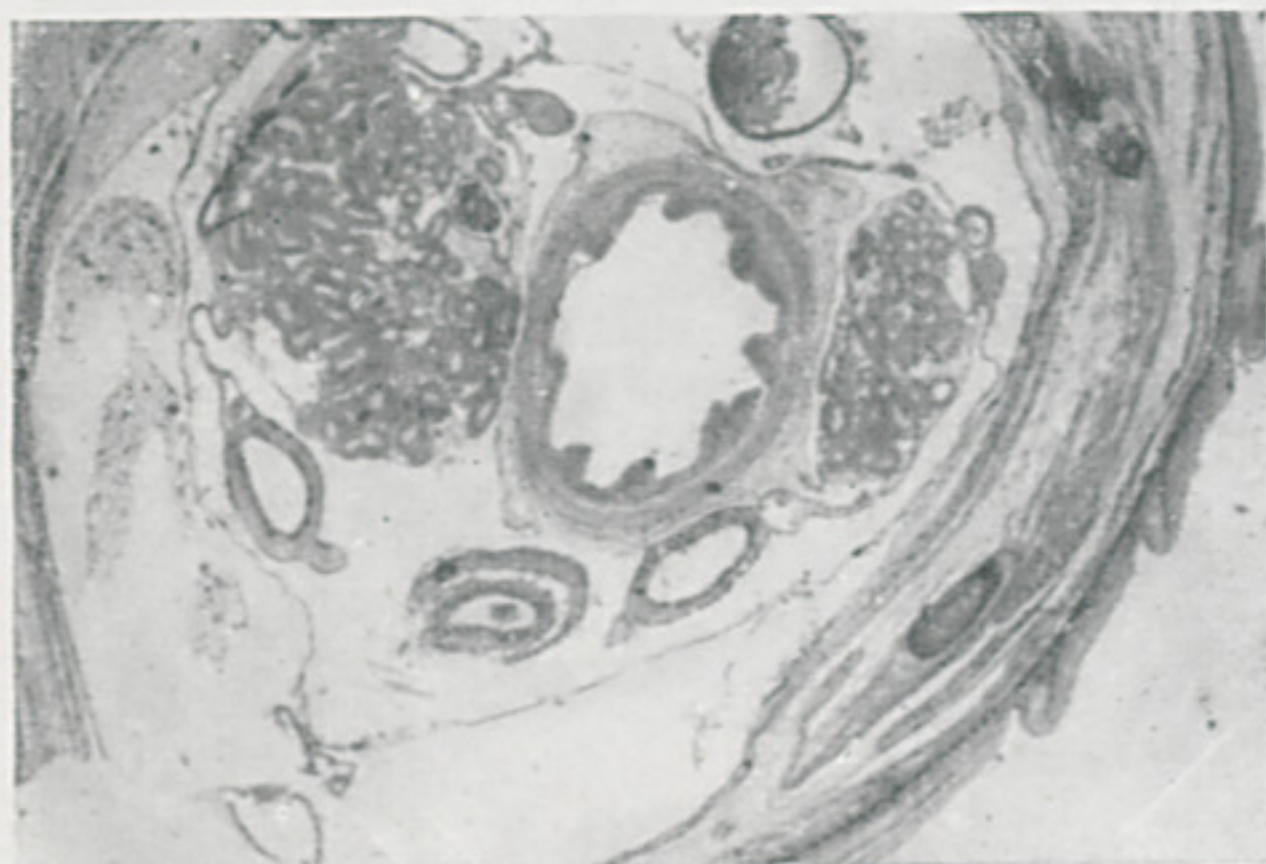


Fig. R

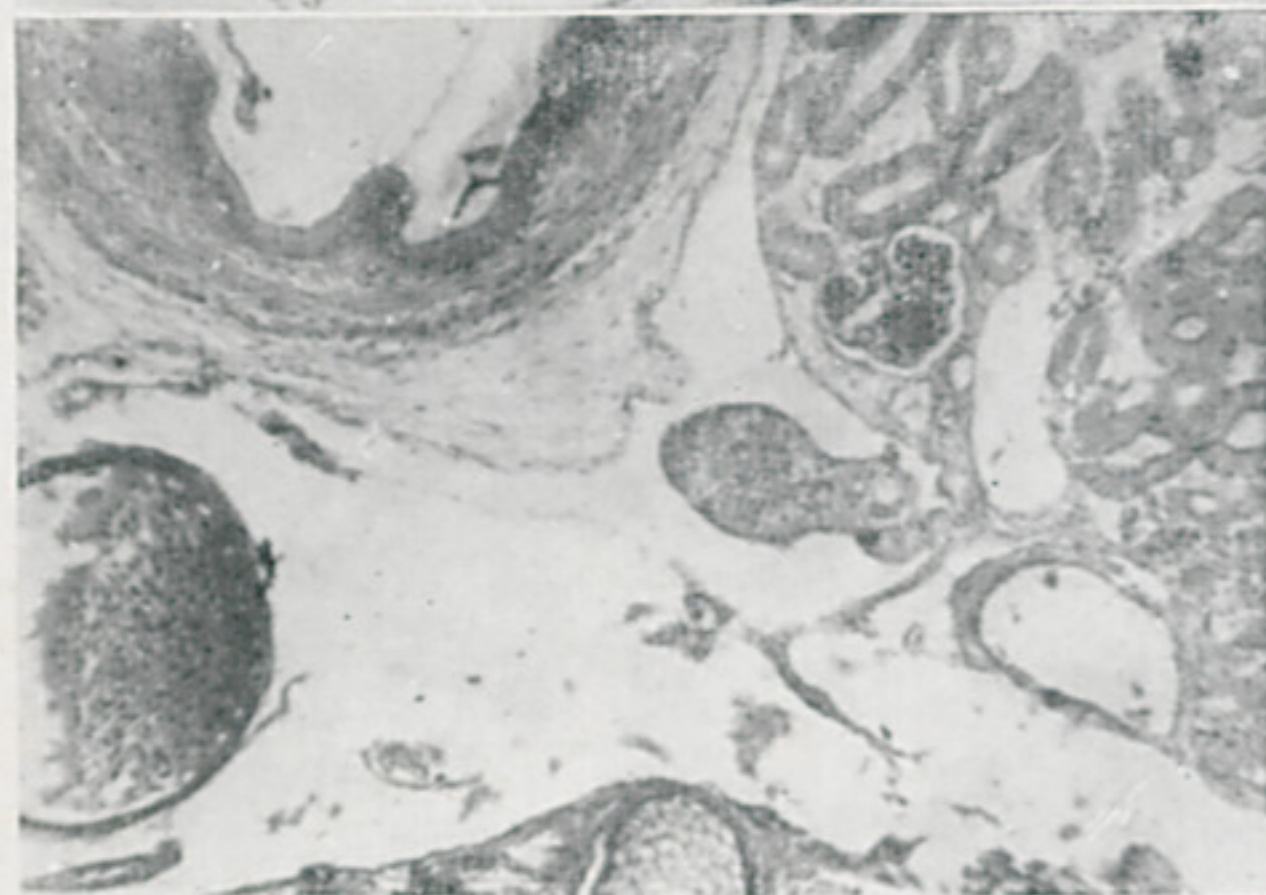
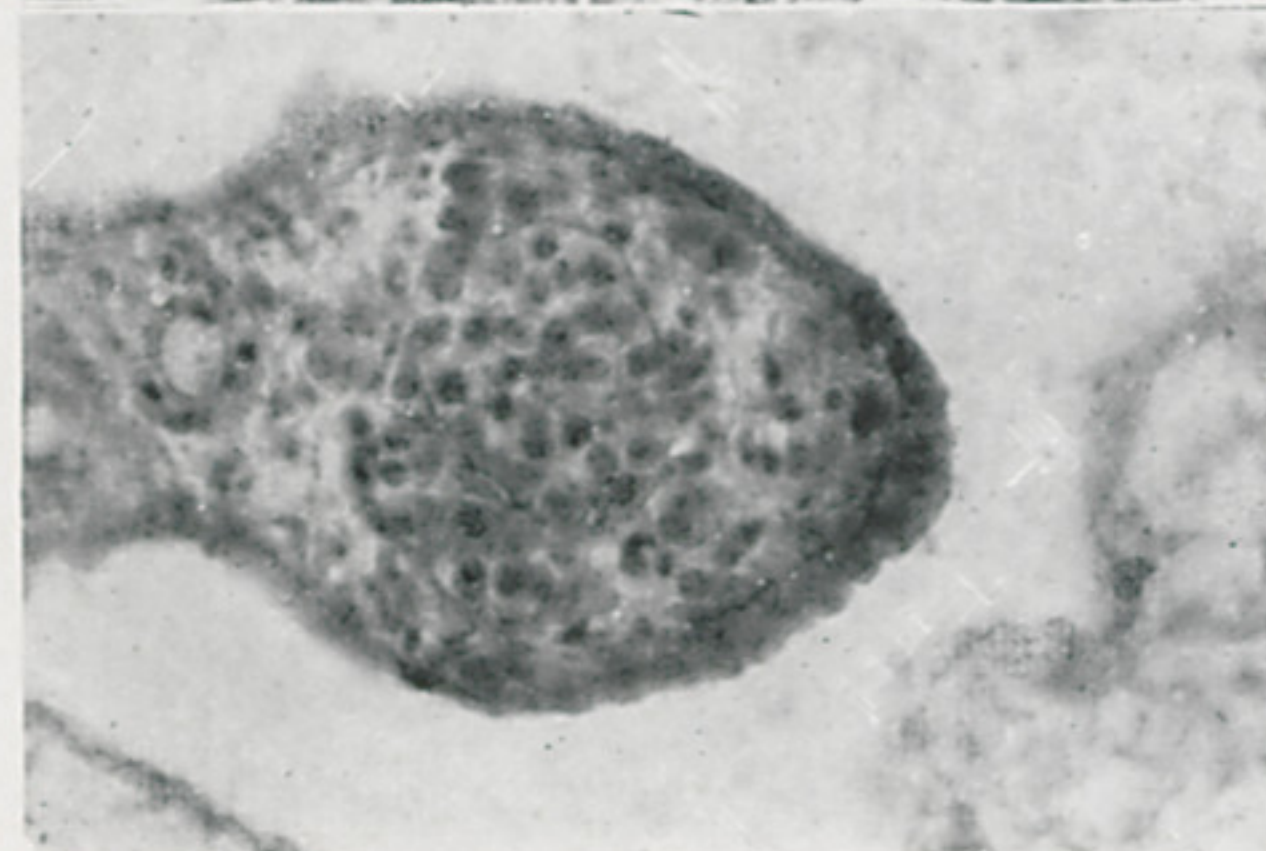


Fig. S





*Embryos n.º 740* (mother 15.115) "a". The gonads have the structure of a well differentiated testicle, and lack mullerian ducts. Cortical epithelium is monolayer and lacks gonocytes. Only at the cranial region of the left testicle there is a little cortical zone with pluristratified epithelium limited to a few sections and without any gonocytes. Like the epithelium, medulla tubule actively proliferating but with few gonocytes, (Plate 10 I).

*Embryo n.º 741* "b, c", same mother as the precedent. This one, as well as embryo n.º 742 "c", has the same testicular structure as n.º 740. Rare gonocytes and no mullerian duct.

*Embryo n.º 733* "d", the gonad is typically feminine, connected with the mesonephros by a peritoneal peduncle (plate 10 J). Cortex pluristratified, with gonocytes. Medullar cords solid, well separated from the cortex by a connective layer. Sometimes, the cords show a tubular structure clearly evident in longitudinal section (plate 10 L). Gonocytes distinctly localized in the cortex, although some rare ones are spread into the cavity of the medullar cords. The gonocytes of the cortex have a well differentiated layer of follicular cells. Mullerian ducts well developed (plate 10 M). This embryo was the only one showing lack of hemipenial structure.

*Embryos n.º 806 "c", n.º 807 and 808.* All with testicular gonads as n.º 741 and 742. Strong proliferation of the medullar canals, but with few gonocytes. Some gonocytes spread into the extra medullar conjunctive tissue, near to the epithelium of the coelomatic cavity. Mullerian ducts totally lacking. In all these embryos the interrenal presented zone of epithelial cords dorsally and laterally to the mesonephros and in some sections there are isles of paragangliar tissue.

6 — *Embryos of the intersexual mother n.º 15.111 — 160 mm.*

*Embryo n.º 727* "a". This is the embryo with the most developed hemipenis. Testicles well evident, but not more developed than those of the embryos from mother n.º 15.115. The tubular structure of the medulla is perhaps more clearly defined. Few gonocytes. Absence of mullerian ducts (plate 11-N-O-P).

*Embryo n.º 728* "e". Hemipenis only on left side. Ovary with pluristratified cortex at the central region. At the sides the epithelium is monostatified. Rare gonocytes in the cortex and in the conjunctive tissue, but not in the medullar cords. Longitudinal section as in the embryo n.º 733, (Plate 12 Q-R-S). Müllerian ducts well developed.

*Embryo n.º 731* "b". Hemipenis only on right side, not well developed. Gonads with feminine structure, with pluristratified cortex well separated by



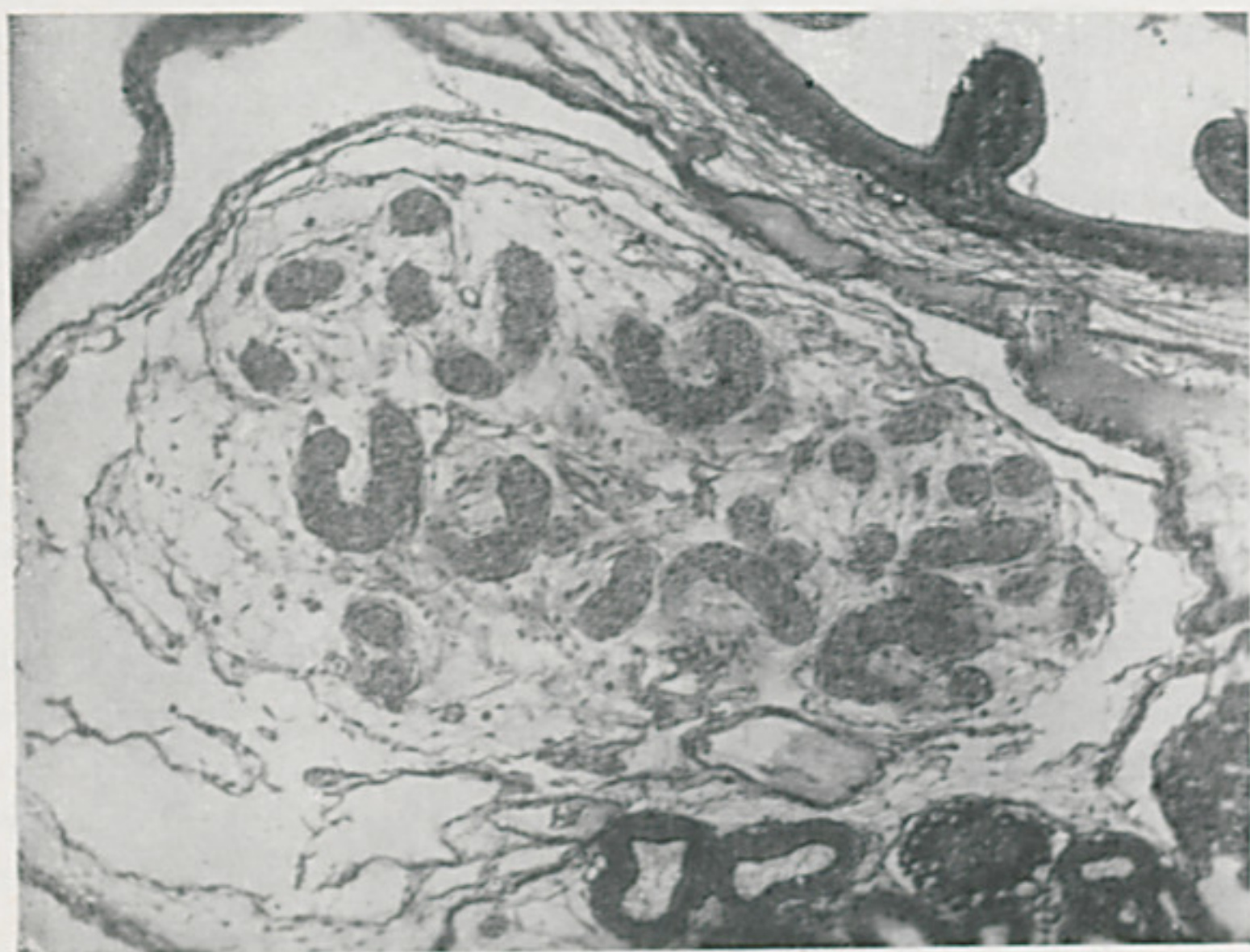


Fig. T



Fig. U



Plate 14

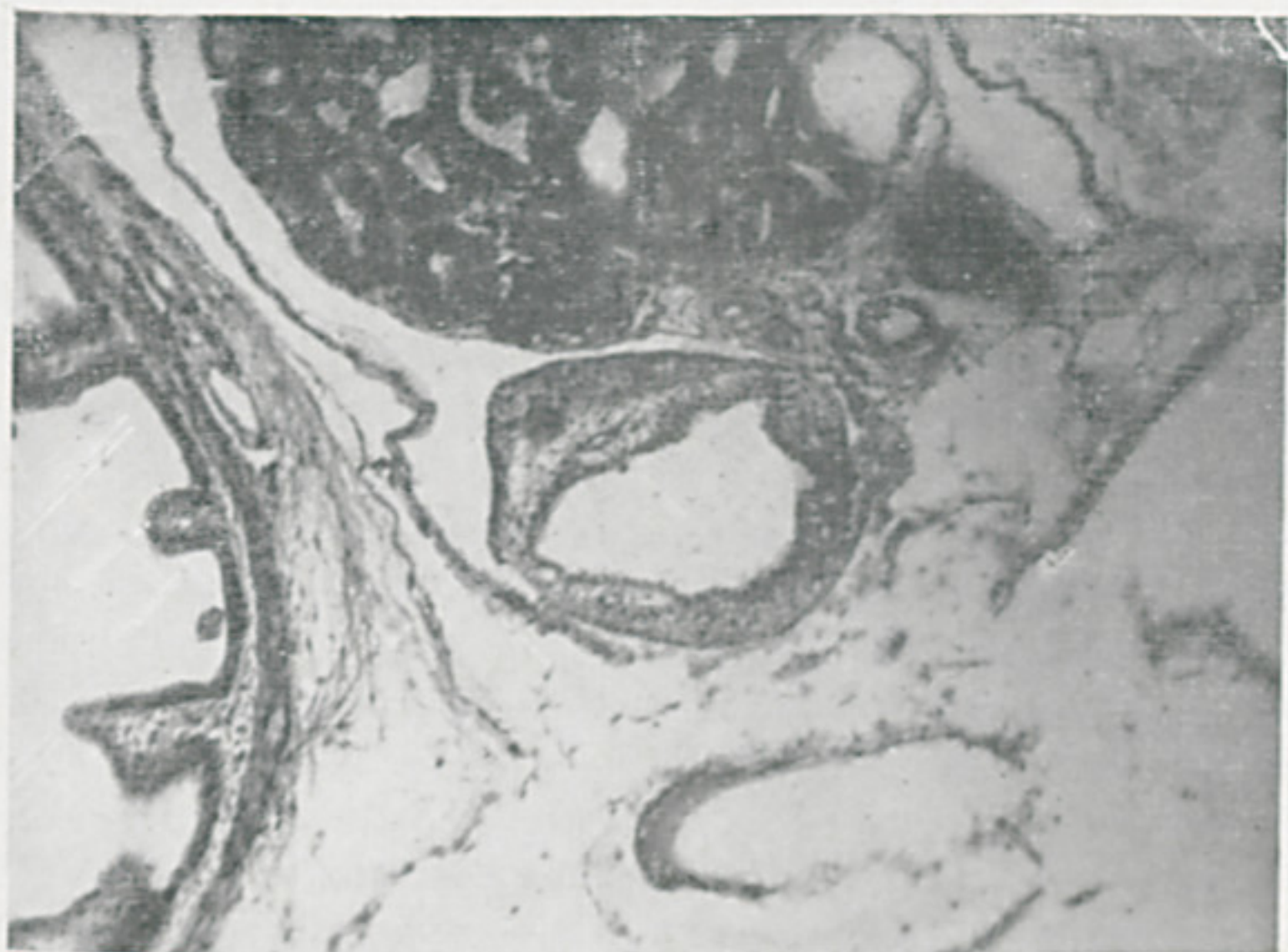


Fig. V

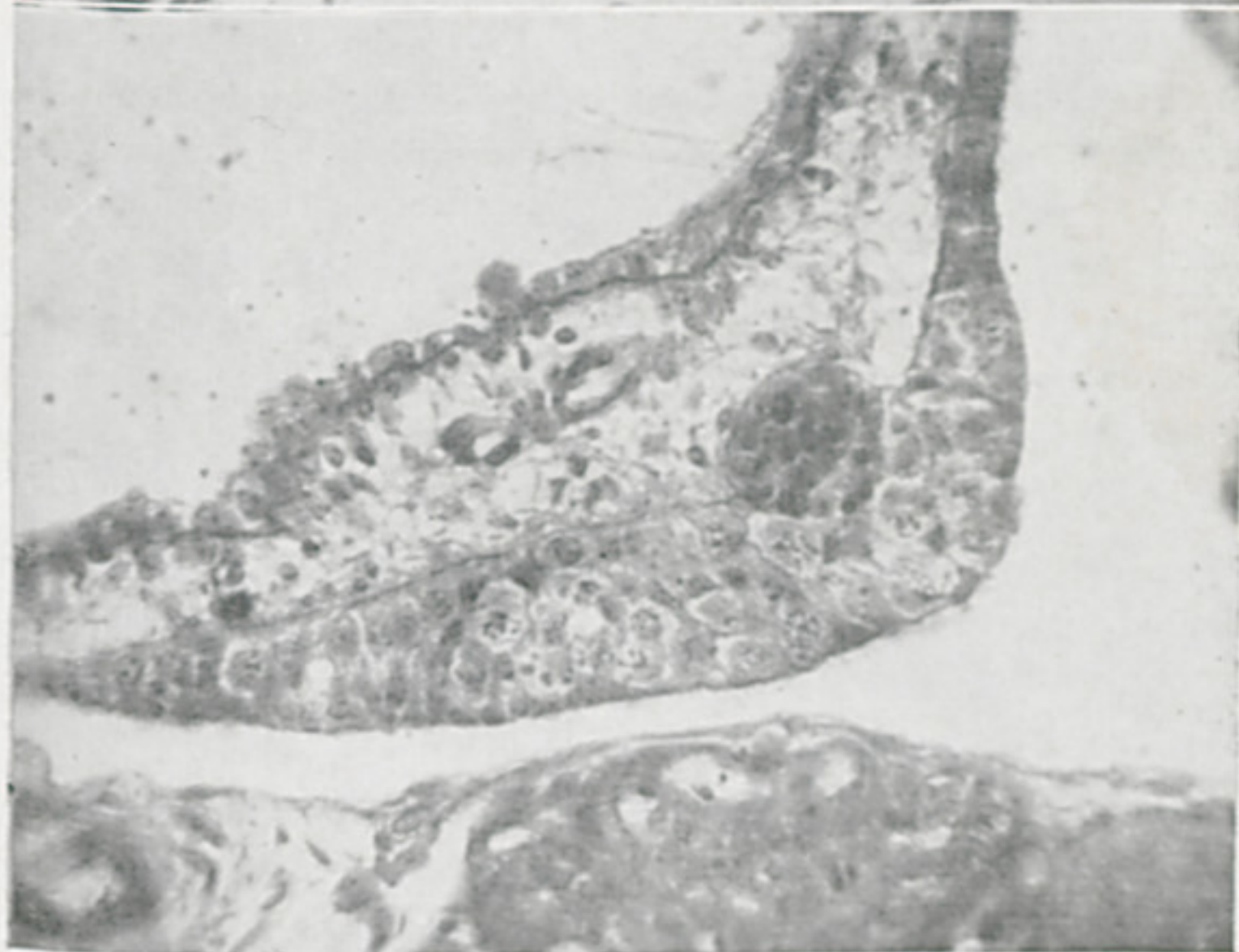


Fig. W



a layer of conjunctive tissue from the non-tubular medullar cords. Rare gonocytes in the cortex as well as in the medullar cords. Two well developed mullerian ducts.

*Embryos n.º 804 and 805.* Testicles with the tubular structure of the medulla with rare gonocytes. No mullerian ducts. Hemipenis well developed on both sides.

7 — Embryo of *Xenodon merremii* of 180 mm.

*Embryo n.º 729* (with well developed hemipenis) (plate 13 T-U). Testicles well developed, with solid medullar cords isolated in the middle of a loose conjunctive. Gonocytes frequently with mitotic stages. No vestiges of the cortex.

*Embryo n.º 730* (without hemipenis). Ovary well developed with a great central cavity surrounded by endothelium. Cortex pluristratified thicker at the ventral region, with many gonocytes at the leptotene and pachytene stages (plate 14 V-W). It is to be noted that the ovary at this stage is more precocious than the testicles in which no gonocyte appear to have begun the meiotic evolution.

8 — *Embryos of Crotalus durissus terrificus* (24 hours after birth). One male and one female embryos of this lot have been dissected and the gonads fixed together with the kidney.

*Newborn n.º 832* (male). Testicles well differentiated, bound to the kidney. Medullar cords without lumen, with few gonocytes in mitotic proliferation.

*Newborn n.º 837* (female). Ovary sac-like with the endothelium of the cavity folded. Cortex only at the ventral side with few gonocytes.

9 — *Embryos of Bothrops alternatus* (24 hours after birth). Gonads fixed separately as indicated for *Crotalus*.

*Newborn n.º 848* (male). Testicle with solid cords rich in gonocytes arresting stage.

*Newborn n.º 854* (female). Ovary sac-like with cavity endothelium folded. Cortex limited to inner side proximal to the kidney. Many gonocytes with different nuclear sizes. Leptotene well evident.

*Newborn n.º 865* (female) Like preceding one.

*Newborn n.º 859* (male). Testicles very large. Medullary cords solid, but rich in gonocytes of variable size, some of them in mitosis.

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Note — The numbers 15.111 and 15.115 refer to the enumeration used in the Collection of the Instituto Butantan. The number 763 and those following refer to the histological preparation at the Institute of Biology at Belo Horizonte.



C — Discussion of the embryological researches

Summing up what we have observed in the embryos it is possible to come to the following conclusions:

1 — In the embryos of 30-70 mm of *insularis*, as well as of "jararaca", probably during the first half of the pregnancy the gonads are not sexually differentiated and localization of the gonocytes in the genetical ridge is already at the beginning. At this phase it is interesting to note that the medullar tissue is still connected with the interrenal blastem.

2 — In the embryos of *Xenodon*, probably during the first half of the pregnancy period, the gonads are sexually differentiated but the testicles retain an evident remain of cortex, as the ovaries retain a noteworthy portion of the medulla, both sexes bearing gonocytes in both parts of the gonad.

3 — In the embryos of *insularis* of 130 mm., probably no far from the birth, the sex is perfectly differentiated. The differentiation of the ovaries is slightly delayed, retaining a medulla as the solid cord stage; the central cavity of the ovary is not yet opened. The testicles are well constituted still at the solid stage of the medullar cords. Only one testicle was found retaining a residue of cortex at one apex.

4 — In newborn snakes, as observed in *Xenodon merremii*, *Crotalus durissus terrificus* and *Bothrops alternatus*, the gonads are perfectly differentiated from the histological point of view full of gonocytes, and the ovaries have gonocytes at the beginning of the meiotic prophase, while the testicles bear gonocytes still in mitosis.

Although this series of stages has not been fashioned with a unique species of snakes, the seriation appears to be quite complete. No newborn of *insularis* has been sacrificed for histological researches, because of the hope to raise them to maturity or to some crossings with related species of *Bothrops*. Unfortunately, they usually died shortly after birth and no good material for histological work could be preserved. The observation seems to confirm Risley's statement that some residue of the blastem of the opposite sex is retained in the full differentiated gonads in both sexes, but that the ovary is, in general, provided with medullar blastem for longer time. At birth, however, it seems that the ovary is ready to initiate meiosis before the testicles.

The embryological researches in the problem of the intersexuality of *insularis* are thus clearly indicating that the individuals bearing hemipenis on one side only are histologically females, while some embryos with a poorly developed but bilateral hemipenis, were definitely males. The sole individual completely deprived of a hemipenis, was definitely female. The intersexes are



not only females from the point of view of histology of the ovary, but bear two well differentiated mullerian ducts that never exist in embryos with testicles.

We cannot, at present, deny that the residue of cortex which can be found in some embryos could subsequently develop into an ovarian cortex, and the medulla degenerate, thus giving a true sex inversion. It seems that this fact can really be accomplished in some Reptilians by hormone treatment in the embryonal stage. Nothing is known about this possibility in snakes nor does it appear to be possible in other reptiles in a more advanced period of development of the gonads (Risley). The fact that only the cloacal region is sensitive to hormone treatment in this later period seems to be interesting for the case of the *insularis*, in which the only masculine character of the intersexes is precisely the development of a hemipenis at the sexual sensitive region of the cloaca.

The statistical analysis shows the feminine nature of the intersexes, and we discussed the genetical value of the somatosexual characters of the pholidosis. However, we must not forget that the statistical analysis shows also an overlapping of the dispersion field of all the sexes and intersexes. We cannot deny, due to this fact, that some males with the pholidotic characters more feminine, and thus genetically not so strong males, could eventually during the development invert the gonadic constitution with an overgrowing of the cortical residuals (see discussion for the diminution of snakes and increase of intersexes). The same can be said of some genetical females, more deviated towards maleness from the statistical point of view in which the medullar region of the gonads or part of the adrenal tissue could eventually take an advantage and at least influence the inversion of the cloacal territory into a hemipenis (even in the embryonic period). This is all theoretical speculation that we must consider as a possibility, according to the data at our disposal. We shall discuss later the problem of the nomenclature of the sexual abnormalities of *insularis*, but we can state here that it is not an easy task, and at present the term "intersexuality", specially in the broad sense of the classification of E. Padoa (58), is still the best to be used.

#### 5 — *A case of true hermaphroditism.*

During the visit to "Queimada Grande" island from november 24 to december 3, 1953, one specimen of *Bothrops insularis* was found that presented a special interest and is here discussed in more detail. This snake (Instituto Butantan, n.º 15.843), dissected in december 15, 1953, presented both right and left hemipenis well developed, and normal left and right ovaries, but at the left side there was a testicle of about normal size. The cloaca received on the right side the mullerian duct as a normal oviduct. On the



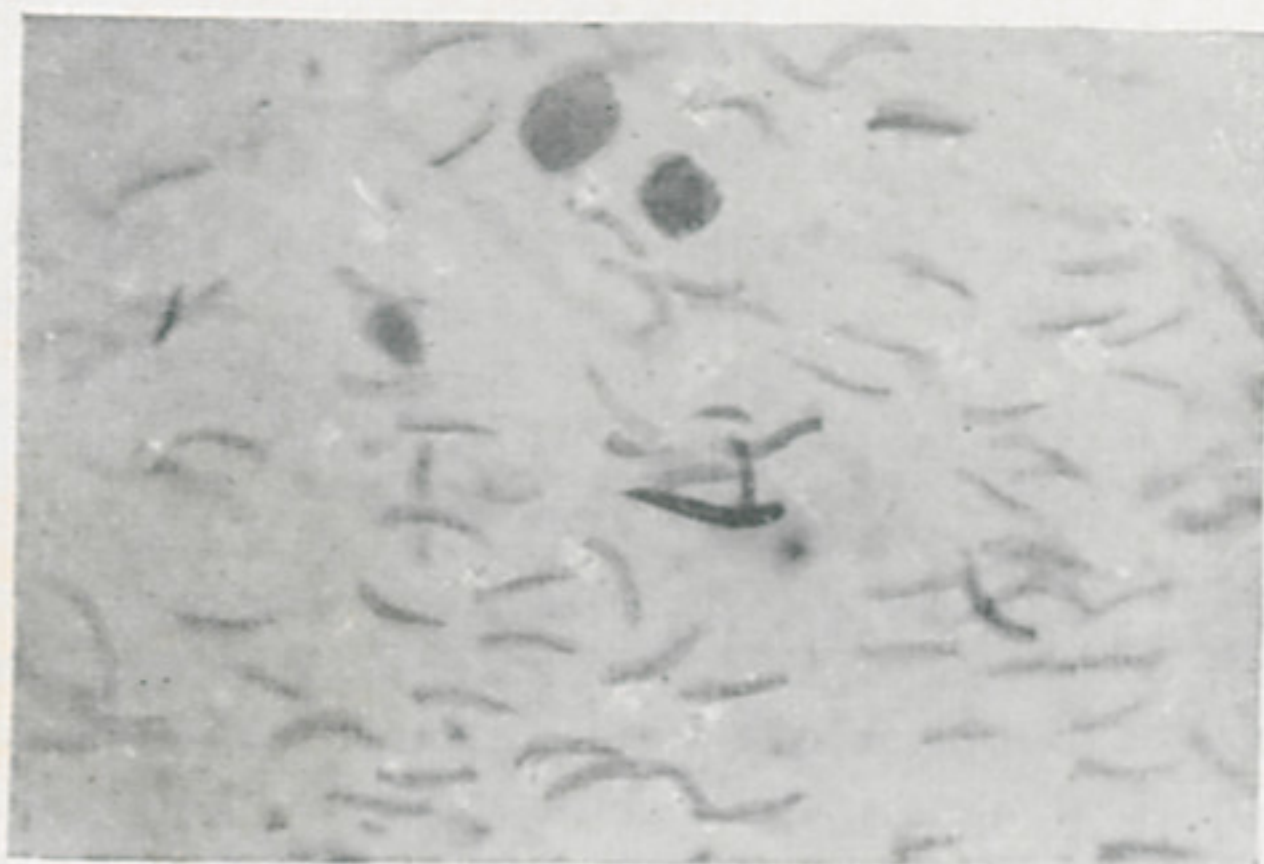


Fig. X

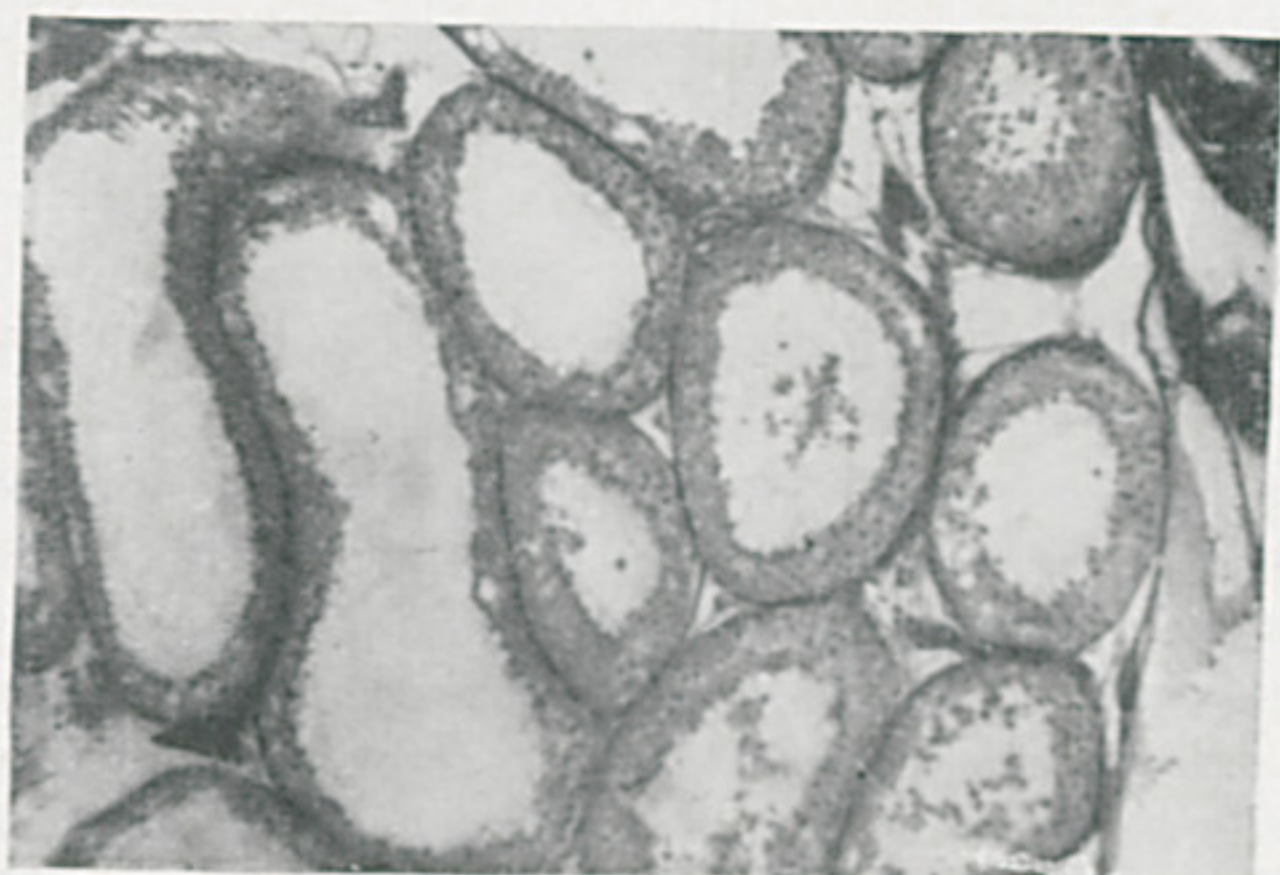


Fig. Z

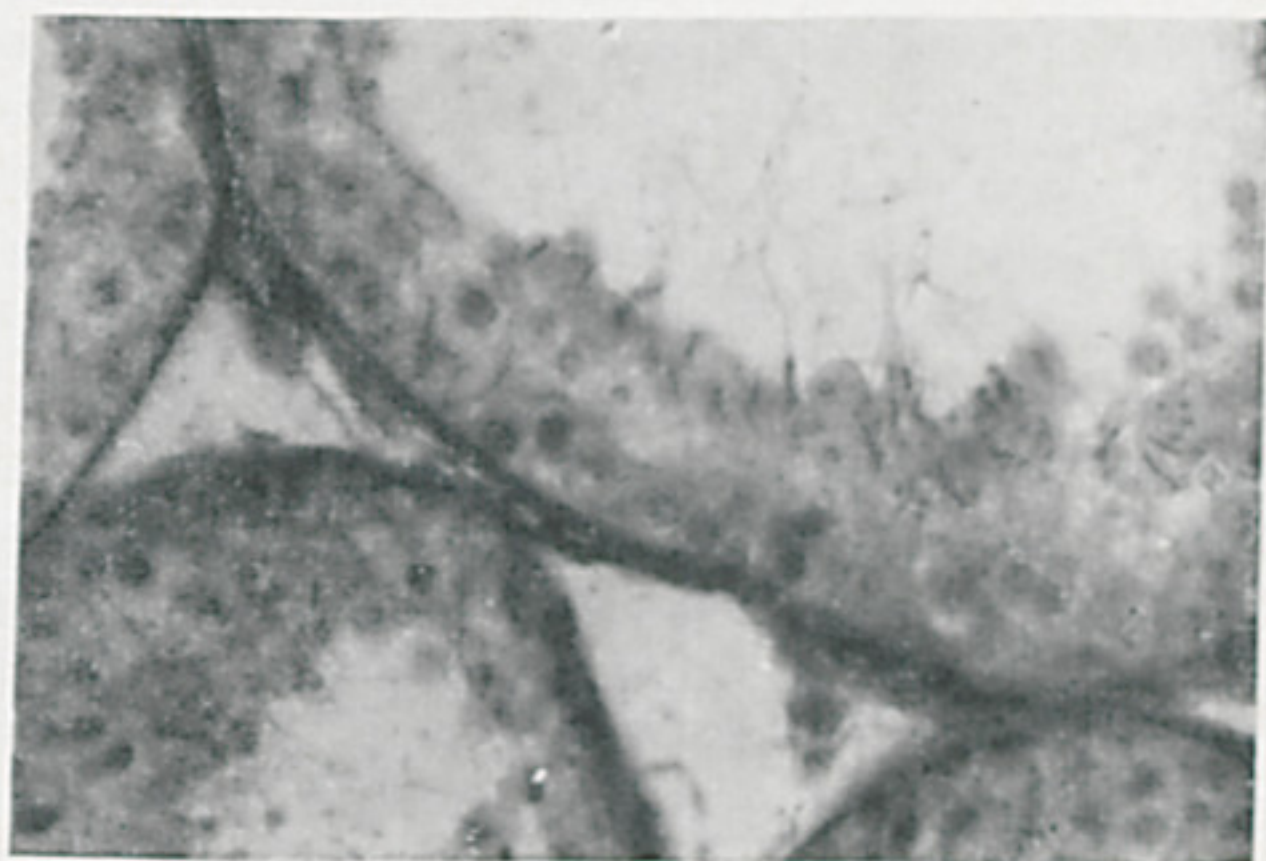


Fig. Z a



left side both an oviduct and a Wolffian duct reached the cloaca. Smears of the contents of the cloaca, and also of the surface of sections of testicles made during the dissection, showed the presence of mature spermatozooids. The histological analysis of this testicles, fixed in Bouin, showed that it was at the end of the breeding season, just as testicles of normal *insularis* and *Bothrops jararaca* males, fixed at the same time for control.

At the end of the breeding season, only few spermatogonia are found on the periphery of the cross section of spermatid tubules. Spermatocytes and spermatides, of various stages, some of them more or less pycnotic and without any meiotic division, are found at this stage. In the lumen of the canalicles clumps of ripe spermatozooids are found, as well as spermatozooids attached to the Sertolian cells (plate 15 Z-Za). From this picture we can infer that with great probability the testicle of this snake was functioning normally.

We must remind here of a special feature of the spermatogenesis of snakes described previously by Schreiber (67) that was evident in these specimens. Plate (15 x) show the smear of the spermatozoid stained with Giemsa and Hematoxylin-eosin. Among the normal spermatozooids there exists a few larger ones although perfectly normal in shape, and more susceptible to staining.

A statistical analysis of the length of the spermatozoid heads shows that the giant ones belong to a separate size class. There are more or less 3,8% of, these giant spermatozooids (461 measured). We are not giving a special emphasis to this spermatozoid "dimegaly" because it has been found also in other species of snakes such as *Crotalus durissus terrificus*, *Bothrops jararaca*, *Bothrops insularis*, *Bothrops atrox*, *Xenodon merremii*, *Tomodon dorsatus*, *Oxyrhopus trigeminus* and *Erythrolamprus aesculapii* by Schreiber (67). Until new similar instances appear, no excessive importance should be given to this case. Such cases of hermaphroditism are known in many other vertebrates, in which a part of the ovary develops into a testicle by a predominance of medullary cords, thus, an ovotestis or separate testicles, being formed. The other intersexes of *insularis* never appear to bear testicles and the only disturbance in the phenotypic sex is a far greater incidence of the hemipenis in the population than any other sex abnormality in other animals.

The case of n.º 15.843 is quite different from the others that have been described now in *insularis*, because of the simultaneous presence of ovaries and testicles. This snake is situated in a strange position on the diagrams. As for the ventral scales, it is close to the male group, but as for the subcaudals it is more feminine. Nevertheless, for the tail length this snake is definitely more masculine in the histogram (Figs. 12 and 13).



We cannot classify clearly this case as one of gynandromorphism, owing to the fact that although a testicle was present on one side only, the ovaries and hemipenis are on both sides. It is clear that the case is one of hermaphroditism, but we cannot say whether it was "sufficient", that is whether the spermatozooids of the same snakes were the ones present in the cloaca, owing to the fact that in snakes the spermatozooids found in the cloaca a long time after copulation. We must wait for more cases of this type, and leave this one as an open problem. The evolutionary side of the problem will be discussed later. We must remember the case of the pig in the New Hebrids islands described by Baker (4, 5). This author found a great variety of "sex intergrade" in pigs both in England and in New Hebrids. There are different cases of intersexes in pigs. Some are true hermaphrodites in which an ovary is present on one side with an ovotestis on the other. In the case of the *Sus papuensis*, however, the fact is somewhat different. All the intersexes bear testicles only (in different degrees of atrophy) but they have the external female genitals, although always lacking vestiges of uterus, vagina or ovaries. This case is closer to that of *insularis*, with the difference of the genetic sex of the intersexes (males in pig and females in *insularis*). Only the anatomy of the external genitals is concerned in both cases. The difference between *Sus papuensis* and *Bothrops insularis*, however, is that the intersexes of the snake are functionally fertile females, while in the pig there is an atrophy of the testicle and the intersexes are steriles.

The high frequency of the intersexes of *Sus papuensis* together with the fact that the abnormality is localized in an insular population could lead to a comparison with the Queimada Grande snake. But we must remember also that the high frequency of pig intersexes is maintained on the island by a careful breeding of intersexes producing by the natives for religious traditions. We cannot ascertain whether natural selection or genetic factors are responsible for the high frequency of intersexes in the case of *insularis* because the population lives in entirely natural conditions.

#### 6 — Caryometric Researches for Ploidy Disturbances

As in *Drosophila* an intersexual condition has been found in triploid vertebrate individuals (20-21). Triploid larvae of *Rana pipiens* as well as triploid newts suffer a transformation of the female gonad into a testicle during the development. The triploid genome alters the equilibrium among the sex factors and shifts the development of the gonads towards maleness. The criterion for detecting poliploidy used by Fankhauser (20-21) has been the nuclear size and the number of nucleoli in the somatic tissue. We tried the same criterion in *Bothrops insularis* in an attempt to see whether intersexes could eventually be related to a disturbance in ploidy.



The nuclear sizes of liver cells of males, true females and intersexes have been measured. The caryometric technique used was the one largely developed at the Institute of Biology of the University of Minas Gerais (68). No differences in nuclear size which could be ascribed to differences in ploidy have been detected between normal sexes and intersexes. The liver of *Ophidia* does not present the adult endopolyploidic growth of the nuclei as in mammals (Jacob's series). So, a unique size class of cells constitutes the liver parenchyma. If triploid or other ploidy disturbances would be the genetic basis of the intersexuality in *insularis*, it would be clearly represented in the somatic tissues by a quite different caryometric picture as the one we found in the histograms here presented.

The supposition that a disturbance in ploidy would eventually occur in *insularis* had a basis on the higher percentage of giant spermatozooids. Although this fact is not specific for the *insularis*, but found in almost all *Ophidia*, the *insularis* examined has a rather higher frequency of these giant spermatozoid. The possibility that these giant spermatozoid were fertile, and being either non-reduced, or haploid with dimerous chromosomes, could eventually give a triploid zygote, with consequent disturbances in sex development. As we have seen from researches in Amphibians, by Fankhauser (20,21) the study of somatic cells can give a good information on the quantitative state of the genome. So, we can, practically, dismiss our working hypothesis of the triploidy as the cause of the intersexuality in *Bothrops insularis*.

#### 7 — Classification of the Sexual Abnormality

The facts here examined indicate that in *Bothrops insularis* there is an abnormal condition in sex development that has as fundamental manifestation the existence of a male copulatory organ (hemipenis) in a certain number of females. We have no cytological proof to assert that these individuals are genetically females. Very little is known on the cytological determination of sex in *Ophidia* (56). From the caryometric analysis of somatic tissues, we can only infer that there are no differences in ploidy between the normal sexes and the anomalous individuals.

An indication about the genetical nature of these intersexes is given by the statistical analysis of quantitative characters and specially of the pholidotic differences. Specially remarkable is the number of ventral scales, that with great probability is a true "somatosexual" trait whose embryological determination is as early as the mesoderm segmentation into somites. A marked difference between males and females is shown by this character, although with some dispersion that gives a large overlapping territory. The statistical significance of these differences is shown with very good clearness



by the application of the "discriminant functions", which takes into consideration the whole group of sex differentiating characters.

The statistical analysis demonstrates that the intersexes belong to the *variations field of females* in almost all the quantitative characters here examined. Specially the number of ventral scales as well as the subcaudal ones which are considered by the herpetologists as a good statistical difference between sexes in almost all snakes. In some cases the variation of the quantitative characters of the intersexes towards femaleness was more distinct in the intersexes than in the true females. We must infer, thus, that from the statistical point of view, the intersexes are genetically females. The same conclusions are drawn from the embryological researches. The gonads of the embryos that bear hemipenis on one side (that would be certainly classified as intersexes if adults) are definitely females. The only embryo found without any trace of hemipenis (true female) has an embryonary ovary that was absolutely similar to the ovary of the intersexes. That in other Reptiles as in *Bothrops insularis*, the female gonad is delayed in the sexual differentiation, is a fact which must be taken into consideration. Remains of the medullar cords are still present almost at birth. A little residue of cortex in some male gonads is also a fact that must be borne in mind. Risley (63-66) determines sex inversion in reptilian embryos by stimulating with the proper hormones the residue of the structures characteristic of the opposite sex. In more advanced stages, Risley (63-66) obtained a result that can be also of some importance in the study of the *insularis*. The treatment of female embryos with male hormones, after the gonadic differentiation, leads to a development of maleness only of the region of the cloaca and of the copulatory organs.

Because of these facts, we cannot deny that some individuals, specially those belonging to the overlapping territory of the statistical variability (and that could eventually have more labile genetical sex determination), could shift during the development towards the other sex. We cannot, at present, say anything about the endocrine state of these individuals, specially about the state of development and physiology of the adrenal cortex (interrenal). The most disturbing fact in our problem is the unilaterality of the hemipenis in more than half the intersexes, and the extremely rare occurrence of, the left side hemipenis. These facts speak for a more specific genetical sex determination of the skin territory of the cloacal region. This territory is probably sensitive to both male and female hormones, but it appears that the threshold for the reaction to different hormones during development is genetically determined. Those facts appear to be rather related to the genetic differences in the various territories of the skin of gynandromorphic birds. In this connexion it may be thought that the sensitive territory of the cloacal



skin is different in *Bothrops insularis*, as compared to other species of snakes. The cases of unilateral hemipenis could thus represent true mosaics of the genetical determination of this territory.

We can thus speak of a genetical factor that exists in some of the females of *insularis* and which determines the development of the hemipenis. This factor can act on one side only. The presence of a masculine character in females can also be explained in another way, according to Wolffs discoveries (75-80) in ducks concerning the copulatory organ and the syrinx. The presence of the copulatory organ is, in this animal, the "neutral type", and exists as "anlage" in both sexes during the embryonic stage. After the differentiation of the gonads, the female hormones stop the development of the copulatory organ in the females.

The same thing seems to happen with the syrinx, and the masculine type is also the "neutral" one in all the early embryos. In the female embryos there is a successive involution that corresponds to the "feminine type" of that organ. A comparison with the case of the *insularis* can be made, with the difference that the bud of the hemipenis is present almost in all the embryos but not in some of them ("true females") as another difference must be considered the absence of the bud of this organ in about half the feminine embryos. This fact probably speaks in favour of a mechanism that differentiates sexually the cloacal skin territory sharply and earlier than in the case of the copulatory organ of the ducks. The case of the hemipenis of the *insularis* could be compared more closely with the behaviour of the mullerian ducts in the ducks in which we can find a hormonal "ambience" in the masculine embryos inducing the involution of the mullerian ducts on both sides, and the feminine environment inducing the involution of the mullerian duct on one side only.

We know, at present, nothing about the intimate mechanism of the lateral differentiation of the reactivity to the hormonal environment both for the mullerian ducts of the duck and the cloacal territory of the *insularis*. We may thus suppose that the case of true females, in which no hemipenis develops, is the extreme result of hormonal inhibition of the "neutral" type of development (hemipenis). We should in this case remember the differences in horn development in some races of sheep in which the skin region from which the horns arise is probably under the command of both genetic and hormonal stimulation.

We do not know anything about endocrine activity of the embryonic gonads in *Bothrops insularis*, and we must remember that at least in the cases described (embryos of 130 mm) the hemipenis is very well developed in males and a little reduced in intersexes, and that the gonads are not yet completed



in this histological differentiation. Concerning the only female embryo without hemipenis, we cannot say with certainty from the histological degree of differentiation of the ovary that either there is a complete inhibitory effect on the "anlage" of hemipenis or there is a genetic lack of the hemipenis.

The classification of the genetical abnormalities has been sharply defined by Goldschmidt, (32-34) which cleared the great confusion in this field due, fundamentally, to the medical analysis of these abnormalities in man. Goldschmidt recognized only two types of abnormalities: intersexuality and gynandromorphism. The first was defined as a mosaic of the sex factors in the time, the second as a mosaic in space. We cannot discuss here the whole problem, but it is a fundamental task to try to set the case of *Bothrops insularis* into the right class of abnormalities. The first case observed by Hoge and Belluomini (37) was classified as case of gynandromorphism. Really, the first cases of unilateral hemipenis in females seemed to fall in this category, but further observations of the absence of the male gonads in the anomalous individuals suggested another classification. The only case observed bearing the ovary on only one side and at the other side both an ovary and a testicle, is rather complicated, and we must wait for further confirmation.

The classification as intersexes of the anomalous *insularis* successively adopted by the authors, (38) was justified by the study of the gonads of the embryos, and by a discussion of the general problems. This classification holds still better after the new facts revealed by more embryological researches (residues of opposite sex in both sexes).

The newest classification of the sex abnormalities given by Padoa (58) is more suited to the case of *insularis*. He classified all deviations from the normal phenotypic state of the sexes as "intersexual". He further considers the factors of the deviation as genetical and hormonal. In both cases he distinguishes: — "gynandromorphs" and "transexuals". The first ones are determined by genetical or hormonal factors that are variable in the topographical localization, but do not vary during development. The transexuals are the cases in which the genetical or hormonal factors vary during the period and act in successive steps of the development.

The sexually abnormal *insularis* can thus be called "intersexes" as a general denomination.

The fact that only the cloacal region is concerned and that this territory itself can be different on the two sides of the body, suggests a gynandromorphic situation of the genetic and development factors of the copulatory organ. It may be a somatic mosaic of different factors between the general gonadic territory and the cloacal region which can be different on one or on both sides.



In the medical classification, the *insularis* case could be easily considered as a "feminine pseudohermaphroditism" owing to the fact that only the external sex organs are masculinized. But this does not give any indication about the intimate essence of the phenomenon. Only if we could demonstrate an increase of the hormonal environment towards maleness before the determination of the histological structure of the gonad, we could call it a slight intersexuality case. We can thus leave the term "intersexuality" for *insularis* as a general indication of a disturbance of the phenotypic expression of the sex.

As stated above, the residues of the two sex "anlage" in the gonads leave the doubt that a true transexuality can occur during the development, specially in individuals situated in the overlapping field of the statistical variability of males and females.

We are induced to introduce a new term in the classification of the *insularis* case. If we consider simply the existence of a gene or group of genes that determines the formation of the hemipenis and can be active not only in the males but also in a certain number of females (eventually with some modifying genetic and endocrine factors), just as in some species the males are provided with some female secondary sex characters (mammary gland), we can use the term "ARRENOIDISM" (existence of male characters). The introduction of this concept can possibly lead to a deeper analysis of the distribution and variation of these genes in the population in the course of 30 years period of observations. This would be an attempt to study the population genetics of a Vertebrate in a very insulated condition, and would be treated in a forthcoming publication.

### SUMMARY

*Ecological data:* — *Bothrops insularis* (Amaral) 1921 is a crotalid snake that lives on a small island (Island of Queimada Grande, SP) of the South Atlantic Ocean, near Santos, S.P. Brazil. It has never been found on the continent. The subject of the present study is the presence of a male copulatory organ (hemipenis) in a certain number of females called intersexes, a character that was never found in other feminine reptiles. The presence of such a special character in a small and strictly isolated population has been studied from two main points of view: statistically and embryologically.

*Statistical researches:* — the following procedure has been followed:

1 — *Sex ratio of males*, pure females and intersexes, focussing the fact that this population of snakes has been studied during a period of about 30 years, in two groups of samples with an interval of about 25 years. The capturing data show a shift in the sex ratio during this period, indicating



that some genetical or ecological factors are acting in the population. A reduction of the number of males, an increase of the intersexes and a stationary frequency of the females is the picture of the sexual modification of the snake population. One hundred and seventy six captured snakes have been reported as intersexes, 15 of them were pregnant and no pure females were found between them. Only intersexes are fertile, although with a clear diminution of the number of living embryos in each brood as compared with other systematically related species living on the continent. The sex ratio of the embryos, although calculated with very few individuals, appears to have a predominance of males.

2 — *Statistical study of some quantitative characters* of these snakes (head, body and tail length; number of dorsal, ventral and subcaudal scales) has been made considering separately the males, the pure females and the intersexes; the frequency of these characters was analysed in the two samples of the collection: the first one between 1914 and 1920, and the second one between 1946 and 1953. A new method of statistical analysis has been applied besides the current ones — the “discriminant functions” — and this analysis emphasizes that the intersexes belong to the female field of variability. Some of the quantitative variable factors belong to “somatosexual” characters that are differentiated by the genetical constitution of the sex hormones. For these facts the intersexes must probably be considered as genetical females, with some genetical factor that determines the development of the copulatory organs (more or less developed) on both sides or on one side only. A slight, non-statistically significant shift of the mean towards maleness is to be taken into consideration, because all the characters have the deviations in the same direction.

3 — *Embryological researches* carried out on embryos from intersexual mothers, as well as on some embryos and new-borns of a few continental species of snakes *Bothrops jararaca*, *Bothrops alternatus*, *Crotalus durissus terrificus* and *Xenodon merremii*, revealed that in *Bothrops insularis*, the intersexual state can be detected since half way of the pregnancy period, by the presence of a more or less developed copulatory organ in almost all the embryos. The embryonic differentiation of the sex gland does not discord from the general picture of all the vertebrates although, as some authors demonstrated and we confirm for *Bothrops insularis*, the reptilian ovary retains the medullary cords for a longer time, and some residues of cortex are found in well differentiated testicles. The embryos bearing ovaries and well differentiated mullerian ducts can be divided in pure females (without any vestiges of hemipenis) and intersexes, bearing bilateral or unilateral hemipenis, generally less developed than those of male embryos.



For these facts, the intersexuated embryos can be classified, from the gonadic point of view, as females, in agreement with the result of the statistical research. An attempt to investigate the possibility of some ploidic disturbances in the intersexes (caryometric analysis of somatic tissues) demonstrates that this fact is not verified, because the nuclear size of males, pure females and intersexes is absolutely equal. A single specimen, among 367 examined, was quite different, bearing besides the bilateral, well developed hemipenis, a testicle on the right side and both ovaries. The testicle was histologically well differentiated, with ripe spermatozooids. This only case of true hermaphroditism must be left, for the present in a separate class of abnormality. The presence of residual medulla in the embryonal gonad could eventually give rise to an unusual post-embryonal development of a testicle, the transformation being total on one side and partial on the other. The pholidotic characters of this specimens are clearly visible in the overlapping field of the statistical variability, indicating, perhaps, an intermediate stage of the genetical sex factors. This case must, however, be considered as exceptional in view of the fact that a great number of true males, pure females and intersexes belong to the same overlapping territory of the statistical variability of somatosexual characters. A rather high frequency of giant spermatozoid has been found in the testicle of this specimen, but this is a fact that previous researches on the dimegaly of the head size of spermatozoid demonstrated also in many other species of snakes.

#### 4 — *Conclusions*

The facts above summarized can lead to the statement that a snake living on a very small, overpopulated island, (in comparison with other snakes living in neighbouring areas of the continent) has an intersexual condition that manifests itself by the presence of the male copulatory organ in most of the females. A noteworthy diminution of the fertility of these intersexes was observed.

The frequency of these intersexes in the population increase markedly during 30 years of observations, together with a sensible diminution of adult males. These facts claim the possibility that in a near future the population will be constituted almost only of intersexes and few females, the males, eventually disappearing. A unique case of true hermaphroditism could eventually show the possibility that this species is evolving towards a state of hermaphroditic reproduction, but the fact that an extremely little number of cases of functional hermaphroditism ("sufficient hermaphroditism") is known among vertebrates, and the rarity of this case, speak for an anomaly rather than a trend towards a general condition of the population.



If no hermaphroditism would develop in the population, the possible extinction of this species should be considered. The few data on sex ratio of embryos demonstrate that before the birth males are rather more frequent than females and intersexes, thus the disappearance of the males in the adult population observed in the 30 years period could probably be caused by a differential mortality of newborn males. A sex inversion of the males into intersexes, in the post-natal period is not yet demonstrated, although it cannot be excluded in at least a part of the individuals (overlapping field of somatosexual characters).

A review of the classification of the abnormality of phenotypic sex in *Bothrops insularis* authorizes the maintenance of the term "intersexuality", in the broadest sense given by PADUA. Some facts suggest that a gynandromorphic mosaic of the genetical determination of the gonadic territory and the cloacal skin territory could explain the existence of the female gonads and ducts together with the male copulatory organ; some unilateral somatic differentiation of the sensitivity towards male factors explain the unilateral distribution of the copulatory organ of the population. The facts described in this paper are compared with those found by Wolff concerning the sexual determination of the syrinx and penis of ducks.

The new term "arrenoidism" is here proposed. It defines the phenotypic appearance of a male character in females, as result of an action of a definite gene, or genes, for the hemipenis, having a certain frequency in the population, and eventually showing a different expression in the adult hormonal environment.

Some problems are posed concerning the origin of this species of snakes on the island, related systematically to some continental *Bothrops*, as well as the possibility of extinction of the species owing to the trend observed in a 30 years period of increasing sexual abnormality in the population.

### RESUMO

*Dados ecológicos:* — *Bothrops insularis* (Amaral) 1921 é uma serpente Crotalidae conhecida até o presente momento, como habitando exclusivamente a Ilha da Queimada Grande, no Atlântico Sul, perto de Santos, Brasil.

Este trabalho relata a presença de órgão copulatório de machos (hemipenis) em certo número de fêmeas (chamadas intersexos) fato esse ainda não assinalado em outros répteis. Essa pequena população, estritamente isolada, foi estudada sob dois aspectos: estatístico e embriológico.

*Estudo estatístico:* — Foi feito da seguinte maneira: 1) A proporção de machos, fêmeas puras e intersexos dessa população foi observada pela coleta



de exemplares durante um período de cerca de 30 anos, separados em duas amostras por um intervalo de 25 anos aproximadamente. Os resultados das capturas mostram uma mudança na proporção dos sexos durante esse período, indicando que fatores genéticos ou ecológicos, estão atuando sobre a população. A redução do número de machos, o aumento dos intersexos e a frequência estacionária das fêmeas, representa o quadro da modificação sexual dessa população de serpentes. De 367 serpentes estudadas 176 são intersexos, dos quais 15 deles foram encontrados em estado de prenhes. Nenhuma fêmea pura foi encontrada em tal estado. Apenas os intersexos parecem apresentar fertilidade. Todavia, o número de embriões é menor, comparando-se com espécies afins existentes no Continente. A proporção sexual dos embriões, embora calculada sobre poucos indivíduos, revelou uma predominância de machos.

2) *O estudo estatístico de caracteres quantitativos* dessas cobras (comprimento da cabeça, corpo e cauda), (número de escamas dorsais, placas ventrais e subcaudais) foi feito analisando, separadamente, machos, fêmeas puras e intersexos. As frequências desses dados foram estudadas pela comparação dos dois grupos de amostras coletadas: o primeiro grupo capturado entre 1914 e 1920, e o segundo entre 1946 e 1953. Um novo método estatístico foi aplicado além dos usados correntemente: — a “função discriminante” e esta análise acentuou que os intersexos pertencem ao campo de invariabilidade das fêmeas, embora os histogramas revelem uma grande área de “overlapping”. Alguns dos dados quantitativos pertencem aos “caracteres somatosexuais”. São diferenciados pela constituição genética dos sexos, antes do início da atividade funcional das glândulas sexuais. De acordo com estes fatos os intersexos podem ser considerados como geneticamente fêmeas. Devem possuir alguns fatores genéticos que determinam o desenvolvimento de órgãos copulatórios (mais ou menos desenvolvidos) de ambos os lados ou de um lado só. Uma pequena, mas não estatisticamente significativa mudança da *média* para com os caracteres dos machos, deve ser tomada em consideração pois todos os caracteres apresentam o desvio na mesma direção.

3 — *Estudos embriológicos* feitos nos embriões provenientes de mães intersexuadas, assim como em embriões e recém-nascidos de algumas outras espécies de serpentes (*Bothrops jararaca*, *Bothrops alternatus*, *Crotalus durissus terrificus*, *Xenodon merremii*) revelam que em *Bothrops insularis* o estado intersexuado pode ser observado desde a metade do período de prenhes, pela presença de um órgão copulatório, mais ou menos desenvolvido, em quase todos os embriões. A diferenciação embrionária das glândulas sexuais não difere do quadro geral dos vertebrados, porém, segundo alguns autores demonstraram, e nós pudemos confirmar em *Bothrops insularis*, o ovário dos répteis retém os cordões medulares por um tempo mais longo e alguns resíduos da



cortex são achados em testículos bem diferenciados. Os embriões, contendo ovários e ductos mullerianos bem diferenciados, podem ser divididos em fêmeas puras (sem vestígios de hemipenis) e intersexos, tendo hemipenis uni ou bilateral, geralmente menos desenvolvidos do que aqueles dos embriões machos.

Estes fatos permitem classificar os embriões intersexuados, sob o ponto de vista das gônadas, como fêmeas, concordando com os estudos estatísticos.

Investigada a possibilidade de haver distúrbios poliploidicos nos intersexos, os estudos realizados (análise cariométrica de tecidos somáticos) demonstraram que o tamanho do núcleo dos machos, fêmeas puras e intersexos, é absolutamente igual.

Um exemplar único, entre 367 examinados, era diferente, apresentando ambos os hemipenis bem desenvolvidos, um testículo do lado direito e ambos os ovários. O testículo estava histologicamente bem diferenciado, com espermatozóides maduros. Foi encontrado um número relativamente elevado de espermatozóides gigantes. Esse fato já foi demonstrado em várias outras espécies de cobras em pesquisas anteriores sobre a dimegalia do tamanho da cabeça do espermatozóide. Este foi o único caso de verdadeiro hermafroditismo assinalado.

É lembrada a possibilidade da presença de resíduos medulares em gônadas femininas vivas embrionárias, eventualmente darem origem a desenvolvimento post-embrionário em sentido masculino, bem como o desenvolvimento dos resíduos corticais dos testículos, poderia levar a transformação total ou parcial destes embriões em fêmeas. Os caracteres da foliose deste exemplar estão claramente situados no campo do "overlapping" da variabilidade estatística, indicando, talvez, um estado intermediário dos fatores genéticos do sexo. Deve-se acrescentar, porém, que grande número de machos, fêmeas e intersexos, pertencem ao mesmo território de "overlapping" da variabilidade estatística dos caracteres somatosexuais.

#### 4 — Conclusões

Os fatos acima mencionados levam a constatação de que uma serpente que vive numa pequena ilha, superpovoada pela referida *B. insularis* (comparada com as serpentes que vivem em áreas vizinhas do continente) tem uma constituição intersexuada que se manifesta pela presença de órgãos copulatórios machos na maioria das fêmeas. Foi observada uma diminuição de fertilidade nesses intersexos.

A frequência destes na população aumentou lentamente durante 30 anos juntamente com uma diminuição sensível dos machos. Estes fatos permitem prever que, num futuro próximo, a população tenderia a ser constituída quase



somente de intersexos e de poucas fêmeas, desaparecendo eventualmente todos os machos, o que levaria à extinção da espécie. Um único caso de verdadeiro hermafroditismo poderia, eventualmente, mostrar a possibilidade de que esta espécie tenderia a evoluir para um estado de reprodução hermafrodita. O fato de apenas ser conhecido número reduzido de casos de hermafroditismo (hermafroditismo suficiente) entre vertebrados, fala mais a favor de se considerar este caso como uma exceção.

Os poucos dados sobre a relação sexual dos embriões parecem demonstrar que, antes do nascimento, os machos são mais frequentes do que as fêmeas e intersexos. O desaparecimento dos machos, observado na população durante um período de 30 anos, poderia, provavelmente, ser causado por mortalidade maior dos mesmos quando recém-nascidos. Uma inversão dos machos em intersexos, no período post-natal, não foi ainda demonstrada. Entretanto não pode ser totalmente excluída devido o "overlapping" apresentado pelos caracteres somatosexuais.

Uma revisão da classificação da anomalia do fenotipo no sexo de *Bothrops insularis*, autoriza o uso do termo "intersexualidade", de acordo com o conceito mais amplo dado por Padoa. Alguns fatos sugerem que o mosaico ginandromórfico da determinação genética do território gonático e do território da pele da cloaca, poderia explicar a existência de gônadas femininas e ductos de Muller ao lado dos órgãos copulatórios masculinos. Outra possibilidade seria uma diferenciação somática unilateral ou bilateral da sensibilidade para com os fatores masculinos, o que explicaria a distribuição do órgão copulatório na população.

São feitas algumas comparações entre os fenômenos aqui observados e os estudados por Wolff na determinação sexual dos territórios formativos da "syrinx" e dos penis nos patos.

Um novo termo, "arrenoidismo", é aqui proposto. Ele define o aparecimento de caracteres de machos em fêmeas como resultado da ocorrência de um gen para o hemipenis, existente na população.



# EXPLANATION OF THE FIGURES

(Microphotographs)

## Plate 7

- A — Embryo of *Bothrops insularis* of 35-40 mm (769) X 100  
B — " " *Bothrops insularis* " 35-40 mm (770) (longitudinal) X 100  
C — " " *Bothrops insularis* " 35-40 mm (770) X 450

## Plate 8

- D — " " *Bothrops insularis* " 60-70 mm (773) X 35  
E — " " *Bothrops insularis* " 60-70 mm (773) X 450  
F — " " *Bothrops insularis* " 60-70 mm (773) X 1000 Gonocytes in the cortex.

## Plate 9

- G — " " *Xenodon merremii* " 90 mm (386) Testicle X 35  
H — " " *Xenodon merremii* " 90 mm (386) Residual of cortex X 450

## Plate 10

- I — " " *Bothrops insularis* " 130 mm (740) "a" Testicle X 100  
J — " " *Bothrops insularis* " 130 mm (733) "d" X 35. Ovary  
L — " " *Bothrops insularis* " 130 mm (733) "d" X 100. Ovary  
M — " " *Bothrops insularis* " 130 mm (733) "d" X 450. Ovary

## Plate 11

- N — " " *Bothrops insularis* " 160 mm (727) "a" 35. Male, no Mullerian ducts  
O — " " *Bothrops insularis* " 160 mm (727) "a" X 100. Testicle.  
P — " " *Bothrops insularis* " 160 mm (727) "a" 450. Gonocytes in medullary cords.

## Plate 12

- Q — " " *Bothrops insularis* " 160 mm (728) "e" X 35. Ovary Mullerian ducts visibles.  
R — " " *Bothrops insularis* " 160 mm (728) "e" X 100  
S — " " *Bothrops insularis* " 160 mm (728) "e" X 450. Cortex and medullary cords.

## Plate 13

- T — " " *Xenodon merremii* " 180 mm (729) X 100. Testicle with medullary cords.  
U — " " *Xenodon merremii* " 180 mm (729) X 450. Medullary cords with gonocytes.

## Plate 14

- V — " " *Xenodon merremii* " 180 mm (730) X 100. Ovary  
W — " " *Xenodon merremii* " 180 mm (730) X 450. Ovary. Cortex with gonocytes.

## Plate 15

- X — Hermaphrodites (N.º 15.843 Col. I. B.). Smear of the content of the cloaca. Spermatozoa. In the middle a giant spermatozoid. X 1000 (761).  
Z — Testicle of hermaphrodite (N.º 15.843 Col. I. B.) (761) X 35  
Za — Testicle of hermaphrodite (N.º 15.843 Col. I. B.) (761) X 450.

Notes: — The number as 761 are the number of hystological series.  
The indications "a", "d", and "e" refer to the series of embryo of the same mother.



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