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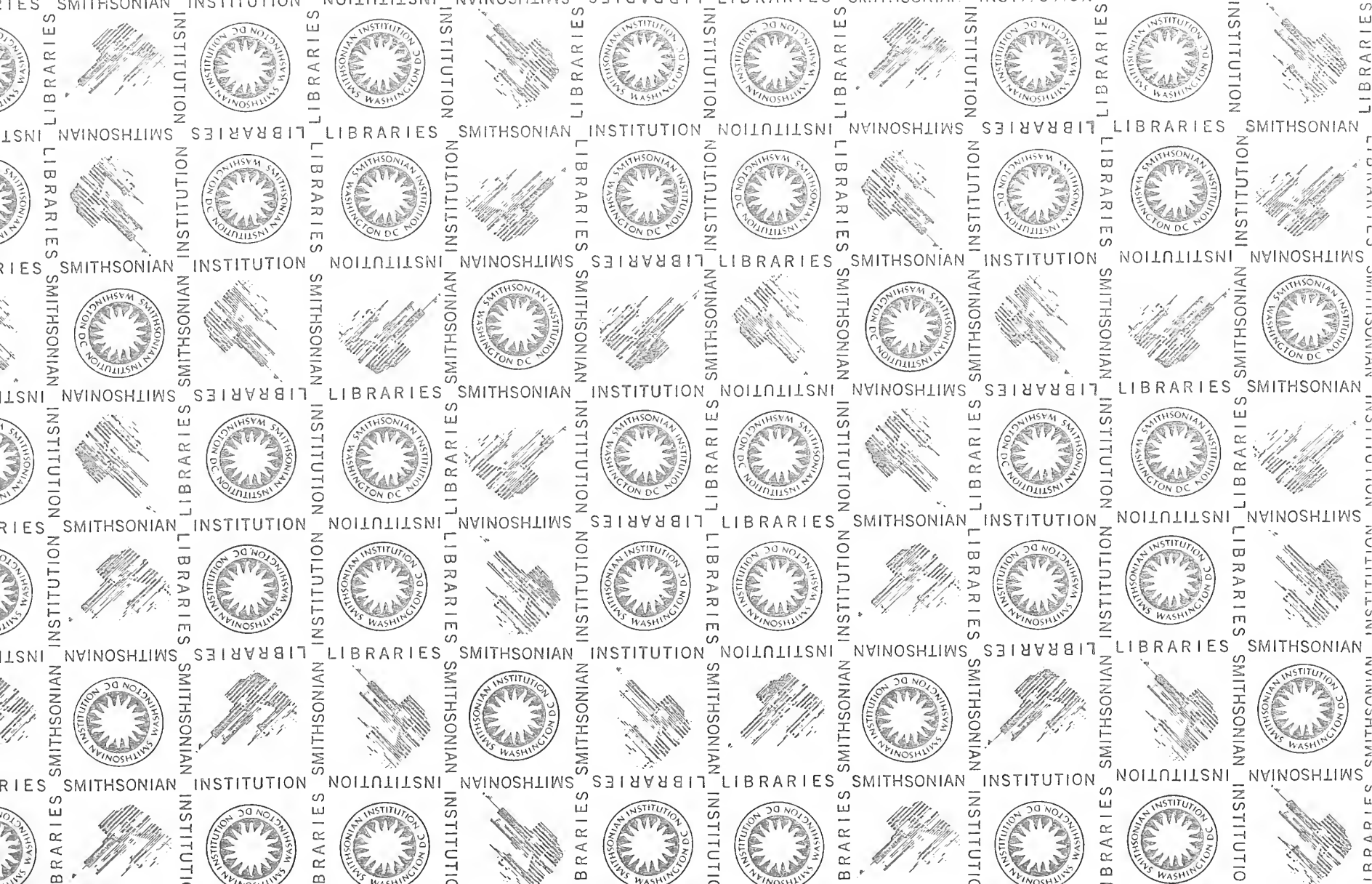
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ZOOLOGICA

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Sex Determination and the Restriction of Sex-linked Pigment Patterns to the *X* and *Y* Chromosomes in Populations of a Poeciliid Fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras[1]

KLAUS D. KALLMAN[2]

(Plates I-II; Text-figure 1)

X. maculatus is polymorphic for sex chromosomes and sex-linked pigment patterns. Females of natural populations may be of the genotype WY, WX, or XX and males XY or YY. Fish from the two rivers were tested for their sex-genotypes, because earlier but limited data had indicated that the X is absent from rivers in British Honduras. Of 8 males and 30 females tested, X chromosomes were only found in one male (XY) and female (XX). One WY female was fertilized by an XY male, before she was collected. Of the 29 females with a W, all but two exhibited one or more sex-linked pigment pattern controlled by at least three loci. The 27 females possessed a total of 41 factors. None was W-linked. In preserved collections from these rivers, the frequency of males with macromelanophore patterns was two to three times that of females. This difference is in good agreement with the hypothesis that in natural populations the W chromosome does not carry pigment factors. This is not true for the X chromosome. Since crossing over beween the W and Y has been observed in the laboratory, it must also occur in natural populations. In the absence of selection, crossing over should tend to equalize the frequency of marked W and Y chromosomes. A selective advantage is postulated for high coloration in males and a disadvantage in females. The significance of the W as a vehicle for strict maternal transmission of characters is discussed.

INTRODUCTION

THE SOUTHERN platyfish, *Xiphophorus maculatus*, has been the subject of many investigations, because of its unusual pigmentary and sex chromosome polymorphism. In *X. maculatus*, which ranges from southern Mexico near Veracruz to British Honduras, females may be of the sex genotypes *XX*, *WX*, or *WY* and males may be *XY* or *YY*. The sex chromosomes have not been identified cytologically, but an abundance of data concerned with sex ratios and the inheritance of sex-linked pigment patterns attest to the reality of the *W*, *X*, and *Y* chromosomes (Bellamy 1922, 1924, 1928; Bellamy and Queal 1951; Fraser and Gordon 1929; Gordon 1927, 1937, 1946, 1947, 1952; Kallman 1965).

The geographical distribution of the *W* and *X* chromosome has been the subject of some controversy and misunderstanding. Based upon experiments with platyfish obtained through commercial sources it was thought that sex determination in this species was of the *WY-YY* type (Bellamy 1922, 1924, 1928; Breider 1942; Gordon 1927, 1937; Kosswig 1938). In 1947 Gordon discovered the *X* chromosome in Mexican populations of platyfish and later stated that *X. maculatus* with an *XX-XY* mechanism inhabited rivers in Mexico and were geographically isolated from populations with a *WY-YY* system in British Honduras (Gordon 1954). This theory has been widely accepted in many review papers and monographs on sex deter-

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mination, but it is now outdated. More recent experiments with fish collected in several major drainages have shown that *W* and *X* chromosomes occur together in roughly 70 percent of the total known range of this species (Kallman 1965). The two female determining chromosomes, *W* and *X*, have been found in the Rio Grijalva, Rio de la Pasion (Rio Usumacinta system), Lake Peten, and Rio Hondo. Only in populations of the Rio Jamapa, Papaloapan, and Coatzacoalcos in the western part of the species range, has the *W* not been demonstrated; the *X* is not known from the New River and Belize River in British Honduras. Because relatively few fish were tested, the failure to demonstrate the *W* or *X* chromosomes from these rivers may be due to a sampling error. Kallman (1965) and Kallman and Atz (1967) have suggested that the sex chromosome mechanism of *X. maculatus* probably has arisen from a *XX-XY* type as is present today in *X. v. variatus, X. v. xiphidium, X. milleri,* and *X. p. nigrensis.* The sex chromosomes of the four species are homologous (Kallman and Atz 1967; Zander 1968). Some sort of selective advantage must have been and perhaps still is associated with the *W* chromosome, since it is widespread today.

To understand the evolution of the sex determining system of *X. maculatus,* it is important to establish whether the *X* is absent from any major river system. Such populations could have arisen as the result of the replacement of the *X* by the *W;* or they could have been founded by fish already possessing the *WY-YY* mechanism. This paper is concerned with sex determination of the platyfish populations inhabiting the Belize River and Sibun River of British Honduras.

Material and Methods

The three collecting stations can be found on Text-fig. 1. Fish from the Sibun River were collected at a locality called Freetown, in a small weedy pond on the left side of the dirt track that branches off the Western Highway about 2 km beyond Hattieville. Fish were caught by repeated sweeps with a 10 feet long, 4 feet wide (one quarter inch mesh) nylon seine along 20 meters of shore line. Because of dense shrubbery other parts of the bank were not accessible. The location, hereafter designated Freetown, was visited on Jan. 20 and 23, 1966. The data presented in Table 7 represent the combined count of the two collections. The ten females and two males which were brought alive to the Genetics Laboratory were given pedigree number 1899 (Table 1 and Table 2) and with the exception of male 1899-12 (Table 1) were collected during the second visit.

Text-fig. 1. Map of British Honduras showing the environs of Belize City. Collecting stations in the Belize River drainage are on the north side of the road leading to Bermudian Landing (above the "u" of Bermudian) and Gabourel Creek near Stanley airport. Collecting station for the Sibun River is at Freetown.

Fish from the Belize River were caught in a shallow, broad lagoon, located in a cow pasture on the right side of the dirt road running from the town of Boom towards the ferry crossing at Bermudian Landing. The exact location is "Mamre Farm," approximately 2 km east of the ferry. The data in Table 7 represent the combined count of two collections made on Jan. 21 and 24. The fish used for breeding purposes were collected on Jan. 24 and were assigned pedigree number 1900 (Table 1 and Table 3). This collecting station will be referred to as "Bermudian Landing."

The second collecting spot along the Belize River was Gabourel Creek, a ditch one to two meters deep that extends from the eastern limit of the main runway of the Belize airport (Stanley Field) towards the Belize River 1 km away. All fish were caught on Jan. 22, 1966, in close proximity to where the creek runs below the access road to Stanley Field; those kept alive for genetic experiments were given pedigree number 1901 (Table 1 and Table 4).

The sex genotypes of the males collected in the Sibun River and Belize River were identified by mating them to *XX* females of the Genetics Laboratory reference stocks (Kallman 1965). Males of the genotype *YY* sire all male broods. Males with the *XY* constitution give rise to offspring of both sexes in equal frequency; the paternal sex-linked pigment pattern, if present, is inherited by one sex only.

The sex chromosomes of the females from the two rivers were identified by either one of two methods. Some fish were mated to *XY* males of the reference stocks in which the *X* and *Y* chromosomes were differently marked. Females with the *WY* genotype give rise to a 1:1 sex ratio with both the *X*- and *Y*-linked paternal pigment patterns exhibited by one half of the offspring of both sexes. A 1:1 sex ratio is also obtained with *XX* females, but the *X*-linked pattern of the male parent is inherited by females and the *Y*-linked pattern by males only. *WX* females produce broods with a ratio of 3 ♀♀: 1 ♂♂: the *X*-linked paternal pattern is inherited by two-thirds of the female but none of the male offspring; the *Y*-linked pattern of the male parent is inherited by every male offspring but by only one third of the females.

Other females of the Sibun River and Belize River were mated to males known to be *YY*. Females with the genotype *XX* give rise to all male broods. Both *WY* and *WX* females give rise to a 1:1 sex ratio, but can be told apart by mating a male offspring of the F_1 generation to a *XX* female of the reference stocks. The male offspring of *WX* females are *XY* and sire broods that consist of both sexes; those of *WY* females are *YY* and give rise to all male progeny.

The identification of the sex genotypes of newly-collected females is greatly facilitated by the presence of sex-linked pigment patterns. Therefore most fish shipped to the Genetics Laboratory were marked. In this respect the breeding data reported in this paper represent a biased sample. In the field the fish of each seine haul were immediately examined by the author for pigment patterns. Fish were kept alive if they exhibited a pattern or combination of patterns net yet present in the collection. Usually not more than two fish with idential markings were selected from each location.

The following sex-linked pigment patterns, many of which are new for *X. maculatus* and which will be described in a forthcoming paper, were present in the fish from the Belize River and Sibun River.

Macromelanophore pattern:

N — Nigra: irregular black blotches along flank.

TABLE 1. SEX CHROMOSOME CONSTITUTION OF EIGHT MALE *Xiphophorus maculatus* FROM THE SIBUN RIVER AND BELIZE RIVER, BRITISH HONDURAS

Pedigree and genotype of parents			*Pedigree of offspring*	*Phenotype of offspring*		
♀♀	♂♂			♀♀		♂♂
Hp-2	X_+X_+	1899-11 $Y_+Y_{Ly}{}^1$	1919	none	15 Ly	22+
Hp-2	X_+X_+	1899-12 $Y_{Ly}Y_{Ir}$	1935	none	13 Ly	9 Ir
Hp-2	X_+X_+	1900-11 $Y_+Y_{Mr\delta d}$	2016	none	8 Mr Sd	12+
Hp-2	X_+X_+	1900-12 $Y_{Sp}Y_{Ty}{}^2$	1922	none	35 Sp	52 Ty
Jp	$X_{Dr}X_{Dr}$	1900-13 Y_+Y_{YoSp}	1964	none	29 Dr	43 Yo Dr Sp
Cp	X_+X_{Sp}	1900-14 $Y_{Dr}Y_{Br}$	2005	none	10 Br	13 Sp Br
Jp	$X_{Dr}X_{Dr}$	1901-11 $Y_{Mr}Y_{Ir}$	1970	none	45 Dr Ir	51 Dr Mr
Jp	$X_{Dr}X_{Dr}$	1901-12 $X_{Ay\delta r}Y_+$	1921	21 Dr Ay Sr	20 Dr	

Further evidence that *Ay Sr* of 1901-12 is X-linked.

1921-1	$X_{Dr}X_{Ay\delta r}$	1970-12 $X_{Dr}Y_{Mr}$	2010	12 Dr	15 Dr Ay Sr	1+ 14 Dr Mr	10 Mr Ay Sr	1 Ay Sr
Hp-2	X_+X_+	1901-12 $X_{Ay\delta r}Y_+$	1977	20 Ay Sr		21+		

Sd — Spotted-dorsal: irregular spots of macromelanophores in the dorsal fin.

f-Sd — Forward spotted-dorsal: irregular spots of macromelanophores in the dorsal fin accompanied by heavy spotting on flank anterior to dorsal fin.

Sr — Stripe-sided: macromelanophores arranged in horizontal rows along the flanks.

Sp — Spot-sided: small irregular spots of macromelanophores along the flank. Genetic tests, to be published elsewhere, have shown that two *Sp* alleles, designnated as Sp^7 and Sp^8, give rise to somewhat different spotted patterns. The differences are heritable. As far as is known the Sp^7 and Sp^8 alleles are also different from the *Sp* alleles of all other platyfish populations (Pl. I and Pl. II). Sp^7 is present in pedigrees 1937, 1968 (Table 2) and 1930, 1931 (Table 3). Sp^8 is present in pedigrees 1922, 1964 (Table 1) and 1927 (Table 2). In these experiments the differences between the spotted patterns were not important and the particular *Sp* alleles have usually not been identified.

Red and yellow body and fin patterns:

The red and yellow patterns of *Xiphophorus maculatus* are formed by xanthophores and xantho-erythrophores. Goodrich, Hill, and Arrick (1941) and Öktay (1964) have identified the red pigments as pterin compounds while the nature of the yellow pigment is still in doubt. According to preliminary results, certain xanthophores contain colorless pterins and carotenoid pigments (Öktay 1964).

CPo — Caudal peduncle orange: Three factors present in the population give rise to somewhat similar patterns. All of them exhibit incomplete penetrance and vary in their expression. In general the coloration is stronger in males than in females. The factor present in peds. 1937 (Table 2) and 1904 is concerned with background coloration; the factors in peds. 1909, 1929, 1972 (Table 3), 1914, 1915, and 1920 (Table 4) may give rise to bright golden-yellow or red pigmentation. Genetic tests have shown the patterns of peds. 1904, 1909, and 1920 to be controlled by different factors (Kallman unpublished). In this study, however, the patterns were treated as if they were caused by the same allele.

Ar — Anal red: anal fin or gonopodium assumes a red or orange coloration. The *Ar* patterns of the Belize and Jamapa populations are caused by different alleles. They are both present in ped. 1936 and 2050 (see below). The differences between the two alleles will be presented in a forthcoming paper.

Mr — Mouth red: in males lower jaw and gular region red; pattern poorly expressed in females.

Ay — Anal yellow: area above anal fin covered by yellow pigment cells; in some fish other parts of the body are also affected.

Br — Body red: this pattern is especially strongly developed along the flank behind the operculum. The tissue behind the two ventral most scale rows is not pigmented.

Vo — Ventral orange: orange coloration along the ventral most scale rows from area of the heart to insertion of anal fin. The pattern bears a superficial resemblance to Gordon's (1951a) pattern "ruby throat," *Rt*, but *Vo* lacks the characteristic bands of red pigments running dorsally. *Vo* is not expressed in Belize females.

Dr — Dorsal red: dorsal fin orange red.

Ty — Tail yellow: caudal fin a rich golden yellow.

Iris pattern:

Iy — Iris yellow and *Ir* — iris red: Most platyfish of natural populations have silvery grey irises with only traces of yellow pigment. Fish have been collected with irises that were either bright red or yellow. Genetic tests (Kallman unpublished) have shown that this pigmentation is controlled by two sex-linked factors. *Iy* gives rise to yellow pigment in females and young males, but in older males red pigment cells may also develop. *Ir* causes the appearance of red pigment cells in the irises of males and females. Both patterns are more strongly developed in males. The phenotypes of the most strongly pigmented *Iy* fish and the most weakly pigmented *Ir* fish overlap. The *Ir* factor was present in 1901-11 (Table 1 and Table 4) and 1899-9 (Table 2): *Iy* was found in 1899-11 (Table 1), 1899-1 and -8 (Table 2), 1900-2 and -7 (Table 3) and in ped. 1918 (Table 4). The male parent of ped. 1935 and ped. 1968 (Table 1 and Table 2) was heterozygous for both *Iy* and *Ir*.

The factors controlling iris coloration, red or yellow body and fin pigmentation and macro-

melanophore patterns are controlled by at least three major loci. According to Gordon (1937) and MacIntyre (1961), the macromelanophore system may represent a pseudoallelic series, since crossing over within the macromelanophore locus has been observed. An exceptional recombinant in which the *Sp* and *Sr* factors of the Jp 163 B stock may have become linked on the *Y* chromosome has been obtained in ped. 1981 (Table 2). Evidence which will be presented in greater detail elsewhere has shown that *Dr* of Jamapa and *Mr* of Belize are not allelic. Of all known pigment genes, the iris locus is located closest to the sex differential segment.

The origin and the sex chromosome constitution of the reference stocks with the exception of the *Up* strains has been described elsewhere (Kallman 1965). Strain *Up*-2 (*WY-YY*) has been derived from fish collected at Sebol, near the source of the Rio de la Pasion. Fish of pedigrees 1334, 1340, and 1375 are the progenitors of the stock (Kallman 1965, Table 17). Strain *Up*-1 (*XX-XY*) has been derived from fish of pedigree 1424 (Kallman 1965, Table 17). The following sex-linked patterns of the reference stocks were used as marker genes of their sex chromosomes:

Hp-2	♀♀ X_+X_+	♂♂ X_+Y_{Sd}
Cp	$X_{Sp}X_+$	
Gp		$X_{Sp}Y_{Sd}$
Up-1	$X_+X_{f\text{-}Sd}$	
Up-2		$Y_{Sr}Y_{Iy}$
Jp 163 A	$X_{Dr}X_{Dr}$	$X_{Dr}Y_{Sr}$
Jp 163 B	$X_{Sp}X_{Sp}$	$X_{Sp}Y_{Sr}$ or $X_{Sp}Y_{ArSr}$

The method for maintaining fish, recording data and assigning pedigree numbers has been explained previously (Kallman 1965).

Results

Sex chromosomes of males: A total of eight males collected from the Sibun and Belize River drainages were crossed to *XX* females of the reference stocks. The *YY* genotype of seven males was clearly indicated by their all male offspring (Table 1). Such broods are characteristic of *XX* ♀♀ x *YY* ♂♂ crosses (Kallman 1965, Table 23). When five of the males were mated to *WY* females from the Belize River and Sibun River, young of both sexes were obtained in frequencies that did not significantly deviate from the theoretical 1:1 ratio (ped. 1927, 1968, Table 2; 1931, 1934, Table 3; 1920, Table 4). However, an eighth male, 1901-12, was *XY:* he gave rise to males and females in equal numbers; *Ay* and *Sr* were inherited in females only (ped. 1921, Table 1). Since the other seven males collected were *YY*, more evidence for the *XY* genotype of 1901-12 was desired. Therefore, this male was tested with an *XX* female of a second strain (Hp-2). Again the results are only consistent with the assumption that 1901-12 was *XY* with *Ay* and *Sr* linked on the *X* chromosome (ped. 1977, Table 1). Additional data was obtained from a cross involving a female descendant of male 1901-12 (ped. 2010, Table 1). The single wild type female of ped. 2010 is presumably due to nonexpression of *Dr* which was poorly developed in many females. The exceptional *AySr* male of ped. 2010 is a crossover which establishes that *Dr* of Jamapa is not allelic to *Mr* of Belize (Kallman, unpublished).

Sex chromosomes of females: The sex chromosomes of 18 females were identified by crossing them with *XY* males of the reference stocks (1899-1, -5, -7, -8, -9, Table 2; 1900-1, -3, -5, -6, -8, -10, -23, -24, -25, Table 3; 1901-1, -2, -3, -4, Table 4). The *X*- and *Y*-linked pigment patterns of the male parent were inherited by offspring of both sexes, thereby establishing the *WY* genotype of the 18 females. Moreover, with few exceptions, the maternal pigment patterns were inherited by males only. The young of eight other females (ped. 1916, 1927, 1968, Table 2; ped. 1905, 1909, 1934, 1931, Table 3; ped. 1920, Table 4) were either sired by *YY* males in the laboratory or by unknown males which had inseminated the females before they were collected. The evidence that the sex chromosome constitution of these eight females was *WY* is presented in Table 5: when a male offspring of each of the eight pedigrees was crossed to *XX* females, broods consisting only of males resulted. In ped. 1913 and 1937 (Table 2) the patterns of the P_1 female were inherited by males only but no further crosses were made with these fish. Females 1899-2 and -6, therefore, possessed a *W* chromosome and either an *X* or *Y* on which the pigment genes were located. The genotype of female 1900-22 was *WY*, although this is not readily apparent from the inheritance of pigment patterns and sex ratio of ped. 1952 (Table 3) in which an exceptional class of offspring was present (the *Sd* males). This cross is further analyzed below (Table 6).

The genotype of female 1900-21 was *XX*. This is indicated by the 1:1 sex ratio and the inheritance of *Sd* by all males but none of the females of ped. 1923 (Table 3). Two crosses provide further evidence for the *XX* genotype of 1900-21. A *Sd* male of ped. 1923 without the *Ar* pattern was crossed with *Jp 163 B*. In their progeny (ped. 2033) *Sd* was inherited only by males. Therefore, the unmarked sex chromosome of female 1900-21 was X_+.

Jp $X_{Sp}X_{Sp}$ x 1923-11 X_+Y_{Sd}
Ped. 2033: 23 *Sp*♀♀; 13 *SpSd*♂♂

TABLE 2. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF TEN FEMALES (ped. 1899 Collected from the Sibun River, British Honduras (Freetown).

Pedigree and genotype of parents				*Pedigree of offspring*			
♀♀		♂♂			♀♀		
1899-3	W_+Y_+†	unknown		1916	8 Ay*	7 +	
1899-5	W_+Y_+	Gp	$X_{Sp}Y_{Sd}$	1933	23 Sp	18 Sd	1
1899-7	W_+Y_{Ay}	Hp-2	X_+Y_{Sd}	1932	16 Sd	19 +	
1899-2	$W_+?_{Ay}$	unknown		1913	8 Dr*	19 +	
1899-9	W_+Y_{Ir}	Jp	$X_{Sp}Y_{Sr}$	1981	24 Sp	14 Sr	
1899-4	W_+Y_{Sd}	1900-13‡	Y_+Y_{VoSp}	1927	17 Sp[2]	16 +	
1899-6	$W_+?_{AySp}$	unknown		1937	11 CPo*	11 Sd*	17
1899-10	W_+Y_{AySp}	1899-12‡	$Y_{Iy}Y_{Ir}$	1968	23 Ir	11 Iy	
1899-1	W_+Y_{IyAy}	unknown		1906a	7 Mr Sd*	9 +	
1899-1		Hp-2	X_+Y_{Sd}	1906b	12 Sd	9 +	
1899-8	W_+Y_{IyAy}	Jp	$X_{Sp}Y_{Sr}$	1928	17 Sp	11 Sr	

†The sex chromosomes listed for the ten females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1899-12 and 1900-13 are identified in Table 1.
*Some patterns inherited from unknown male parent.
[1] An exceptional offspring with a new macromelanophore pattern linked to *Ar* on Y chromosome (Kallman unp
[2] *Vo* is not expressed in females; 7 males were sacrificed at the age of 5 months before the pattern was apparent.
[3] Some fish also exhibit some red coloration in iris.

TABLE 3. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIFTEEN FEMALES (ped. 19 Collected from the Belize River, British Honduras (Bermudian Landing)

Pedigree and genotype of parents				*Pedigree of offspring*			
♀♀		♂♂			♀♀		
1900-1	W_+Y_{CPo}	unknown		1904a	14 +	9 N*	5 Sd*
1900-1		Jp	$X_{Sp}Y_{Sr}$	1904b	11 Sp	9 Sr	
1900-5	W_+Y_{Sr}	unknown		1911a	13 +	1 Sd Mr*	
1900-5		Hp-2	X_+Y_{Sd}	1911b	21 +	18 Sd	
1900-22	W_+Y_N	Gp	$X_{Sp}Y_{Sd}$	1952	24 Sp	3 Sd	
1900-24	W_+Y_N	Hp-2	X_+Y_{Sd}	1953	20 Sp	15 Sd	
1900-8	W_+Y_{Ay}	unknown		1917a	9 +	10 Mr Sd*	
1900-8		Gp	$X_{Sp}Y_{Sd}$	1917b	1 Sp	10 Sd	
1900-2	W_+Y_{Iy}	unknown		1905	29 +	1 Ir*	1 Mr*
1900-10	W_+Y_{Ar}	Jp	$X_{Dr}Y_{SrAr}$	1936	18 Sp	20 Sr Ar[1]	
1900-9	W_+Y_{AySp}	1900-14‡	$Y_{Dr}Y_{Br}$	1931	7 +	18 Br	
1900-23	W_+Y_{AySp}	Jp	$X_{Dr}Y_{Sr}$	1930	22 Sr	17 Dr	13 Dr
1900-6	W_+Y_{CPoSr}	Hp-2	X_+Y_{Sd}	1929	34 +	35 Sd	
1900-4	W_+Y_{CPoSr}	unknown		1909	53 +		
1900-25	W_+Y_{CPoN}	Jp	$X_{Dr}Y_{Sr}$	1972	10 Dr	14 Sr	
1900-3	W_+Y_{BrSd}	unknown		1908a	15 +	11 Dr*	
1900-3		Jp	$X_{Sp}Y_{Sr}$	1908b	19 Sp	18 Sr	
1900-7	W_+Y_{IyAy}	1900-11‡	Y_+Y_{MrSd}	1934	9 +	10 Mr Sd	
1900-21	X_+X_{Ar}	Hp-2	X_+Y_{Sd}	1923	19 +	10 Ar	

†The sex chromosomes listed for the 15 females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1900-11 and 1900-14 are identified in Table 1.
*Some pattern inherited from unknown male parent.
[1] Anal red, *Ar*, pattern of 1900-10 and of the 35 male offspring is different from that present in the 20 *Sr* females.
[2] One male of each class developed some orange coloration in the iris.

Phenotype of offspring

	♂♂	
7 Ay*	10 +	
9 Sp	30 Sd	
1 Ay Sd	15 Ay	
0 Ay Dr*	16 Ay	1 Iy Ay*
6 Ir *Sp*	11 Ir Sr	1 Ir M[1]
3 Vo Sp Sd	28 Sd	7 Sp Sd[2]
1 Ay Sp CPo*	5 Ay Sp Sd*	19 Ay Sp
1 Iy Ay Sp	28 Ir Ay Sp	
5 Iy Ay Mr Sd*	14 Iy Ay[3]	1 +
2 Iy Ay Sd	12 Iy Ay	
9 Iy Ay Sp[3]	19 Iy Ay Sr[3]	

Phenotype of offspring

	♂♂	
6 CPo	2 CPo N*	
7 CPo Sp	7 CPo Sr	
1 Sr		
3 Sr	12 Sd Sr	
2 N Sd	9 Sp N	13 Sd
0 N Sd	9 N	
0 Ay Mr Sd*	9 Ay	
5 Ay Sd	5 Ay Sp	
6 Iy	14 Ir*	2 Mr* 5 +
6 Sp Ar[1]	19 Sr Ar[1]	
5 Ay Sp Dr	12 Br Ay Sp	
4 Ay Sp Sr	13 Dr Ay Sp	
5 Sd CPo Sr	25 CPo Sr	
3 CPo Sr		
2 CPo N Sr	20 CPo N Dr	
3 Br Sd	12 Br Sd Dr*	
3 Br Sd Sr	10 Br Sd Sp	
9 Iy Ay[2]	7 Iy Ay Mr Sd[2]	
7 Ar Sd	20 Sd	

A female of ped. 1923 with *Ar* was crossed to a *Jp* male. All female offspring but none of the male offspring (ped. 2050) inherited *Sp*, while *Sr* was inherited by males only. Therefore the genotype of 1923-1 must have been *XX*, one *X* derived from *Hp-2*, and the other, marked by *Ar*, from 1900-21.

$$1923\text{-}1\ X_{+}X_{Ar} \quad x \quad Jp\ X_{Sp}Y_{ArSr}$$

Ped. 2050: 18 *Sp*♀♀; 13 *SpAr*♀♀; 33 *ArSr*♂♂

In ped. 2050 the *Ar* progeny fell into two non-overlapping classes. The 13 *Ar* females and 19 of the *Ar* males exhibited a red pattern quite unlike that present in *Jp* fish with *Ar* or in Jp x Belize hybrids that inherited the *Ar* factor of Jamapa.

The unknown male which had already fertilized female 1900-2 (Table 3) at the time of capture, must have possessed the *XY* genotype. A wild-type female offspring (1905-2) was crossed to 1904-11, a $Y_{CPo}Y_{N}$ male. They produced young of four classes (ped. 2124): 7 N♀♀, 8 + ♀♀, 3 N♂♂ and 2 *CPo*♂♂. The eight wild-type females presumably carry the *CPo* allele which is poorly expressed in many females. When a *N* male (2124-12) was bred with a Jp $X_{Dr}X_{Dr}$ female, all nine *Dr* offspring were females, all 13 *Dr N* were males (ped. 2245). Similarly a male with the *CPo* pattern (2124-11) sired 15 *Dr* females and 30 *Dr CPo* males (ped. 2234), when mated with a Jp female. These results indicate that *CPo* and *N* are *Y*-linked (already confirmed for *CPo* by ped. 1904 b, Table 3). The other sex chromosome of males 2124-11 and -12 must have been an unmarked X, traceable to the unknown male that fertilized 1900-2. The genotype of the wild-type female, 1905-2, was *WX*.

Among the pedigrees listed in Tables 2, 3, and 4 are several exceptions that require further explanation. If the genotype of female 1900-2 were $W_{+}Y_{Iy}$, only males of pedigree 1905 should have inherited the iris pattern (Table 3). However, there were seven female young with iris coloration and seven male offspring without any. Pedigree 2084 (Table 6) demonstrates that *Iy* is Y-linked in females of ped. 1905. The females must have inherited the iris pattern from one of the unknown males that had inseminated female 1900-2 before she was collected. One of the males (1905-11, Table 5) with wild-type iris coloration was tested. He sired 32 *Mr* males and 29 *Iy* males: the *Iy* allele remained unexpressed in this male parent. However, it cannot be assumed that the other exceptional males (ped. 1905) were also due to nonexpression. The two males of peds. 1906a and 1907b (Table

TABLE 2. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF TEN FEMALES (ped. 189
Collected from the Sibun River, British Honduras (Freetown).

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*		
♀♀		♂♂			♀♀		♂♂
1899-3	W_+Y_+†	unknown		1916	8 Ay*	7 +	7 Ay*
1899-5	W_+Y_+	Gp	$X_{Sp}Y_{Sd}$	1933	23 Sp	18 Sd	19 Sp
1899-7	W_+Y_{Ay}	Hp-2	X_+Y_{Sd}	1932	16 Sd	19 +	11 Ay Sd
1899-2	$W_+?_{Ay}$	unknown		1913	8 Dr*	19 +	10 Ay Dr*
1899-9	W_+Y_{Ir}	Jp	$X_{Sp}Y_{Sr}$	1981	24 Sp	14 Sr	16 Ir Sp
1899-4	W_+Y_{Sd}	1900-13‡	Y_+Y_{roSp}	1927	17 Sp^2	16 +	13 Vo Sp Sd
1899-6	$W_+?_{AySp}$	unknown		1937	11 CPo*	11 Sd*	11 Ay Sp CPo°
1899-10	W_+Y_{AySp}	1899-12‡	$Y_{Iy}Y_{Ir}$	1968	23 Ir	11 Iy	11 Iy Ay Sp
1899-1	W_+Y_{IyAy}	unknown		1906a	7 Mr Sd*	9 +	5 Iy Ay Mr Sd*
1899-1		Hp-2	X_+Y_{Sd}	1906b	12 Sd	9 +	12 Iy Ay Sd
1899-8	W_+Y_{IyAy}	Jp	$X_{Sp}Y_{Sr}$	1928	17 Sp	11 Sr	19 Iy Ay Sp^3

Phenotype of offspring		
♂♂		
10 +		
30 Sd		
15 Ay		
16 Ay	1 Iy Ay*	
11 Ir Sr	1 Ir M^1	
28 Sd	7 Sp Sd^2	
5 Ay Sp Sd*	19 Ay Sp	
28 Ir Ay Sp		
14 Iy Ay^3	1 +	
12 Iy Ay		
19 Iy $Ay Sr^3$		

†The sex chromosomes listed for the ten females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1899-12 and 1900-13 are identified in Table 1.
*Some patterns inherited from unknown male parent.
[1] An exceptional offspring with a new macromelanophore pattern linked to *Ar* on Y chromosome (Kallman un
[2] *Vo* is not expressed in females; 7 males were sacrificed at the age of 5 months before the pattern was apparent.
[3] Some fish also exhibit some red coloration in iris.

TABLE 3. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIFTEEN FEMALES (ped. 1
Collected from the Belize River, British Honduras (Bermudian Landing)

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*		
♀♀		♂♂			♀♀		♂♂
1900-1	W_+Y_{CPo}	unknown		1904a	14 +	9 N*	5 Sd^3
1900-1		Jp	$X_{Sp}Y_{Sr}$	1904b	11 Sp	9 Sr	16 CPo
1900-5	W_+Y_{Sr}	unknown		1911a	13 +	1 Sd Mr*	7 CPo Sp
1900-5		Hp-2	X_+Y_{Sd}	1911b	21 +	18 Sd	11 Sr
1900-22	W_+Y_N	Gp	$X_{Sp}Y_{Sd}$	1952	24 Sp	3 Sd	13 Sr
1900-24	W_+Y_N	Hp-2	X_+Y_{Sd}	1953	20 Sp	15 Sd	12 N Sd
1900-8	W_+Y_{Ay}	unknown		1917a	9 +	10 Mr Sd*	10 N Sd
1900-8		Gp	$X_{Sp}Y_{Sd}$	1917b	1 Sp	10 Sd	10 Ay Mr Sd*
1900-2	W_+Y_{Iy}	unknown		1905	29 +	1 Ir*	1 Mr 5 Ay Sd
1900-10	W_+Y_{Ar}	Jp	$X_{Dr}Y_{SrAr}$	1936	18 Sp	20 Sr Ar^1	16 Sp Ar^1
1900-9	W_+Y_{AySp}	1900-14‡	$Y_{Dr}Y_{Br}$	1931	7 +	18 Br	5 Ay Sp Dr
1900-23	W_+Y_{AySp}	Jp	$X_{Dr}Y_{Sr}$	1930	22 Sr	17 Dr 13 Dr	14 Ay Sp Sr
1900-6	W_+Y_{CPoSr}	Hp-2	X_+Y_{Sd}	1929	34 +	35 Sd	25 Sd CPo Sr
1900-4	W_+Y_{CPoSr}	unknown		1909	53 +		53 CPo Sr
1900-25	W_+Y_{CPoN}	Jp	$X_{Dr}Y_{Sr}$	1972	10 Dr	14 Sr	12 CPo N Sr
1900-3	W_+Y_{BrSd}	unknown		1908a	15 +	11 Dr*	13 Br Sd
1900-3		Jp	$X_{Sp}Y_{Sr}$	1908b	19 Sp	18 Sr	13 Br Sd Sr
1900-7	W_+Y_{IyAy}	1900-11‡	Y_+Y_{MrSd}	1934	9 +	10 Mr Sd	9 Iy Ay^2
1900-21	X_+X_{Ar}	Hp-2	X_+Y_{Sd}	1923	19 +	10 Ar	7 Ar Sd

♂♂		
2 CPo N*		
7 CPo Sr		
12 Sd Sr		
9 Sp N	13 Sd	
9 N		
9 Ay		
5 Ay Sp		
14 Ir*	2 Mr* 5 +	
19 Sr Ar^1		
12 Br Ay Sp		
13 Dr Ay Sp		
25 CPo Sr		
20 CPo N Dr		
12 Br Sd Dr*		
10 Br Sd Sp		
7 Iy Ay Mr Sd^3		
20 Sd		

†The sex chromosomes listed for the 15 females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1900-11 and 1900-14 are identified in Table 1.
*Some pattern inherited from unknown male parent.
[1] Anal red, *Ar*, pattern of 1900-10 and of the 35 male offspring is different from that present in the 20 *Sr* female
[2] One male of each class developed some orange coloration in the iris.

A female of ped. 1923 with *Ar* was crossed to a *Jp* male. All female offspring but none of the male offspring (ped. 2050) inherited *Sp*, while *Sr* was inherited by males only. Therefore the genotype of 1923-1 must have been *XX*, one *X* derived from *Hp-2*, and the other, marked by *Ar*, from 1900-21.

1923-1 X_+X_{Ar} x Jp $X_{Sp}Y_{ArSr}$

Ped. 2050: 18 *Sp*♀♀; 13 *SpAr*♀♀; 33 *ArSr*♂♂

In ped. 2050 the *Ar* progeny fell into two non-overlapping classes. The 13 *Ar* females and 19 of the *Ar* males exhibited a red pattern quite unlike that present in *Jp* fish with *Ar* or in Jp x Belize hybrids that inherited the *Ar* factor of Jamapa.

The unknown male which had already fertilized female 1900-2 (Table 3) at the time of capture, must have possessed the *XY* genotype. A wild-type female offspring (1905-2) was crossed to 1904-11, a $Y_{CPo}Y_N$ male. They produced young of four classes (ped. 2124): 7 N♀♀, 8+ ♀♀, 3 N ♂♂ and 2 *CPo* ♂♂. The eight wild-type females presumably carry the *CPo* allele which is poorly expressed in many females. When a *N* male (2124-12) was bred with a Jp $X_{Dr}X_{Dr}$ female, all nine *Dr* offspring were females, all 13 *Dr N* were males (ped. 2245). Similarly a male with the *CPo* pattern (2124-11) sired 15 *Dr* females and 30 *Dr CPo* males (ped. 2234), when mated with a Jp female. These results indicate that *CPo* and *N* are *Y*-linked (already confirmed for *CPo* by ped. 1904 b, Table 3). The other sex chromosome of males 2124-11 and -12 must have been an unmarked X, traceable to the unknown male that fertilized 1900-2. The genotype of the wild-type female, 1905-2, was *WX*.

Among the pedigrees listed in Tables 2, 3, and 4 are several exceptions that require further explanation. If the genotype of female 1900-2 were W_+Y_{Iy}, only males of pedigree 1905 should have inherited the iris pattern (Table 3). However, there were seven female young with iris coloration and seven male offspring without any. Pedigree 2084 (Table 6) demonstrates that *Iy* is Y-linked in females of ped. 1905. The females must have inherited the iris pattern from one of the unknown males that had inseminated female 1900-2 before she was collected. One of the males (1905-11, Table 5) with wild-type iris coloration was tested. He sired 32 *Mr* males and 29 *Iy* males: the *Iy* allele remained unexpressed in this male parent. However, it cannot be assumed that the other exceptional males (ped. 1905) were also due to nonexpression. The two males of peds. 1906a and 1907b (Table

2 and Table 4) lacking maternal pigment patterns were genetic sex reversals of the *WY* genotype (Table 6). This is evinced by the inheritance of *CPo* and *Sr* in all male and some of the female offspring of ped. 2146 and by the appearance of *Sr* females (*WX*) and *SdSr* males (*XY*) in ped. 2069.

Most probably the *Sd* males of ped. 1952 (Table 3) were genetic sex reversals of the *WY* genotype. None were tested, because an earlier analysis of a similar situation had shown that *WY* males often give rise to many more sex reversals (Kallman 1968). Such results would not help to identify the sex chromosome constitution of female 1900-22. Instead, one fish each of the "normal' classes was tested (Table 6). Ped. 2065 demonstrates that the *N* gene of female 1900-22 was *Y*-linked; ped. 2071 and 2090 show that her unmarked sex chromosome was a W. The eleven *Sr* and two *Iy* males of the last two pedigrees are also sex reversals. Thus fish of ped. 1952 were of the following genotypes: *Sp* ♀♀ – WX; *Sd* ♂♂ and ♀♀ – WY, *SpN* ♂♂ – XY; *SdN* ♂♂ – YY.

The occurrence of males with the exceptional genotype WY is not new for *X. maculatus* and one special case was analyzed recently (Kallman 1968). As in the previous study WY males arose not only among F_1 hybrids between two stocks, but were also found among the progeny when normal female sibs of sex reversals were mated to males of different, totally unrelated stocks (in these crosses to *Jp* and *Up,* peds. 2071 and 2090). Since the WY males of ped. 1952 (*Bp* ♀ x *Gp* ♂), ped. 2071 [(*Bp* ♀ x *Gp* ♂) ♀ x *Jp* ♂] and ped. 2090 [(*Bp* ♀ x *Gp* ♂) ♀ x *Up-2* ♂] obviously have different genotypes, relatively few factors, perhaps only one or two with incomplete penetrance, must cause WY fish to differentiate into functional males. In contrast to the descendants of *Np* x *Cp* crosses (Kallman 1968), no WX males were herein encountered.

Discussion

Breeding data involving fish collected in British Honduras have demonstrated that the majority of females are *WY* and males *YY*. However, one *XX* female and one *XY* male from the Belize River have been identified at Bermudian Landing and one *XY* male at Gaboural Creek. Therefore, the theory that the *X* chromosome is absent from *X. maculatus* populations inhabiting rivers in British Honduras can no longer be maintained. The *X* chromosome has been demonstrated throughout the range of *X. maculatus,* from the Rio Jamapa (Veracruz, Mexico) in the west to the Belize River in the east. There are a few locations where the *X* chromosome has not yet been found. No fish were examined from the Rio Tonala, Mexico. Only one female and two males were tested from the New River in British Honduras (Kallman 1965). Information is also lacking for several small populations of platyfish in the streams and creeks of the narrow coastal plain of British Honduras, south of the Sibun River, but these populations inhabit an area that comprises less than one per cent of the total range of this species.

The results of the crosses herein described suggest the hypothesis that the *W* chromosome of natural populations does not carry pigment factors. This is unexpected because crossing over of pigment genes from the *Y* to the *W* has been reported in domesticated stocks of platyfish (Bellamy and Queal, 1951; Fraser and Gordon 1929; Gordon 1937) and in laboratory stocks derived from wild populations (Kallman 1965). The 27 marked females collected in the Sibun and Belize Rivers (Tables 2, 3, and 4) possessed a total of 41 patterns controlled by sex-linked factors (4 ♀♀ – macromelanophores only; 11 ♀♀ – macromelanophores and red and yellow body patterns; 7 ♀♀ – red or yellow body patterns only; 2 ♀♀ – iris patterns only; 3 ♀♀ – red or yellow body and iris patterns). Of the 41 factors representing at least three loci, not one was *W*-linked. Since the females were obtained from three stations only, the sample may actually be smaller than it appears: several females of a collection could have been related and could have inherited their pigment genes from the same parent. Thus certain marked chromosomes would be represented more than once in the sample. This is probably true of the Sp^8 and *Vo* alleles. The combination Sp^8Vo was present in two *YY* males from Bermudian Landing. No other sexually mature fish with Sp^8 or *Vo* were collected[3]. However, even if each pattern or combination of patterns of each location is counted only once, the three collections combined are still comprised of 19 differently marked females with 28 pigment factors. The absence of *W*-linked patterns, therefore, does not appear to be a sampling error.

Certain red patterns develop poorly in females. Preliminary experiments have shown them to be under androgenic control. There exists the possibility that females might possess pigment factors on the *W* that would go undetected for many generations. It is difficult to surmise the possible function of a pigment factor that would be inherited strictly maternally but which could only be expressed in males. The possibility of

[3] An immature fish with Sp^8 was present in the Gabourel Creek collection. It was too young to have any *Vo* exhibited.

TABLE 4. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIVE FEMALES (ped. 1901)
Collected from the Belize River, British Honduras (Gabourel Creek)

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*					
♀♀		♂♂			♀♀			♂♂		
1901-1	W_+Y_{Ay}	unknown		1907a	4 N*	23 +		1 Ay N*	20 Ay	
1901-1		Hp-2	X_+Y_{Sd}	1907b	14 Sd	12 +		11 Sd Ay	5 Ay	1 Sd
1901-4	W_+Y_{Ay}	unknown		1918a	16 Dr*	19 +	5 Iy*	11 Ay Dr*	3 Ay Iy*	11 Ay
1901-4		Hp-2	X_+Y_{Sd}	1918b	4 Sd	5 +		2 Sd Ay	4 Ay	
1901-2	W_+Y_{CPoSd}	Jp	$X_{Sp}Y_{Sr}$	1914	21 Sr	33 Sp		25 Sp CPo Sd	30 Sr CPo Sd	
1901-3	W_+Y_{CPoSd}	Jp	$X_{Sp}Y_{Sr}$	1915	21 Sr	19 Sp		16 Sp CPo Sd	16 Sr CPo Sd	
1901-5	W_+Y_{CPoSr}	1901-11‡	$Y_{Mr}Y_{Ir}$	1920	19 Mr	9 Ir		21 CPo Sr Mr	15 CPo Sr Ir	

† The sex chromosomes listed for the five females are the only ones that will adequately explain the results.
‡ Sex chromosomes of 1901-11 are identified in Table 1.
* Some patterns inherited from unknown male parent.

TABLE 5. IDENTIFICATION OF SEX CHROMOSOME CONSTITUTION OF EIGHT FEMALES FROM SIBUN RIVER AND BELIZE RIVER
(Crosses between XX females of reference stocks and male offspring of wild-caught females)

Pedigree and sex chromosomes of parents				*Pedigree of offspring*	*Phenotype of offspring*			
♀♀		♂♂			♀♀	♂♂		
Jp	$X_{Sp}X_{Sp}$	1916-11	$Y_{Ay}Y_+$	2119	none	20 Ay Sp	13 Sp	
Jp	$X_{Sp}X_{Sp}$	1927-12	$Y_{Sd}Y_{VoSp}$	2105	none	23 Vo Sp^s**	15 Sd Sp	
Up-1	X_+X_{f-Sd}	1968-11	$Y_{Ir}Y_{AySp}$	2053	none	5 Ay Sp	19 f-Sd Ay Sp	11 f-Sd Ir 10 Ir
Jp	$X_{Sp}X_{Sp}$	1905-11**	$Y_{Iy}Y_{Mr}$	2063	none	32 Mr	29 Iy	
Jp	$X_{Sp}X_{Sp}$	1909-12	Y_+Y_{CPoSr}	2106	none	26 Sp	20 Sp CPo Sr	
Jp	$X_{Sp}X_{Sp}$	1934-11	$Y_{MrSd}Y_{IyAy}$	2130	none	15 Iy Ay Sp	10 Sp Mr Sd	
Jp	$X_{Dr}X_{Dr}$	1931-12	$Y_{Br}Y_{AySp}$	2064	none	29 Dr Br	23 Ay Sp Dr	
Jp	$X_{Sp}X_{Sp}$	1920-11	$Y_{Mr}Y_{CPoSr}$	2057	none	27 Sp Mr	39 Sp CPo Sr	

* Sp^s masks Sp pattern of Jamapa stock.
** Phenotypically *Mr* when sacrificed at age of 20 months.

cryptic patterns does not apply to the macromelanophore alleles that are well expressed in both sexes, nor to iris coloration although it is generally more intense in males (Kallman unpublished). Four red or yellow body and fin patterns, *Br, Dr, Ar,* and *Ay,* develop quite strongly in both sexes, but the recognition of *Mr, Vo,* and certain *CPo* factors in females may be difficult or impossible[4]. *Mr* in females is not visible to the unaided eye but can be seen under higher magnification as an increased concentration of faintly orange pigment cells on the lower jaw. Since the poor expression of *Mr* in females was established soon after the beginning of the study of the Belize fish, all females and their offspring in Tables 2, 3, and 4 were examined for the pattern. Only two *Mr* females were discovered in pedigrees in which the pattern was not expected (ped. 1911, 1905, Table 3). *Vo* and occasionally *CPo* cannot be identified in females.

Breeding data published previously (Kallman 1965) are in accord with the hypothesis that the *W* chromosome of natural populations carries the wild-type allele at the macromelanophore locus. The patterns of 14 *WX* or *WY* females collected in the Belize River in 1949, New River, Rio Hondo (3 stations), Lake Peten, and Rio de la Pasion were controlled by *X*- or *Y*-linked factors.

It is also interesting to note that even in the domesticated stocks of the popular aquarium trade which have the *WY-YY* mechanism, *W*-linked pigment factors are usually absent. This is true of Gerschler's (1914) *X. maculatus* in Germany[5], Bellamy's platyfish at the University of Chicago and University of California (Bellamy 1922, 1928, 1933; Bellamy and Queal 1951), Gordon's fish at Cornell (Gordon 1927, 1937; Fraser and Gordon 1929)[6], Kosswig's and Breider's stocks in Germany (Kosswig 1928;

TABLE 6. EXPLANATION OF EXCEPTIONAL PROGENY FROM TABLES 2, 3, AND 4

Pedigree and genotype of parents			*Pedigree of offspring*	*Phenotype of offspring*			
♀♀	♂♂			♀♀		♂♂	
1905-1	$W+Y_{Jr}$	Jp	$X_{Sp}Y_{Sr}$	2084	9 Sp	16 Sr	12 Ir Sp	11 Ir Sr	
2049-6*	$W+Y_{CPoSr}$	1906a-13	$W+Y+$	2146	7 +	4 CPo Sr	6 CPo Sr		
Jp	$X_{Sr}X_{Sr}$	1907b-11	$W+Y_{Sd}$	2069	9 Sr		20 Sr Sd		
Jp	$X_{Sp}X_{Sp}$	1952-11	$Y_{Sd}Y_N$	2065	none		41 Sp Sd	32 Sp N	
1952-1	$W+Y_{Sd}$	Jp	$X_{Sp}Y_{Sr}$	2071	36 Sp	28 Sr	31 Sp Sd	18 Sd Sr	9 Sr
1952-2	$W+X_{Sp}$	Up-2	$Y_{Sr}Y_{Jy}$	2090	16 Sr	16 Jy	8 Sr Sp	16 Jy Sp	2 Jy 2 Sr

* From 1927 ♀ (Table 2) x 1909 ♂ (Table 3).

[4] No females with the *Ty* factor of the Belize population have been obtained; the expression of this pattern in Belize females remains unknown. A similar pattern, perhaps identical with *Ty* of Belize, occurs in the Up-1 stock of *X. maculatus* derived from fish collected in the headwaters of the Rio de la Pasion. In this stock a yellow to orange tail pattern is expressed in females.

[5] Gerschler described a cross between a *X. maculatus* female with "pulchra" pattern and a male of *X. hellerii*. In the F_1 generation "pulchra" was inherited by males only; actual numbers were unfortunately not given. Since F_1 *maculatus* x *hellerii* hybrids that have inherited the *W* chromosome of *maculatus* usually differentiate into females (see Kallman 1965, Table 26), it is most likely that pulchra was *Y*-linked.

[6] From Gordon's data (1951b) it cannot be determined whether the *W* chromosome of the Bh stock was marked by pigment genes.

TABLE 7. FREQUENCY OF MACROMELANOPHORE PATTERNS (M) IN COLLECTIONS OF PLATYFISH FROM THE BELIZE RIVER AND SIBUN RIVER (BRITISH HONDURAS)*

M	*G*† *1949*‡		*G 1950*		*G 1951*		*G 1952*		*G 1966*		*B*† *1966*		*F*† *1966*	
Patterns	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
+	193	125	165	133	34	20	445	227	60	35	52	59	122	91
Sr		5	1				11	14	1	5	3	4	1	6
N	8	7	15	30	3	3	11	19	1	5	2	4		1
Sd	3	10	3	4	2	2	7	17	3	3	1	2	5	5
Sp[7]											2	6	5	3
Sp[8]							2	2		**		2		
SdN						1	1	2		2				
SdSr														
NSr		1						1						
Total fish	204	148	184	167	39	26	477	282	65	50	60	77	133	106
Total M fish	11	23	19	34	5	6	32	55	5	15	8	18	11	15
% M	5.4	15.5	10.3	20.4	12.8	24	6.7	19.5	7.6	30	13.3	23	8.3	14.2
Assumption I: Difference between males and females due to chance.														
Expect. M	20	14	28	25	7	3	54	32	11	9	11	15	14	11
χ^2	10.84		7.16		4.09		29		8.84		1.74		2.32	
P	0.001		<0.01		<0.05		<0.001		<0.01		>0.1		>0.1	
Assumption II: W chromosome is not marked.														
q***	0.08		0.11		0.13		0.11		0.16		0.12		0.08	
Expect. M ♀♀	16		18		5		50		10		7		11	
χ^2	0.26		0.066		0.001		7.26		2.92		0.16		0	
P	>0.1		0.8		0.98		>0.01		>0.05		>0.5		1	

*The figures for the Gabourel Creek collections from 1949-1952 are different from those listed by Gordon and Gordon (Table 15, 1957); however, both their data and ours show an excess of patterned males. Gordon and Gordon inexplicably mistook several males for females, confused *N* and *Sr* with *Sp*, and failed to recognize *Sd* patterns in several specimens. Difference in total number of fish is due to the fact that they rejected more fish as immatures.

†G = Gabourel Creek, B = Bermudian Landing, F = Freetown.

‡Year of collection.

**One immature fish with *Sp*[8] cannot be sexed.

***q = the frequency of marked Y chromosomes in the population.

Breider 1936)[7] and Kosswig's (1938) Fury strain. In an aquarium store in a small German town, Breider (1942) discovered a single female with a combination of patterns to suggest *W*-linkage and subsequent crosses proved this to be true. Since he was aware that the *W* chromosome is usually unmarked he suggested that the female most likely arose due to crossing over. The rarity of females with marked *W* chromosomes is also indicated by Gordon's report (1937) that inspection of two commercial aquarium establishments yielded only two females with two macromelanophore patterns each which upon breeding proved to be *W*- and *Y*-linked[8]. The absence of marked *W* chromosomes in commercial stocks appears quite significant, since breeders select for fish with the brightest colors or the most patterns.

The seven preserved collections of *X. maculatus* from the Belize River and Sibun River provide additional support for the hypothesis. Without exception, the proportion of males exhibiting macromelanophores in the samples was 2 to 3 times that of females (Table 7). In Table 7 it was first assumed that the number of patterned males and females differed due to chance; that the frequency of marked *W* and *Y* chromosomes was the same. Based upon this assumption, the deficiency of spotted females was highly significant for all but the two small 1966 collections from Bermudian Landing and Freetown, which nevertheless showed the same trend as the other samples.

However, if we assume that the *W* chromosome always carries the + allele, the frequency of patterned females should be less than that of males. From the number of males with macromelanophores the frequency of marked *Y* chromosomes in the population can be obtained (Table 7); this value should equal the percentage of females with patterns. A slight complication is introduced by (1) considering all females as *WY* and males as *YY* and thereby ignoring the few fish with *X* chromosomes, and by (2) possible nonexpression of macromelanophore alleles in young fish. In spite of these possible sources of error, the actual data of six of the seven collections fit the assumption rather well that the *W* carries the + allele at the macromelanophore locus. Only for the 1952 collection is there a deficiency of females, but here too the fit is much better for the second assumption.

Although the data of the preserved collections tell us nothing about the frequency of the red or yellow patterns, breeding experiments indicate that the *W* chromosome of natural populations lacks pigment factors at these loci as well. This is not true of the *X* chromosome. Two of the four *X* chromosomes recovered from the populations of the Belize River and Sibun River were marked, one by *Ar* and the other by *AySr*. Similarly, several *X* chromosomes of males and females collected at locations in Guatemala carried factors for macromelanophore (Kallman 1965) and a variety of red and yellow patterns (Kallman unpublished).

As Gordon and Gordon (1957) already noted, no consistent correlation can be established between the frequency of a population's patterned males versus females and its sex chromosome mechanism. In regions where the *X* chromosome seems to be more common than in the Belize River (or where the *W* chromosome may be absent altogether), the frequency of patterned females may equal that of males (Rio Hondo), may be significantly higher than that of males (Rio Papaloapan) or significantly lower (Rio Jamapa, Rio Coatzacoalcos). It must be admitted, however, that our knowledge of platyfish sex chromosome mechanisms of the Rio Jamapa and Rio Papaloapan (Gordon 1947) and the Rio Coatzacoalcos and Rio Hondo (Kallman 1965) is quited limited, therefore no precise statement concerning the relative frequency of *X* and *W* chromosomes or absence of the *W* is possible. According to data published previously (see Kallman 1965, Table 21) the *X* chromosome in these populations seems to be more common than in those of the Belize River or Sibun River.

The diverse populations of the major river systems cannot be equated. Significant differences exist in the frequency and occurrence of certain macromelanophore and tail patterns (Gordon and Gordon, 1957). The *N* pattern and two tail spot markings are absent from the Rio Jamapa. Other tail patterns are not found in populations of the Rio Usumacinta system and rivers of British Honduras. The frequency of fish with macromelanophore patterns ranges from 0.05 in the Rio Grijalva to 0.35 in the Rio Papaloapan. Recent experiments indicate that the *Sd* and *Sp* patterns of populations inhabiting different river systems are caused by different alleles (Kallman unpublished). The selective value of alleles that give rise to virtually identical patterns may not be the same in

[7] The single female with a marked *W* chromosome reported by Kosswig in 1936 was due to crossing over as explained in a later paper (Kosswig 1937).

[8] It is realized, of course, that *WY* females homozygous for a pattern or heterozygous with *W*-linkage cannot be told apart from heterozygous females with *Y*-linkage by mere inspection. However, in stocks in which the pigment gene is on the *W*, the pattern is exhibited by females only.

the various populations. The selective value may depend upon the frequency of other pigment factors in the gene pool and upon linkage with other pigment genes.

The sex-linked patterns of *X. maculatus* are controlled by at least three major loci in the following sequence: sex differential segment, locus for iris pattern, locus for red or yellow body pattern, locus for macromelanophore pattern (Kallman unpublished). Crossing over between the *W* and *Y* chromosomes or between the *X* and *Y* chromosomes involving the sex differential segment and macromelanophore locus occurs at a frequency of 0.2 to 0.3% (Kallman 1965; Bellamy and Queal 1951). Thus certain combinations of patterns with high selective values will be maintained and could spread through the population. In the experiments reported herein, only three crossovers were observed. So far no sex chromosome with three pigment factors has been identified from natural populations (Table 8), although one has been obtained through crossing over in the *Up* stock (Kallman unpublished).

Although nothing has been published as to what conditions contribute to the remarkable sex chromosome and pigmentary polymorphism of *X. maculatus* (Table 8), it is most likely that, in general, conspicuous pigmentation in males may be advantageous during courtship and agonistic behavior, but of disadvantage as far as predation is concerned. In females dull coloration may be favored. These factors seem to be involved in maintaining the pigmentary polymorphism of another poeciliid fish, *Poecilia reticulata.* Fisher (1930) and Sheppard (1953) pointed out that Winge's data (Winge, 1927) showed overwhelming *Y*-linkage for the incompletely sex-linked pigment patterns, but that in the absence of selection crossing-over should tend to equalize the percentage of pigment factors on the *X* and *Y* chromosomes. They suggested the deficiency of *X*-linked pigment factors indicated bright coloration is an advantage in males and a disadvantage in females. Haskins and Haskins (1950) and Haskins, Haskins, McLaughlin, and Hewitt (1961) have demonstrated in laboratory experiments that in mating competitions brightly-colored males enjoy an advantage over dull-colored ones. In predation experiments the situation was reversed. They were also able to show that *X*-linked patterns were relatively rare in natural populations exposed to fish predators. It must also be emphasized that in *P. reticulata* most of the sex linked pigment patterns are under androgenic control and are not expressed in females even when present in homozygous condition. Thus two mechanisms, one hormonal and the other chromosomal, tend to restrict patterns to males. Rosen and Tucker (1961) have noted that in poeciliid genera with short gonopodia males are usually more highly pigmented than females and display elaborate courtship behavior.

Certain similarities between *P. reticulata* and *X. maculatus* are apparent. Besides the absence of pigment factors from the strongly female-determining *W* chromosome in natural populations of *X. maculatus,* evidence for a selective advantage of bright coloration in males of this species comes from the observation that in the Belize population (and perhaps also in others) certain red patterns are much better developed in males while other patterns are under androgenic control and not expressed in females at all. No such sex difference has been noted in the development of macromelanophore patterns, except that Sp^8 males often exhibit a large black spot above the insertion of the gonopodium. The spot is not present in Sp^8 females (Pl. I, figs. 1 and 2).

Certainly the breeding systems in the eight major drainages are not the same. In populations with an *XX* ♀♀ x *XY* ♂♂ mechanism, the *Y* chromosome, aside from rare cases of crossing

Table 8. Pigment Factors or Combination of Pigment Factors* on the Sex Chromosomes of the Offspring of 38 *Xiphophorus maculatus* Collected from the Belize and Sibun Rivers, British Honduras

Freetown (ped. 1899)	*Bermudian Landing* (ped. 1900)	*Gabourel Creek* (ped. 1901)
	Sr	
Sd	Sd	
	N	N
Mr Sd	Mr Sd	
	Br Sd	CPo Sd
Ay Sp^7	Ay Sp^7	
	CPo Sr	CPo Sr
		Ay Sr (x)
	CPo N	
	Vo SP^8	
	Br	
Dr	Dr	Dr
Ay	Ay	Ay
	Ar	
	Ar (x)	
	Ty	
CPo	CPo	
	Mr	Mr
Iy	Iy	Iy
Ir	Ir	Ir
Iy Ay	Iy Ay	

*All pigment factors located on Y chromosome except those marked by (x).

over, essentially represents a vehicle for strict paternal transmission of characters, which ceases the moment a *W* chromosome arises. Factors on the *W* chromosome, irrespective of the frequency of the *X* in the gene pool, are strictly maternally inherited. In populations with a *XX-XY* mechanism, dull coloration in females and bright coloration in males theoretically could be achieved by restricting all color factors to the *Y* chromosome, or bringing the development of the patterns under hormonal control. Elimination of marked *X* chromosomes would be difficult, however, since they would be passed on to one half of the male offspring; such males would enjoy an advantage over those with an unmarked *X*, if bright coloration in males is at a premium. A system of absolute *Y*-linked pigment factors (holandric genes) would suffer from the disadvantage that crossing over would not be possible and that, consequently, the number of pattern combinations in a population would be fixed and limited. The evolution of a *W* chromosome might have been another means of insuring greater pigmentary polymorphism in males. A W_+ chromosome could spread readily in a population, because it would be inherited by none of the male offspring but by all female descendants of *WY* x *XY*, *WX* x *YY*, and *WY* x *YY* matings, and by two thirds of the female progeny of *WX* x *XY* crosses. W_+X and W_+Y females will be pigmented to a lesser degree than *XX* females and *XY* or *YY* males in populations in which pigment factors are restricted to *X* and *Y* chromosomes. On the other hand, marked *W* chromosomes that arose through crossing over would be exposed to negative selection in every generation and eventually lost. Perhaps this is one of the reasons why at present the W_+ chromosome is widespread and extremely common in some populations.

Summary

The teleost *Xiphophorus maculatus* (Poeciliidae) is polymorphic for sex chromosomes and sex-linked pigment patterns. Females of natural populations may be of the genotype *WY*, *WX*, or *XX* and males *XY* or *YY*. Fish of two populations from the Belize River and one from the Sibun River in British Honduras were tested for their sex chromosome constitution, because earlier but limited data had indicated that the *X* chromosome is absent from rivers in British Honduras (Gordon 1954).

Of six males examined from the Belize River, five possessed the *YY* and one the *XY* genotype. Of 20 females tested, 19 were *WY* and one *XX*. Breeding data showed that one of the *WY* females was fertilized by an unknown *XY* male before she was collected. No *X* chromosome was identified in the two males and ten females from the Sibun River.

Of the 29 females with a *W* chromosome all but two exhibited one or more sex-linked pigment patterns controlled by at least three loci: one concerned with iris coloration, a second with red or yellow body or fin pigmentation, and a third with macromelanophore patterns. The 27 females possessed a total of 41 pigment factors. Of these none was *W*-linked.

An analysis of the frequency of macromelanophore patterns in seven preserved collections from the Belize River and Sibun River showed that consistently the frequency of patterned males was two to three times that of females. This difference is in good agreement with the hypothesis that in natural populations the *W* chromosome does not carry pigment factors. This is not true for the *X* chromosome.

Since crossing over between the *W* and *Y* chromosome has been observed in the laboratory, it must also occur in natural populations. In the absence of selection crossing over should tend to equalize the frequency of marked *W* and *Y* chromosomes. A selective advantage is postulated for high coloration in males and a disadvantage in females. The significance of the *W* chromosome as a vehicle for strict maternal transmission of characters is discussed.

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EXPLANATION OF PLATES

PLATE I

FIG. 1. Female of ped. 1927 with Sp^8 pattern of Belize, 6 months. Macromelanophore spotting on flank extends as far anteriorly as the eye.

FIG. 2. Male of pedigree 2167 (descendant of ped. 1927), 9 months. Macromelanophore spotting on flank produced by Sp^8 extends over much of body. The largest macromelanophore spot is typically found above the insertion of the gonopodium. Black spotting in dorsal fin is caused by the *Sd* factor of Sibun river.

FIG. 3. Female 1899-10, 11 months after capture, with Sp^7 pattern. In contrast to Sp^8, spotting on flank is restricted to area below dorsal fin and caudal peduncle.

PLATE II

FIG. 1. Male of ped. 1968, 5 months. Sp^7 spotting is restricted to posterior part of body. In contrast to Sp^8 pattern only a single spot is found in front of level of dorsal fin and no large black spot develops above gonopodium.

FIG. 2. Female of ped. 1936, a Belize x Jamapa hybrid, with Sp^1 pattern from strain Jp 163 B, 7 months. Sp^1 factor in F_1 hybrids usually causes less than ten pigment spots. Expression becomes further reduced in backcross hybrids to Belize. Large black spot in front of dorsal fin is "shoulder spot" and markings at base of caudal fin are complete-crescent, *Cc*, and dot, *D*, tail patterns.

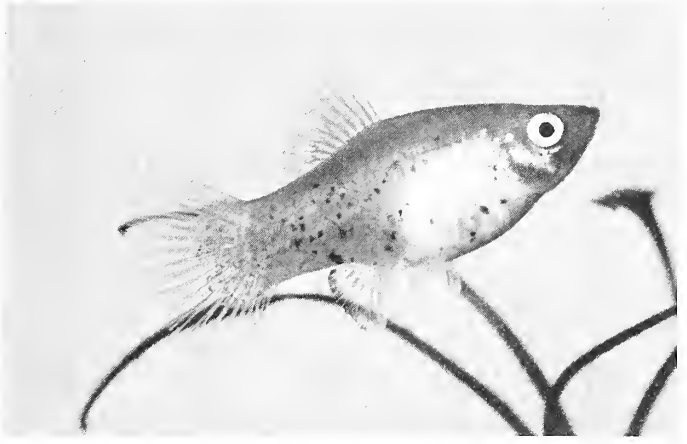

FIG. 1

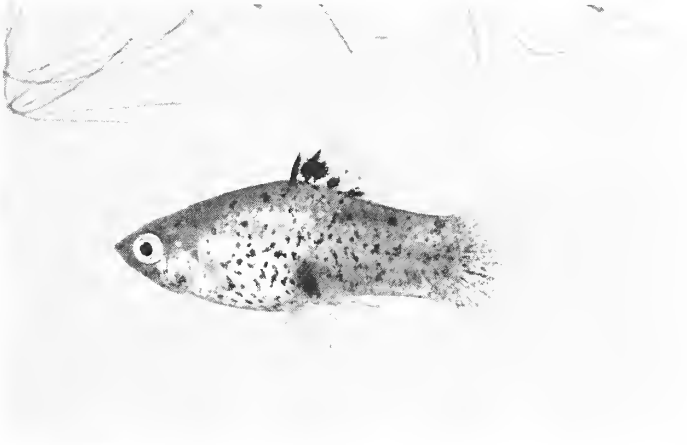

FIG. 2

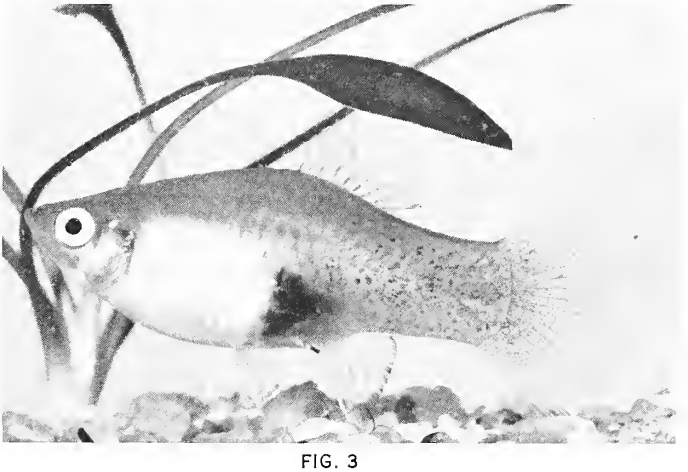

FIG. 3

SEX DETERMINATION AND THE RESTRICTION OF SEX-LINKED PIGMENT PATTERNS TO THE X AND Y CHROMOSOMES IN POPULATIONS OF A POECILIID FISH.

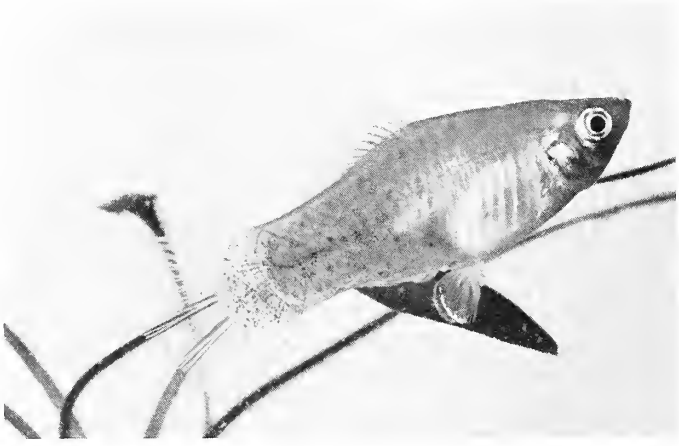

FIG. 1

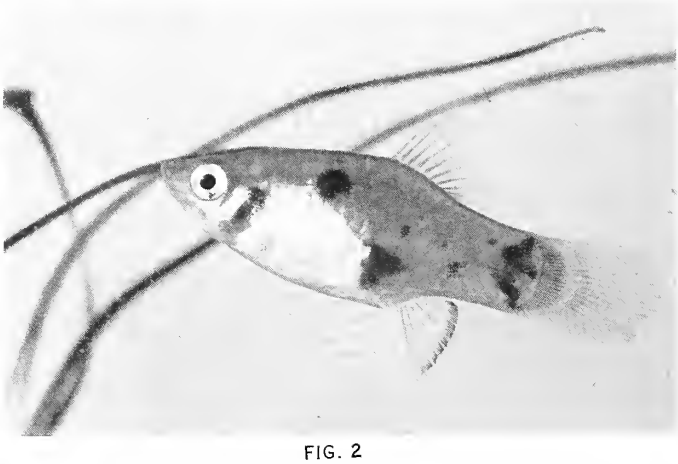

FIG. 2

SEX DETERMINATION AND THE RESTRICTION OF SEX-LINKED PIGMENT PATTERNS TO THE X AND Y CHROMOSOMES IN POPULATIONS OF A POECILIID FISH.

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Metabolism, Energetics, and Thermoregulation During Brooding of Snakes of the Genus *Python* (Reptilia, Boidae)

ALLEN VINEGAR[1], VICTOR H. HUTCHISON[2], AND HERNDON G. DOWLING[3]

(Plates I-II; Text-figures 1-24)

A study was made of various aspects of the metabolism and energetics of pythons in which particular attention was given to brooding females.

Gas exchange was measured at different temperatures with an open respirometric system equipped with an infrared carbon dioxide analyzer and a paramagnetic oxygen analyzer. Temperatures were measured with copper-constantan thermocouples connected to a potentiometric recorder. Gas exchange and temperature were measured and recorded continuously during periods of experimentation. Length-weight data of pythons were recorded in the New York Zoological Park and from information sent by other individuals and institutions. An energy budget was constructed from caloric determinations of ingested and egested material from several pythons.

Determinations of standard metabolism of pythons showed that their metabolic rates increase directly with temperature. At given temperatures metabolic rate per unit weight varies inversely with weight.

Heat production of *Python curtus, P. m. molurus, P. m. bivittatus,* and *P. reticulatus* at 26.0°C to 28.4°C is proportional to the two-thirds power of weight.

Heart beats of *Python molurus* increased from only 3/minute to 5/minute from 17°C to 26.5°C, but increased to 16/minute at 33°C. The pattern of increase paralleled that of oxygen consumption so that the oxygen pulse varied only slightly over the whole temperature range (range of O_2 pulse = $2.19 \times 10^{-2} - 2.96 \times 10^{-2}$ $cc O_2$ $beat^{-1}$ kg^{-1}).

Metabolic response to a 15°C temperature change was determined for *Python molurus* and *P. reticulatus.* Both species showed an initial metabolic rise with increased temperature from about 5 $cc O_2$ kg^{-1} hr^{-1} to 40 $cc O_2$ kg^{-1} hr^{-1} within 2 days but *P. molurus* dropped to 20 $cc O_2$ kg^{-1} hr^{-1} within 5 days, while *P. reticulatus* remained at the high level. When temperature was dropped to the low level, *P. molurus* showed a slight metabolic undershoot before returning to the original metabolic level. *Python reticulatus* did not show this undershoot.

A female Indian python *(Python m. bivittatus)* laid eggs in 1965 and 1966. During both brooding periods she produced sufficient additional heat by muscular contraction so that her body temperature was maintained above ambient. Temperature differentials of up to 5°C were recorded. The muscular contractions began when the ambient was below 33°C, and increased in frequency with decreasing temperature. Correlated with the decreasing temperature and increasing frequency of contractions was an increasing metabolic rate. At temperatures of about 25°C, the metabolic rate was about ten times the standard level. As the frequency of contractions increased, the python changed the

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shape of her coil around the eggs decreasing the surface area-to-volume ratio, thus aiding the maintenance of the temperature differential by decreasing the rate of heat loss.

A female *Python m. molurus* showed false brooding behavior over a period of several months and finally developed respiratory difficulty and was euthanized. Metabolic measurements made during parts of the false brooding corresponded with results from the above brooding python. Evidence from the true and false brooding points to a hormonal involvement in preparation for the brooding metabolic response to temperature.

Evidence is given for the occurrence of physiological thermoregulation during brooding in *Python curtus* and *Chondropython viridis* and its lack in *P. reticulatus* and *P. sebae.*

Energy budgets for three hatchling *Python curtus* over a two year period indicated that in normally growing animals about 10 percent of the food energy consumed was wasted as intestinal or renal waste; about 65 percent was used for maintenance and movement and about 25 percent went into growth.

Available data are presented to support the hypothesis that although *Python molurus* and *P. reticulatus* are sympatric over much of southeast Asia, the limiting factor for northern distribution is the ability of *P. molurus* to thermoregulate by physiological means during brooding and the lack of this ability in *P. reticulatus.*

Introduction

Physiological thermoregulation during brooding in pythons has been suspected since 1832 when Lamarre-Picquot (1835) read a paper to the French Academy which stated that the Indian rock python *(Python molurus)* produces internal heat to aid egg incubation. A committee of the French Academy rejected Lamarre-Picquot's statements as being "hazardous and dangerous." Valenciennes (1841) and Lamarre-Picquot (1842) reported on new observations of the above phenomenon but Dumeril (1842) attributed the heat to decaying eggs. Additional reports of brooding in large pythons were made by Sclater (1862), Wray (1862), Forbes (1881), and Lederer (1944). Reports on brooding of smaller pythons have been made by Noble (1935) and Kratzer (1962). Krogh (1916) was prepared to make metabolic measurements of a brooding python in the Copenhagen Zoo but was denied permission by zoo officials. Benedict (1932) made temperature measurements of a brooding python over the period of one working day. More recent observations of heat production during brooding in pythons were made by Stemmler-Morath (1956).

In evaluating the above literature, the cases of brooding that also reported internal heat production have been found to be questionable on some grounds, e.g., inadequate temperature measuring devices, poorly-controlled experiments or insufficient data to justify the conclusions. Therefore, when Dowling made observations and temperature measurements with adequate temperature measuring devices under controlled conditions on two species of pythons *(Python molurus bivittatus* and *P. sebae),* he was justified in claiming the existence of internal heat production in *P. m. bivittatus* (Brattstrom, 1965; Dowling, 1960; Pope, 1965). These observations led us to the initiation of a more complete study of brooding and metabolism in pythons. This paper represents a part of the results of this study (Hutchison, Dowling, and Vinegar, 1966).

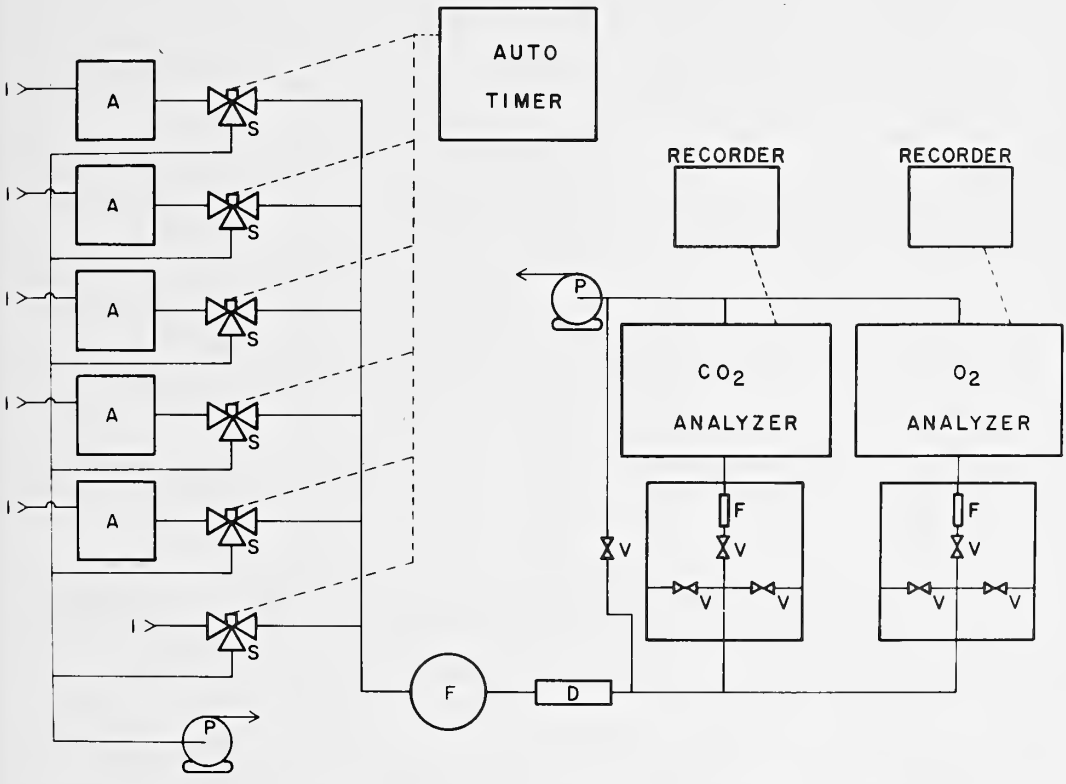
Materials and Methods

Gas exchange was measured by an open system of respirometry. Animals were placed in individual plastic chambers through which room air was drawn. The air drawn from the chamber was analyzed for oxygen with a Beckman Model F3A3 analyzer and for carbon dioxide with a Beckman Model 15A infrared analyzer. Room air was analyzed periodically as a check against the chamber air. Flow rate was monitored with a wet-test flow meter. Air was drawn through the whole system with a pump. The outgoing chamber air passed through a three-way solenoid valve and then to a manifold before going through the analyzers. The solenoid valves were activated in sequence by an automatic sequencing timer; thus several chambers could be sampled automatically in sequence. A diagram of the system is shown in text-fig. 1.

Calculations of oxygen consumption were made using the formula derived by Depocas and Hart (1957) for open circuit systems where CO_2 and O_2 are monitored:

$$\dot{V}_{O_2} = (\dot{V}_I P_{I_{O_2}} - (\dot{V}_I + \dot{V}_{CO_2})\ P_{E_{O_2}})/P_B - P_{E_{O_2}}$$

where $\dot{V}_{O_2}$ is the oxygen consumption of the animal per hour, $\dot{V}_{CO_2}$ is the CO_2 production per hour, $\dot{V}_I$ is the volume of air flowing into the chamber per hour, $P_{I_{O_2}}$ is the partial pressure of oxygen in the air flowing into the chamber, $P_{E_{O_2}}$ is the partial pressure of oxygen in the air flowing out of the chamber, and P_B is the barometric pressure.



TEXT-FIG. 1. Diagram of apparatus for measuring gas exchange. The symbols are as follows: A, animal chamber; D, drying tube; F, flow meter; P, air pump; S, three-way solenoid valve; V, flow control valve.

The room in which the experiments were performed was of double wall construction, with insulating material between the two walls. Temperature was controlled within $\pm 1°C$ with a system, composed of two air conditioners and a heater, which was thermostatically controlled and operated in opposition for fine control of temperature. The larger of the two air conditioners produced the initial drop in temperature for large temperature changes; the smaller unit then was adequate for maintaining the temperature.

Temperatures were recorded from copper-constantan thermocouples connected to a Leeds and Northrup Speedomax G 24-channel potentiometric recorder. Thus, 24 different temperatures could be monitored simultaneously with print-out for all channels appearing every 72 seconds.

Heart rates were recorded by taping small (50 mm^2) pieces of fine mesh bronze screening with wires attached, on the venter anteriorly and posteriorly to the heart of the animal. Electrode jelly was used for making good electrical contact. Each of the two wires attached to the pieces of screening were connected to a miniature phone plug. The phone plug was then inserted into a jack at the end of a long, shielded cable which was attached to a Heathkit Impscope. Heart rates were determined by observing the beats on the Impscope and timing their frequency with a stop watch. Rates were not measured until at least one hour after the electrodes had been placed on the animal.

Information on weights and lengths of pythons were obtained from several sources. Animals at the New York Zoological Park (NYZP) were measured and weighed at varying intervals. Additional information was obtained by writing to other zoos and individuals who kept pythons. Weights also were taken of all pythons at the NYZP prior to all metabolic measurements.

Surface areas of pythons that died at NYZP were determined by carefully skinning the animal without pulling on the skin. The skin was placed on brown paper and traced. Areas of

small skins were determined with a K & E Compensating Polar Planimeter. Large skin areas were determined by weighing samples of the brown paper of known area and extrapolating the area of the skin from the total weight of the tracing. Measurements of the circumference of the animal were made at various points along its length before skinning to determine the degree of stretching; when the skin was placed on the tracing paper these measurements of circumference were maintained as closely as possible. Because the animal is truncated at both ends, it would be expected that the length of the skin would be greater than the actual length of the animal. However, since the length of the skin rarely exceeded the original length by more than a foot – even in the case of large animals – it is assumed that stretching was minimal.

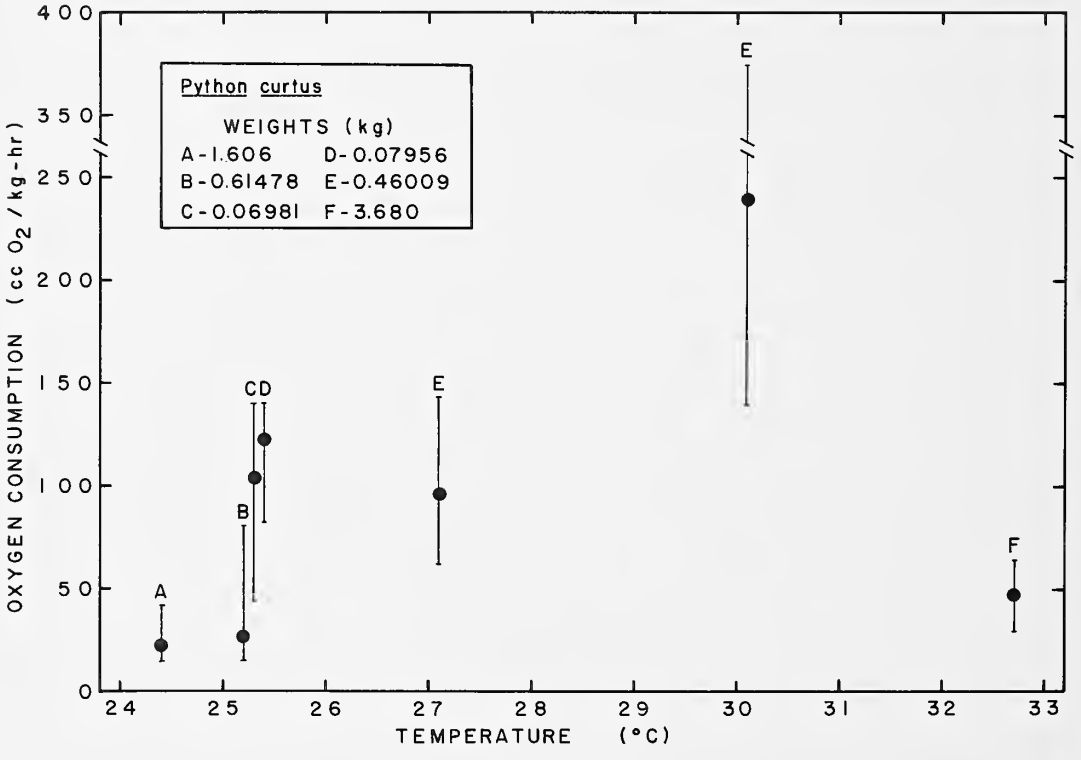
Results and Discussion

Standard Metabolism of Pythons

Oxygen consumption of *Python curtus, P. molurus,* and *P. reticulatus* was measured at several ambient temperatures. Text-figs. 2 to 4 show the results of these measurements for animals of various weights. The data indicate increasing metabolic rate with increasing temperature and higher metabolic rates per unit weight for smaller animals.

Heat Production-Weight Correlation in Snakes

Galvão et al. (1965) dealt with heat production in relation to body weight and surface area in a number of tropical snakes. After converting oxygen consumption to heat production by using 4.8 calories as the equivalent of one ml of consumed oxygen, they plotted hourly heat production as a function of weight. Their experiments were performed at about 21.5°C but full acclimation information is not provided. Data for 16 boids of three species *(Boa constrictor amarali, Eunectes murinus,* and *E. notaeus)* gave the regression equation, calories/hour = 0.04 weight$^{1.09}$. They compared these data with data from Benedict (1932) for 12 boids of four species *(Boa constrictor, Epicrates angulifer, Python molurus bivittatus,* and *P. recticulatus)* measured at 19.5°C to 23.3°C. These data



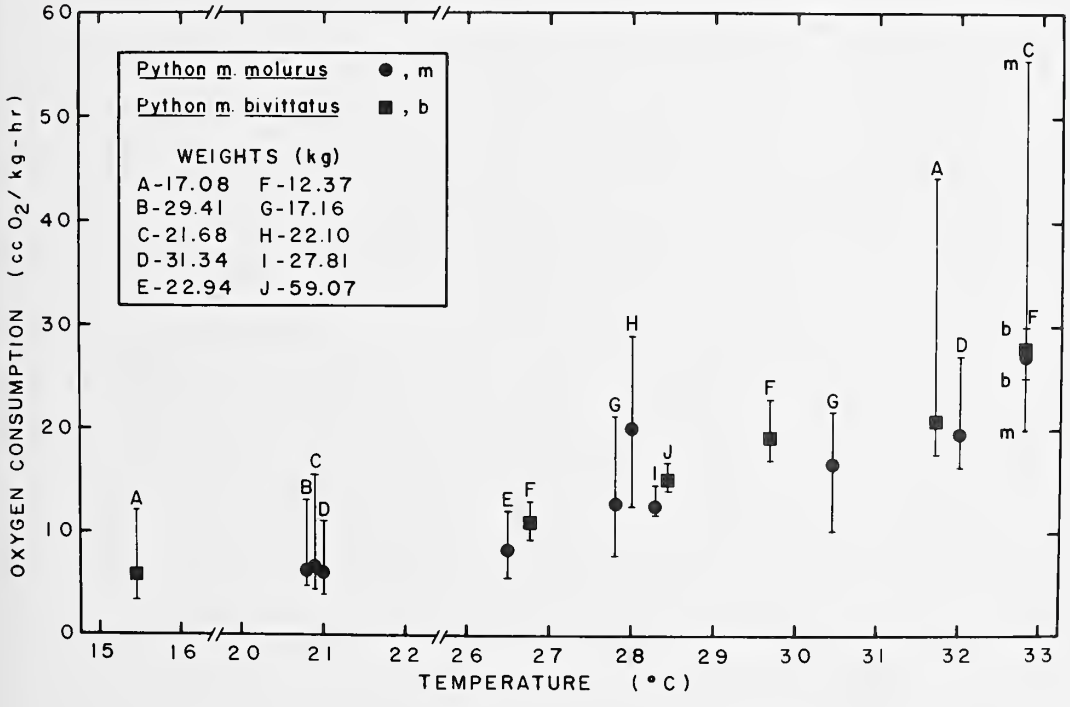
Text-fig. 2. Oxygen consumption of *Python curtus* at various ambient temperatures. Circles, means; vertical lines, ranges.

produced the equation, $C = 0.02W^{1.12}$. The two equations are not significantly different. The regression coefficient is not significantly different from 1.0, indicating that the metabolism is directly proportional to weight and not to the 0.67 power of the weight.

We did not have sufficient data for animals of different weights at 21°C for direct comparison with the above data. Therefore, a regression of metabolism on weight was calculated for data from ten boids of three species *(Python curtus, P. m. molurus, P. m. bivittatus,* and *P. reticulatus)* at 26.0°C to 28.4°C. These calculations resulted in the equation, $C = 1.975W^{0.664}$ where metabolism is, indeed, proportional to weight to the 0.67 power. For a comparison with Benedict's data, values for ten boids at 27.1°C to 29.1°C were taken from his work. These include eight of the animals and all four species used by Galvão et al. (1965) to calculate the regression at 19.5°C to 23.3°C The equation obtained was, $C = 0.395W^{0.852}$. The exponent is not significantly different from 0.664 figure obtained in this study or from the values of 1.09 and 1.12 calculated by Galvão for his data at 21.5°C or for Benedict's data at 19.5°C to 23.3°C. All four regression equations discussed above and the individual values from this study are shown in text-fig. 5.

The relation between metabolism and weight depends on the temperature at which the metabolism is measured. It is also likely that a better comparison could be made, if the data were calculated for single species, rather than lumping together data from several species of two subfamilies (Pythoninae and Boinae).

Baldwin (1930) gave oxygen consumption data at 20°C for 13 specimens of *Pituophis sayi* weighing 272 grams to 835 grams. No data were given on the acclimation conditions. By converting the oxygen data to calories per hour and calculating a regression of heat production on weight, we obtained the equation, $C = 8.961W^{0.458}$. The data are highly variable, the 95 percent confidence limits on b being ± 0.797. A "t" test on b ($S_b = 0.362$) was not significant



TEXT-FIG. 3. Oxygen consumption of *Python molurus* at various ambient temperatures. Circles, means; vertical lines, ranges.

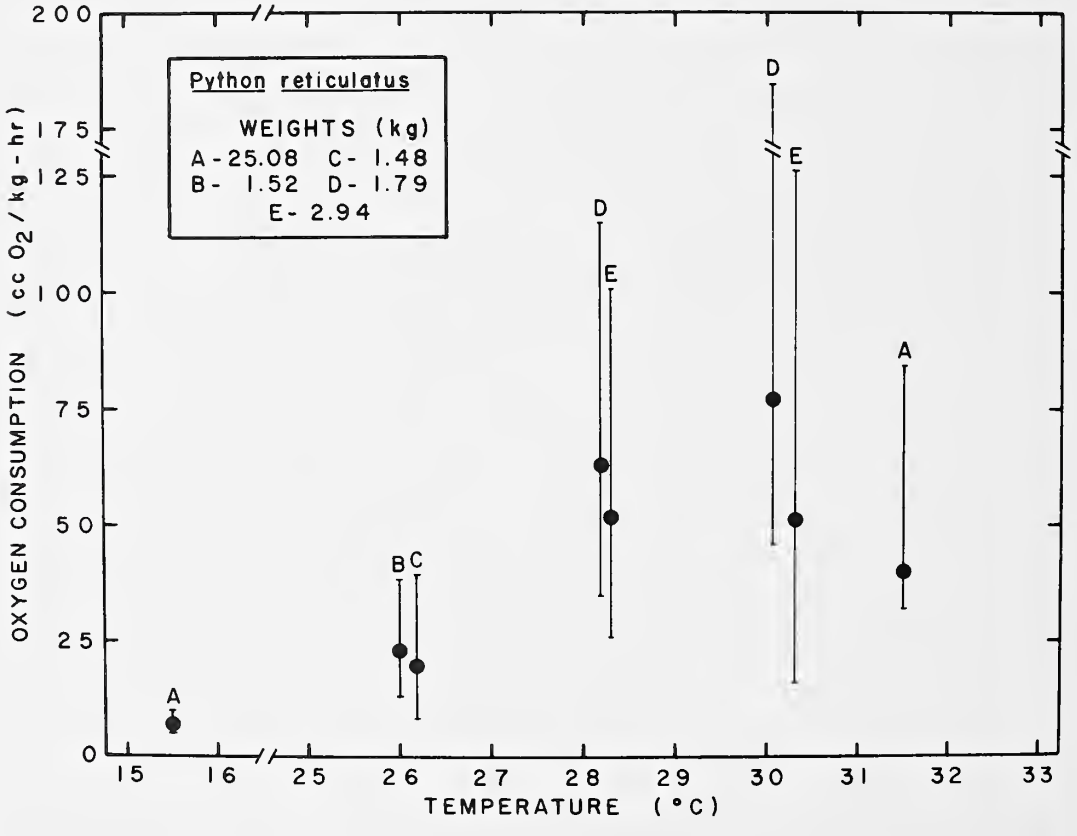
($P < 0.20$). Unfortunately, Baldwin provided no information that gives any clue to the source of the variability of the data.

Heart Rates and Oxygen Pulse

Difficulty was encountered in obtaining heart rates from pythons. The time interval necessary for the animals to settle down after having the EKG leads placed on them also provided sufficient time for them to work the leads off. In spite of this problem heart rates were obtained for *Python molurus* acclimated to several temperatures. Rates varied from about three per minute at 17°C to about five per minute at 26.5°C. At 33°C the rate had increased to about 16 per minute (text-fig. 6). The response of heart rate to temperature apparently parallels the increase of oxygen uptake with temperature. Oxygen uptake increases from about 5 cc kg^{-1} hr^{-1} at 15.5°C to 12 cc kg^{-1} hr^{-1} at 27°C. The rate of increase then rises until the uptake is about 30 cc kg^{-1} hr^{-1} at 33°C (text-fig. 3). This correlation is shown more readily when the data are calculated as oxygen pulse (Table 1); with increasing temperature the heart rate increases proportionately with oxygen consumption and oxygen pulse remained fairly constant.

Jayasinghe and Fernando (1964) found a rate of 40 beats per minute for *Python molurus*. They failed to report either the acclimation or the measurement temperatures. The size of the animals was given as between 10 feet and 20 feet (three meters to six meters). Animals of these lengths could weigh between 10^4 grams and 10^5 grams. Therefore, without specific temperature and weight information, no direct comparison can be made with these data.

Heart rates of a 4313 gram, male boa constrictor, *Constrictor* [= *Boa*] *c. constrictor* were given by Clarke and Marx (1960). The snake was kept at 23°C to 27°C for some time before the measurements were made. Heart rates were taken over a four-hour period during which the temperature was dropped from 23°C to 18°C and the heart rate dropped from 15 beats to 12 beats per minute.



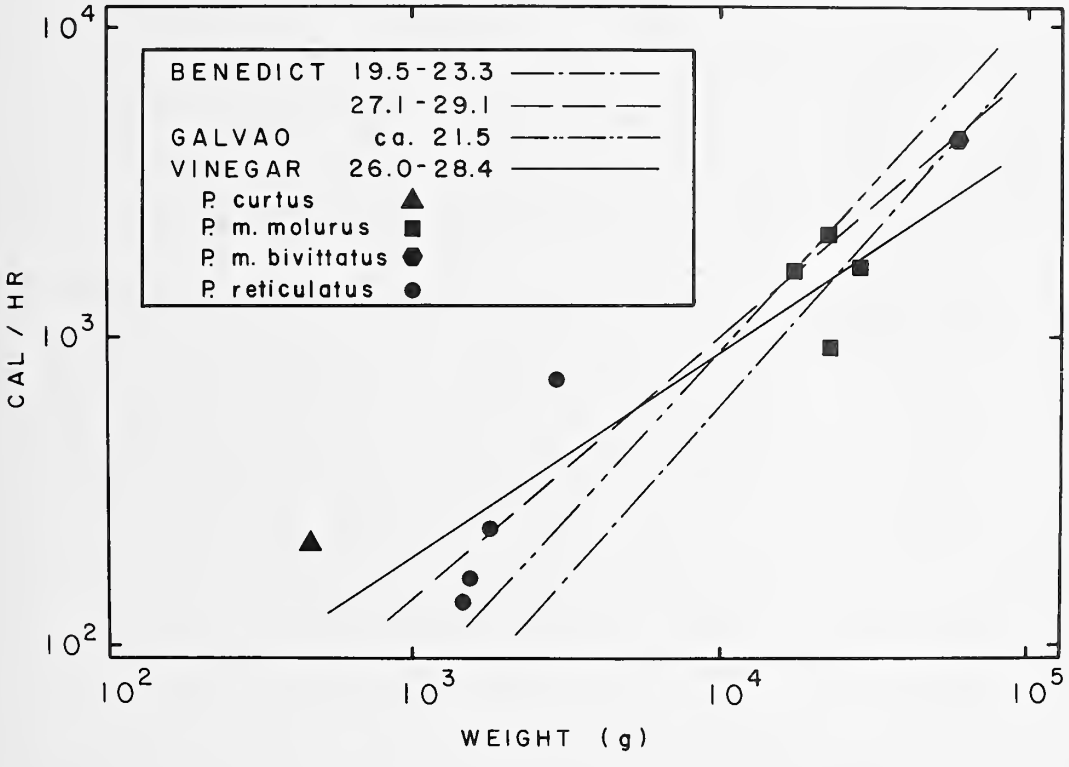
TEXT-FIG. 4. Oxygen consumption of *Python reticulatus* at various ambient temperatures. Circles, means: vertical lines, ranges.

TABLE 1.
HEART RATE AND OXYGEN PULSE DATA FOR *Python molurus*.

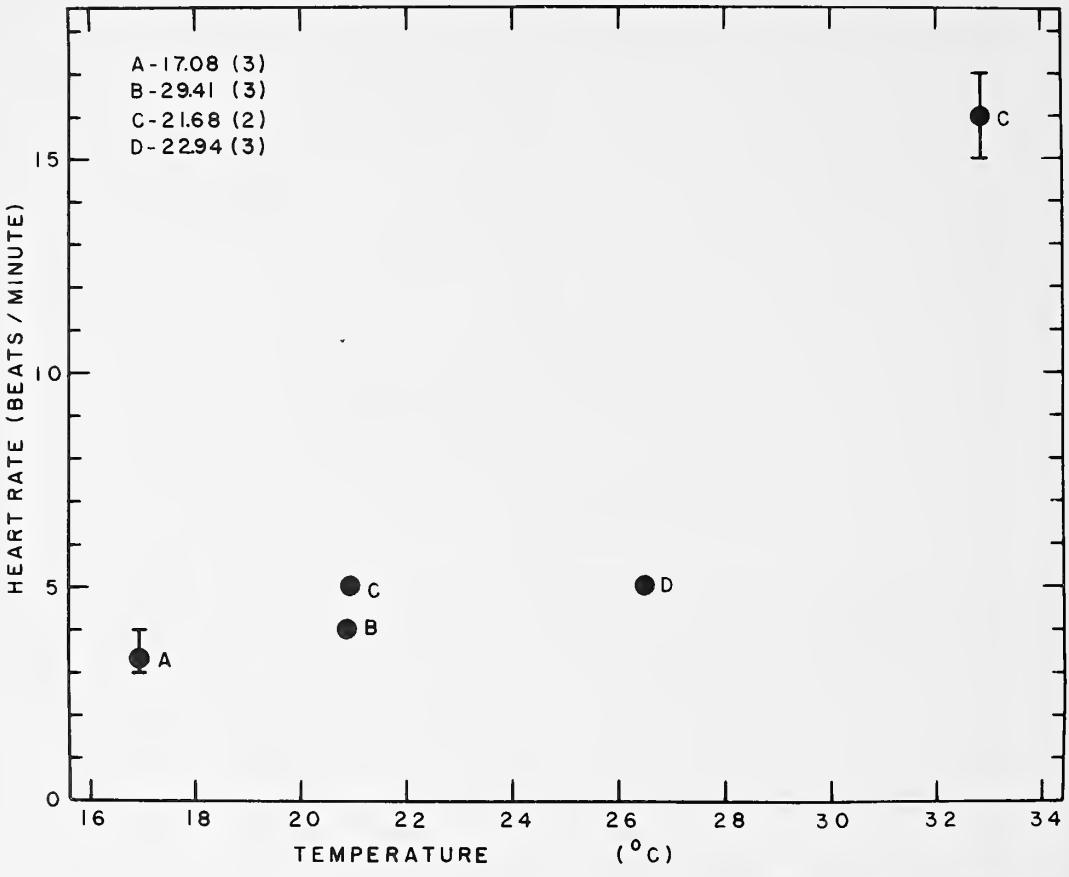
Temp. (°C)	O_2 *Cons.* (cc kg^{-1} hr^{-1})	*Heart Rate* (beats/hour)	O_2 *Pulse* (ccO_2 $beat^{-1}$ kg^{-1})	*Snake Wt.* (kg)
16.9	5.86	198	2.96×10^{-2}	17.08
20.8	6.40	240	2.67×10^{-2}	29.41
20.9	6.58	300	2.19×10^{-2}	21.68
26.5	8.37	300	2.79×10^{-2}	22.94
32.8	27.07	960	2.82×10^{-2}	21.68

Rebach (1969) measured heart rates of boa constrictors weighing from 0.393 kilograms to 8.51 kilograms at temperatures of 20.0°C and 32.2°C. Rates at 20.0°C were 6.4 (5.0-8.0) beats per minute and at 32.2°C were 23.9 (13.0-30.0) beats per minute. When the smaller size of these snakes is considered, the measured heart rates are in good agreement with those of the pythons measured in the present study.

Mullen (1967) measured heart rates of several temperate zone lizards and snakes, all of which are of small size compared to pythons. Mean heart rates for the snakes varied from about 43 beats per minute at 22°C to about 95 beats per minute at 30°C. These rates are considerably higher than the rates found for the Indian python but the greater mass of the python probably accounts for its lower heart rates. Bartholomew and Tucker (1964) demonstrated an inverse correlation between heart rate and weight in varanid lizards; this correlation also occurs in other animals.



TEXT-FIG. 5. Correlation of heat production with weight of boids based on the data of several investigators. Regression equations obtained by the method of least squares are as follows: Benedict (19.5-23.3), $C = 0.02W^{1.12}$; Benedict (27.1-29.1), $C = 0.395W^{0.852}$; Galvao (21.5), $C = 0.04W^{1.09}$; Vinegar (26.0-28.4), $C = 1.975W^{0.664}$. Individual points are plotted for data from this study only.



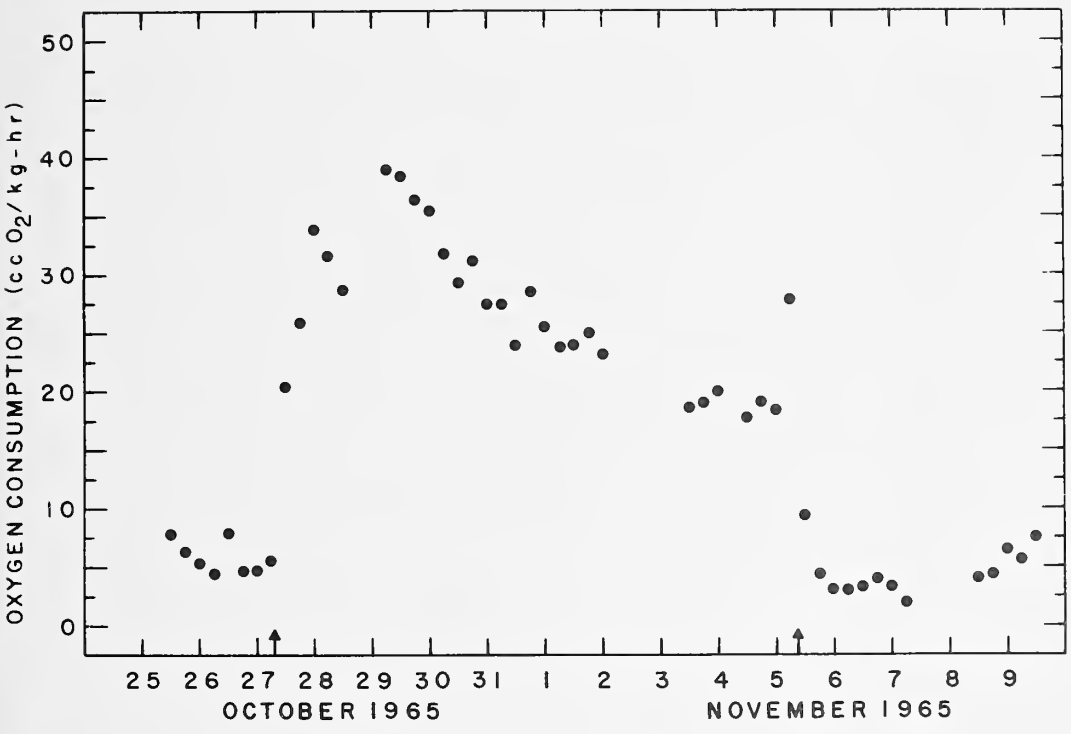
TEXT-FIG. 6. Heart rates of *Python molurus* at several ambient temperatures. Weights and sample size for each point are shown in the upper left of the figure. Circles, means; vertical lines, ranges.

Metabolic Responses to Temperature Change

Specimens of *Python molurus bivittatus* (NYZP No. 630514) and *P. reticulatus* (NYZP No. 640670), 17.08 kilograms and 25.08 kilograms, respectively, were subjected to rapid changes in temperature of about 15°C. The animals were initially acclimated to the lower temperature for a two-week period. Temperature was then increased, kept at the high setting for nine days and finally returned to the low setting. Gas exchange was measured over the whole period (text-figs. 7 and 8). The metabolic response to temperature rise was slightly different in each of the animals. *Python molurus* showed an initial increase from about 5 cc kg^{-1} hr^{-1} to 40 cc kg^{-1} hr^{-1} within two days and then a gradual drop to 20 cc kg^{-1} hr^{-1} within five days. *Python reticulatus* showed the same initial increase but remained at a level of about 35-40 cc kg^{-1} hr^{-1} for the nine day period. Decreased temperature seemed to produce a slight metabolic undershoot in *P. molurus,* while *P. reticulatus* returned immediately to its initial level. Further measurements are necessary before any conclusions can be reached regarding the consistency of these responses.

Brooding Metabolism of *Python molurus bivittatus*

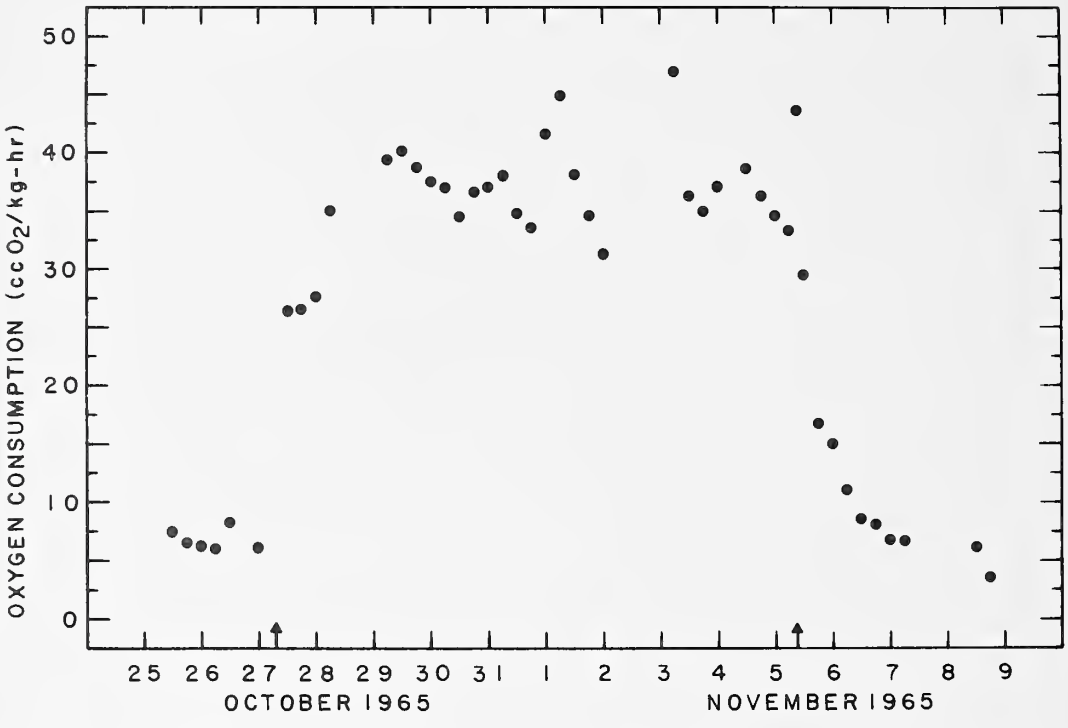
An Indian python *(Python molurus bivittatus,* NYZP No. 630514), 14.25 kilograms in weight and 2.7 meters in length, laid 23 infertile eggs on or about February 15, 1965. The animal was found coiled around the eggs on February 18 and was transferred, without disturbing her or the eggs, to a respiration chamber in a temperature controlled (± 1°C) room. Measurements of gas exchange, temperatures, and contraction rates were made for the 30-day period that the python remained on the eggs. Additional gas exchange measurements were taken 40 days after the end of the brooding period to obtain non-brooding values. Oxygen consumption calculations were based on the weight of the snake at 14.25 kilograms during brooding and at 12.37 kilograms during non-brooding.



TEXT-FIG. 7. Metabolic response to temperature change in a 17.08 kilogram *Python molurus bivittatus*. Temperature changed from 15.4° to 31.7°C on October 27 and back to 15.4°C on November 5 (arrows).

Oxygen consumption during non-brooding was typical of an ectothermic animal, decreasing with decreasing temperature (text-fig. 9, lower curve); but oxygen consumption during brooding was similar to that of endothermic animals (text-fig. 9, upper curve). At about 33°C, the metabolic rate of the python is approximately the same during brooding and non-brooding. However, the oxygen consumption of the brooding animal increased when the temperature was decreased from 33°C to 25.5°C. Thus, 33°C appears to be analogous to the "lower critical temperature" of birds and mammals. The analogy is enhanced further by the onset of muscular contractions which accompany the increased metabolism at temperatures below 33°C. The frequency of contractions increases with decreasing temperature and increasing metabolism (text-fig. 10). A similar correlation exists between the temperature differential between animal and air and the contraction rate (text-fig. 11). A maximum temperature differential of 4.7°C was maintained at an ambient temperature of 24.8°C (text-fig. 12). A full account of the data for this brooding was given by Hutchison, Dowling, and Vinegar (1966).

On February 15, 1966, a specimen of *Python molurus bivittatus* (NYZP No. 630514) was found coiled in a corner of an exhibit cage. One egg, which was opened and found to be infertile was noted beside her. No other eggs were laid at the time. The animal was taken to the laboratory and placed in a respiration chamber. She weighed 17.25 kilograms (including the ejected egg). Twenty-one additional eggs were laid during the night of February 16-17, 1966. Respiration, temperature, and contraction rate data were collected as during the brooding period of the previous year. Several eggs were removed during the course of brooding as they started to turn yellow. On March 28, 1966, the remaining eggs, which were all infertile, were removed from the female as all signs of regular contractions had stopped. Irregular contractions were noted until April 8, 1966.

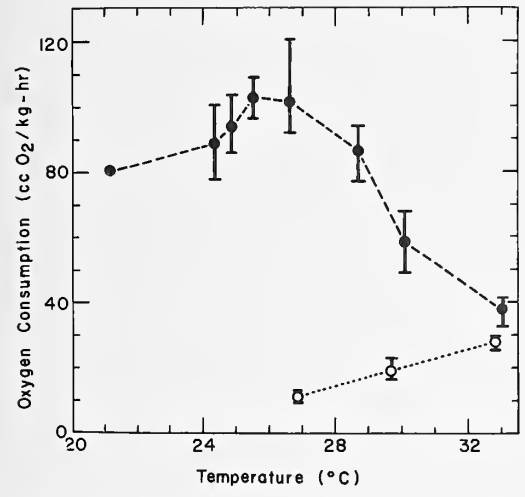


TEXT-FIG. 8. Metabolic response to temperature change in a 25.08 kilogram *Python reticulatus*. Temperature changed from 15.5° to 31.5°C on October 27 and back to 15.5°C on November 5 (arrows).

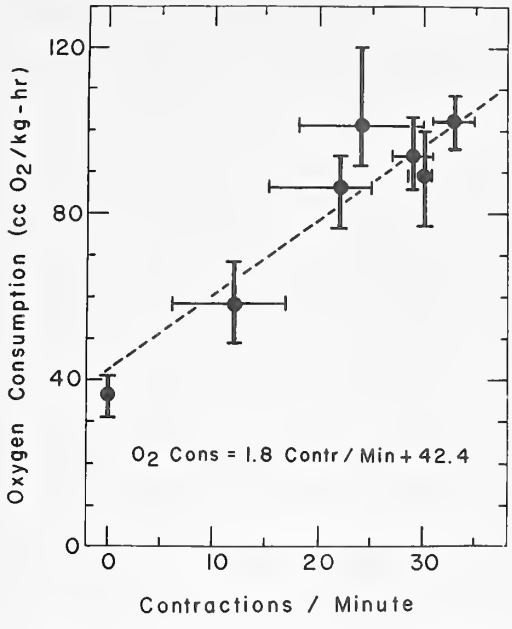
Although tidal volume could not be determined quantitatively, qualitative observations were made. The number of inspirations per minute increased with increasing contraction rate. Contraction and breathing rates were: 2, 2; 9, 4; 33, 6; 37, 6. The normal inspiration rate in a large python is about two per minute. Although the frequency of inspirations did not increase greatly with increasing contractions, the tidal volume did increase. The breaths of six per minute were quite noticeably deeper than those at two to four per minute. Measurements were also made of the height and basal width of the snake's coil at different contraction rates. Calculations of volume and area were made assuming the coil to be a solid cone. A brooding *P. m. bivittatus* is pictured in Plate I. Table 2 shows the calculated data; the surface area calculated includes that part in contact with the substrate. Surface area to volume ratios decreased with increasing contraction rates. The metabolic data were similar to those obtained in the previous year. Text-fig. 13 shows the oxygen consumption-contraction rate data for the two years. Data for contraction rates at various temperature differentials are plotted in text-fig. 14, and body temperature-ambient temperature data are shown in text-fig. 15. During most of the 1966 brooding, a 17.1 kilogram specimen of *P. m. molurus* (NYZP No. 640578), was kept in a second respiration chamber. Its metabolic responses to temperature were also determined. Data for the two animals are shown for an 11-day period at various temperatures in text-fig. 16.

False Brooding Behavior in a Female *Python molurus molurus*

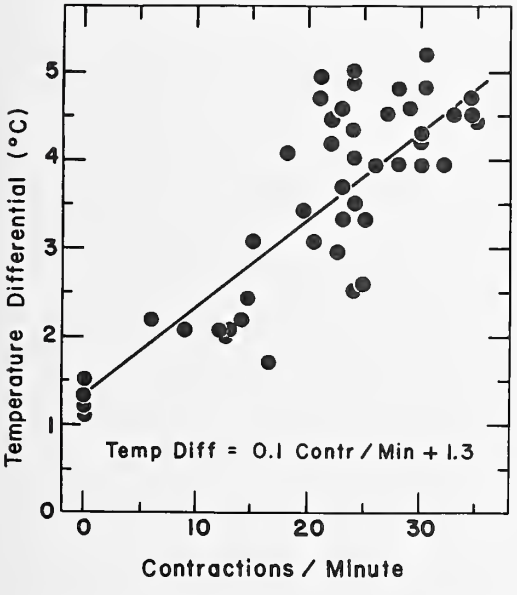
On May 31, 1966, a specimen of *Python molurus molurus* (NYZP No. 640578) was seen contracting at an uneven rate in a manner similar to a brooding python. Since contractions of this type had been seen previous to the last two egg layings of *P. m. bivittatus* (NYZP No. 630514), it was assumed that No. 640578 would probably lay eggs within the following few days. The ambient temperature of the cage and surroundings of the python was about 27°C. It was transferred to the laboratory (at 31°C) and placed in one of the metabolism chambers so that she



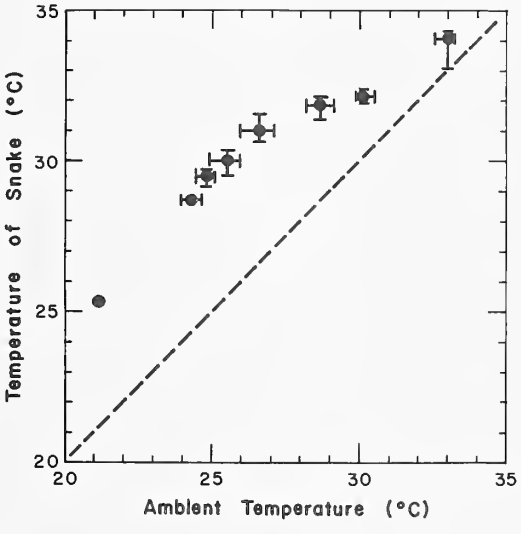
TEXT-FIG. 9. Oxygen consumption of a *Python molurus bivittatus* at different ambient temperatures. Upper curve: animal during brooding. Lower curve: same animal during non-brooding. Circles, means; vertical lines, range. (from Hutchison, V. H., H. G. Dowling and A. Vinegar, 1966).



TEXT-FIG. 10. Correlation of rate of body contractions with oxygen consumption in a brooding *Python molurus bivittatus.* Dashed line and regression equation calculated by method of least squares. Circles, means; vertical lines, range of oxygen consumption; horizontal lines, range of contraction rate (from Hutchison, V. H., H. G. Dowling and A. Vinegar, 1966).



TEXT-FIG. 11. Correlation of contraction rate with temperature differential in a brooding *Python molurus bivittatus.* Line and regression equation calculated by method of least squares. Circles represent individual measurements (from Hutchison, V. H., H. G. Dowling and A. Vinegar, 1966).



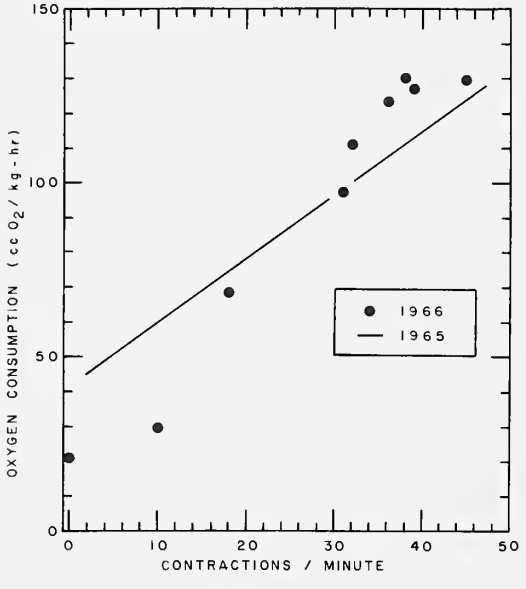
TEXT-FIG. 12. Correlation of body temperature of a brooding *Python molurus bivittatus* with ambient temperature. Dashed line indicates equal ambient and animal temperatures. Circles, means; vertical lines, range of animal temperature; horizontal lines, range of ambient temperature (from Hutchison, V. H., H. G. Dowling and A. Vinegar, 1966).

Table 2.
Coil Size Data for a Brooding Indian Python.

T_B-T_A (°C)	Contraction Rate Per Minute	Coil Ht. (cm)	Coil Width (cm)	Coil Vol. (cm^3)	Coil Area (cm^2)	Area/Vol.
1.6	2-3	11	50	7198.13	4106.34	0.57
3.4	30	16	48	9649.15	3982.02	0.41
4.8	42-45	20	46	11077.26	3862.33	0.35

could lay her eggs there. Gas exchange measurements were made from June 13 to June 16 and July 2 to July 12, 1966. Ambient temperature was changed several times during these periods. No eggs were laid and the animal was removed from the laboratory on July 12, when muscular contractions were no longer observed. Occasional irregular contractions were noted until September 2. Offerings of food were consistently ignored. On October 6 the python was force fed one rat. A mucous discharge was seen coming from the mouth on October 25. The animal seemed to be having trouble breathing and was euthanized with nembutal on November 1. Autopsy confirmed that the animal was a female.

The contractions of the animal were more regular and the response to temperature change more pronounced from June 13 to June 16 than from July 2 to July 12 (text-fig. 17). A decrease in ambient temperature from 31°C to 26°C on June 14 resulted in an immediate increase in metabolic rate. On July 5, a similar decrease from 32° to 25.5°C resulted in an initial decrease and then in an increase in metabolism, although not to the level reached on the earlier date. By this time the metabolic response to temperature change was slight. These observations and the irregular contractions seen in *P. m. bivittatus* (No. 630514) prior to egg laying suggest that physiological thermoregulation in pythons is under hormonal control. The irregular contractions in No. 630514 up to one week prior to egg laying suggest an increase of certain hormone levels to that required for thermoregulation. The sluggish response to temperature change in No. 640578 probably reflects a change in hormone level towards the normal non-brooding condition. This, however, does not explain the brooding behavior and thermoregulation in an animal that has not laid eggs. A possible explanation might be a malignancy affecting the brain area controlling the whole brooding response or the gland responsible for secreting a hormone involved in brooding. Since the normal brooding period is from one-and-a-half to two-months long, and since this animal continued to show irregular brooding behavior for over three months, further support is given to the malignancy hypothesis.

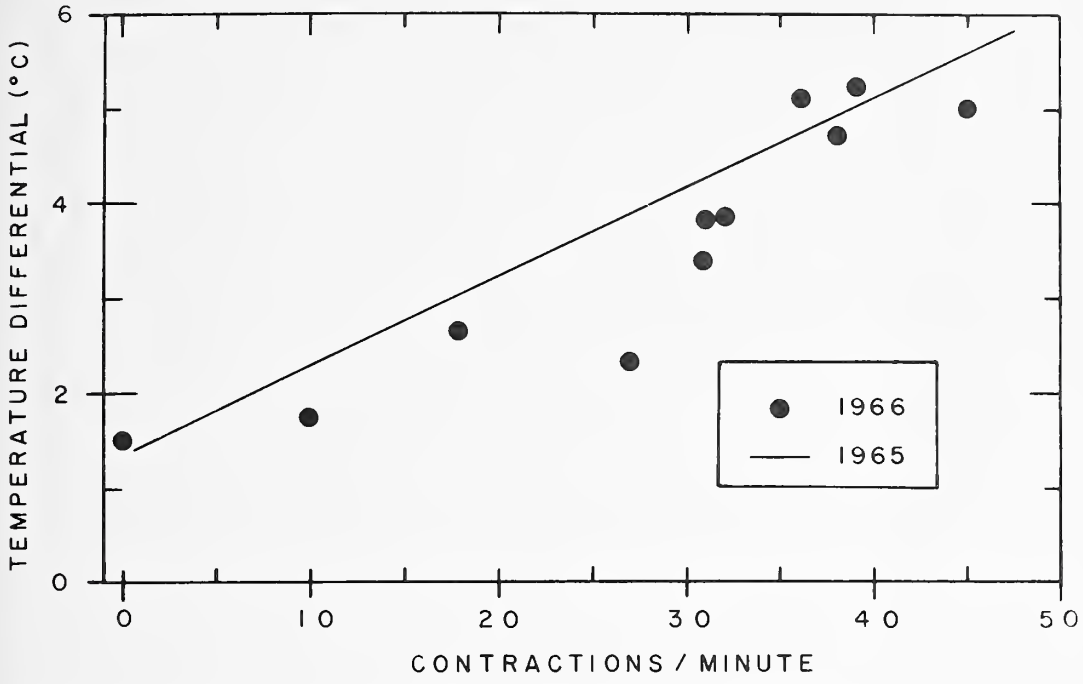


Text-fig. 13. Correlation of rate of body contractions with oxygen consumption in a brooding *Python molurus bivittatus*. Regression line represents data from 1965 shown in Fig. 10. Circles represent data from the same individual for 1966.

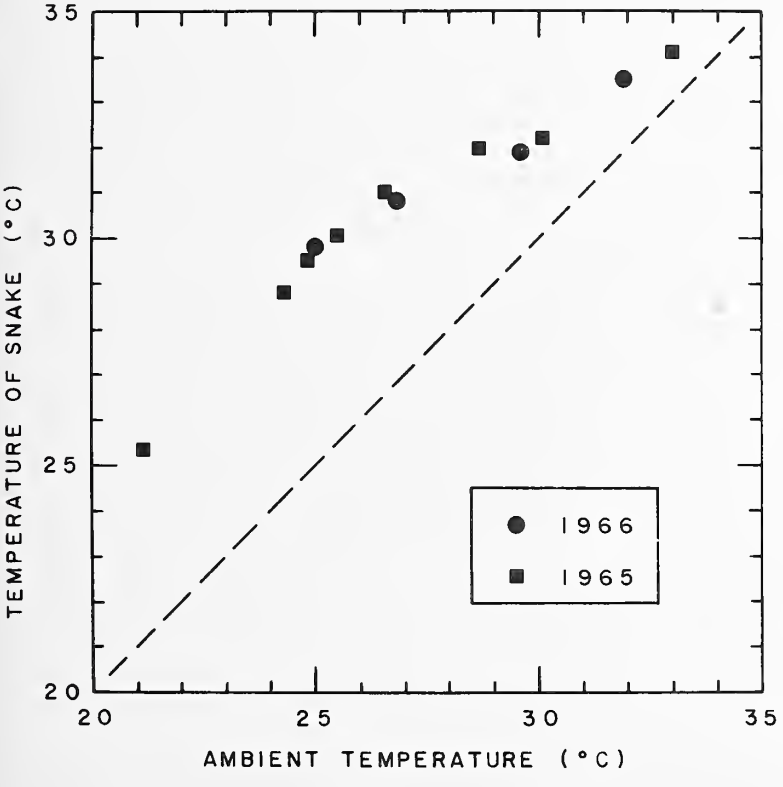
Brooding in Various Python Species

Python curtus

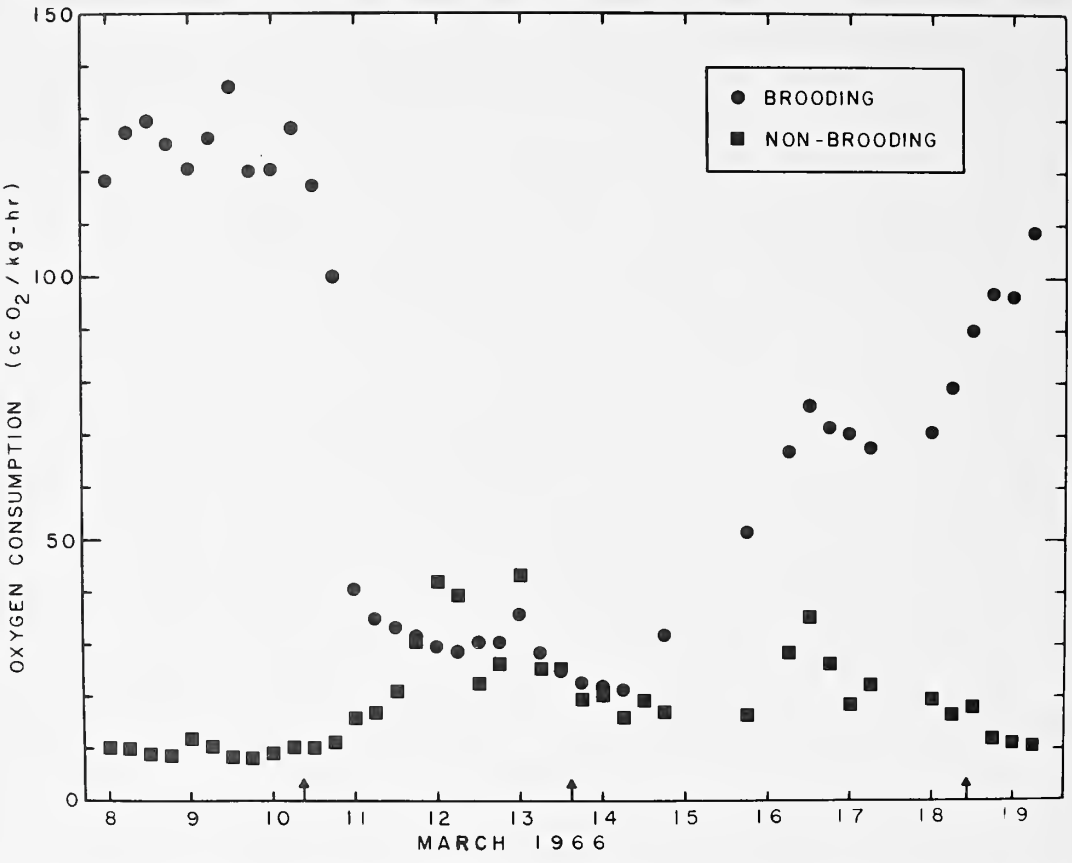
Noble (1935) described the laying of 16 infertile eggs and the brooding of a blood python, *Python curtus*. Temperatures reported were taken with a gas-filled mercury thermometer. All of the body temperatures reported were intermediate between the temperatures of the substrate and the air, and indicated that the snake had not developed an elevated body temperature. However, the substrate temperatures reported were 31.5°C, 31.8°C, and 32.2°C. These temperatures are in the vicinity of the lower critical temperature (33°C) found in the present study for *P. molurus*, therefore the "thermostat" of Noble's python may not have been "calling for heat." No conclusions regarding the ability or inability of *P. curtus* to thermoregulate can be drawn from Noble's observations.



TEXT-FIG. 14. Correlation of contraction rate with temperature differential in a brooding *Python molurus bivittatus*. Regression line represents data from 1965 shown in Fig. 11. Circles represent data from the same individual for 1966.



TEXT-FIG. 15. Correlation of body temperature of a brooding *Python molurus bivittatus* with ambient temperature. Dashed line indicates equal ambient and animal temperatures. Squares, data from 1965; circles, data from 1966.



TEXT-FIG. 16. Oxygen consumption of brooding and non-brooding *Python molurus* under identical ambient temperatures. Temperature changed from 24° to 31.5° on 10 March, to 30° on 13 March and to 23.5°C on 18 March (arrows).

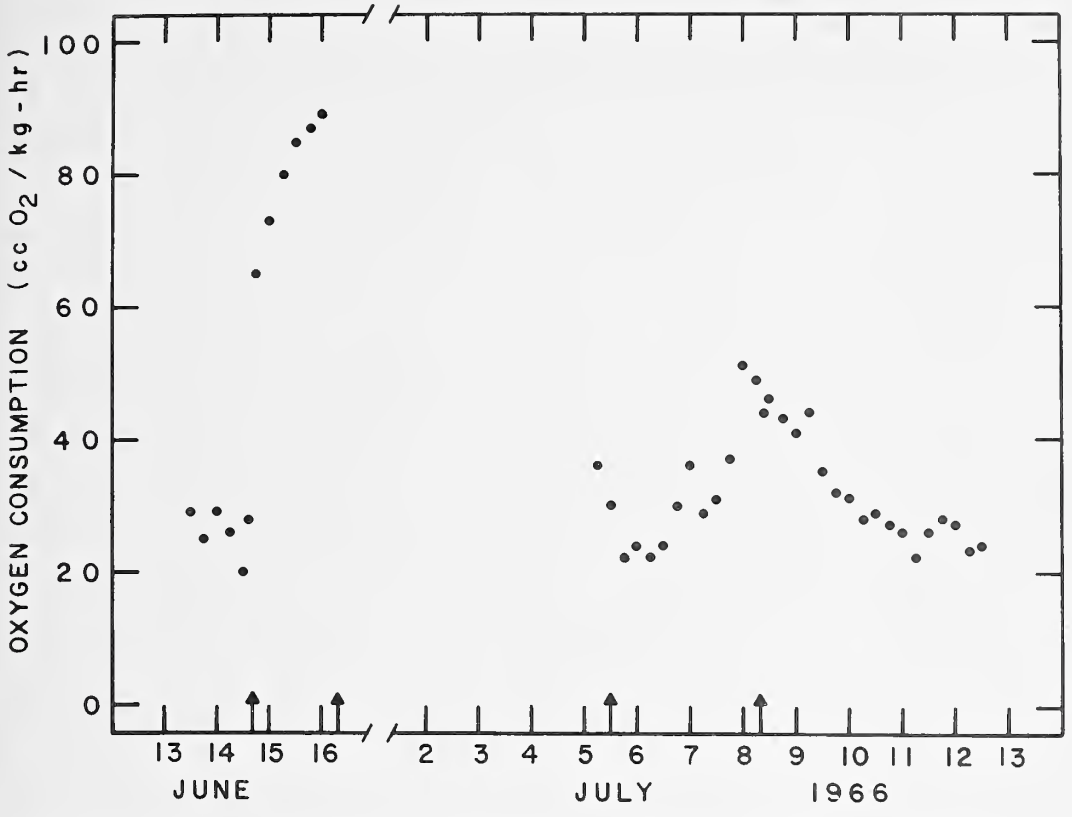
Additional definite information regarding the ability of *P. curtus* to thermoregulate physiologically while brooding its eggs was obtained during the present studies. A female blood python laid fertile eggs on May 29, 1965, while none of the project personnel was available. The animal was removed from her eggs at that time and would not return to them on May 31 when she was placed back with them. Nevertheless, on June 1 when she was placed in a room at about 27°C, she contracted her musculature at a rapid but irregular pace of about 27 contractions per minute. Irregular sporadic contractions had been noticed six weeks prior to egg laying. No further data were obtained from the python because normal brooding information could no longer be obtained. However, this evidence suggests a second species of python that has some ability to respond physiologically to decreases in ambient temperature while brooding eggs.

Chondropython viridis

Evidence exists for a third species having the ability to control its temperature while brooding. Kratzer (1962) reported on mating and brooding of *Chondropython viridis*. After egg laying the female python showed muscular twitching at intervals of two to three seconds. The temperature of the substrate was reported at 28°C and that of the air, 26°C to 30°C. However, these temperatures were obtained a month or more before the eggs were laid. If the temperatures were about the same after laying, the observed contractions would be expected in an animal capable of such a response to temperature decrease.

Python reticulatus

Little information exists regarding brooding in *Python reticulatus*. Wall (1926) described various aspects of the biology of the reticulated python. In his section on brooding he stated that "Experiments prove that the dam's body temperature is not raised during this period." However, he did not state whose experiments these were or under what conditions they were performed. Lederer (1944) made some observations on a brooding animal of this species and



TEXT-FIG. 17. Oxygen consumption of a *Python molurus* showing false brooding behavior. Temperature changed from 30.5° to 26° on 14 June, to 33° on 16 June, to 25° on 5 July and to 30°C on 8 July (arrows).

found that the snake's temperature was close to that of the substrate which was several degrees higher than the air temperature. Unfortunately, the lowest substrate temperature reported was 32.2°C, which is the approximate critical minimum temperature for the Indian python. The animal, therefore, probably was not observed under conditions that would have elicited the thermoregulatory response.

Some information that may be more useful in determining whether *P. reticulatus* is capable of maintaining its temperature above that of the environment by physiological means was provided by a visitor from Malaya to the New York Zoological Park. The information was given to Assistant Animal Manager Peter Brazaitis by the visitor, K. J. Sims. Sims had a 20-foot python that laid 67 eggs in an outdoor enclosure. Since Sims worked up to 14 hours a day, many of his observations. were made in the evening when the temperature was already cooler. During all of his observations he never saw the animal undergo muscular contractions. These observations suggest that the reticulated python lacks any physiological thermoregulatory ability.

On January 29, 1968, a *P. reticulatus,* weighing 73.19 kilograms, laid 51 eggs at the New York Zoological Park. The animal died two days later so that little information was obtained. However, at no time did anyone see any signs of muscular contractions.

No other reports were found of *P. reticulatus* brooding in zoological gardens or in nature that would clarify the brooding situation in this species. The information that is available indicates that this species does not have the same thermoregulatory ability that has been demonstrated in *P. molurus.*

Python sebae

Brooding in *Python sebae* was reported by Sclater (1862). He made simultaneous temperature measurements of a 14-foot male and a 22-foot brooding female python. The eggs were laid on January 13 and were removed from the female on April 4. She had left the eggs only a few times during the whole brooding period. Temperatures between the coils of the male and female on each of four different days were:

74.8°F, 81.6°F; 74.0°F, 83.2°F; 76.0°F, 96.0°F; 77.6°F and 86.0°F (23.8°C, 27.6°C; 23.4°C, 28.5°C; 24.5°C, 35.6°C; 25.4°C, and 30.0°C). Air temperatures during each of the previous measurements were: 58.6°F, 65.4°F, 60.0°F, and 66.0°F (14.8°C, 18.6°C, 15.6°C, and 18.9°C). No conclusions regarding the physiological thermoregulatory ability of *P. sebae* can be reached on the basis of these date. Although the temperatures of the male and female pythons were both above the air temperature, no mention of the floor temperature was made except that the cage was heated by hot water pipes. It seems likely that the animals were warmed by conductive heat from the water pipes (probably during the night when the snakes are more active). The female would have retained heat better than the male because her larger size and coil around the eggs presented less surface area per unit mass for heat loss to the environment.

Fitzsimons (1930), in referring to brooding of pythons, stated: "At this period her blood rises to a temperature of 90° Fahrenheit, which is apparently, Nature's rule for the hatching of infant pythons." The source of this information was not stated. It is apparently a generalization that Fitzsimons made as a result of reading some of the early accounts of python brooding temperatures, since he referred to these accounts without giving the specific sources.

Benedict (1932) gave an account of respiration rates and temperatures of a brooding 4.6 meter specimen of *P. sebae* at the National Zoological Park in Washington, D.C. The measurements were made on one day only. Average respiration rates during the course of the day ranged from 2.0 to 3.1 breaths per minute. Three sets of measurements were made during the day. Temperatures of the gravel around the python ranged from 29.92°C to 32.82°C; of the air 10 centimeters to 15 centimeters above the floor, 29.20°C to 32.04°C; of the air 30 centimeters above the floor, 29.30°C to 32.02°C; of the air 60 centimeters above the floor, 29.14°C to 31.44°C; under the python, 32.07°C to 34.62°C: between folds of python, 33.33°C to 35.18°C. During the three sets of measurements Benedict recorded temperature differences between snake and environment of up to 3°C or 4°C. However, some information given by Benedict casts doubt on the validity of his conclusions. The incubating python was located near a glass window that was a few centimeters from the air in the corridor outside the cage. The air outside the cage was reported as 20.4°C.

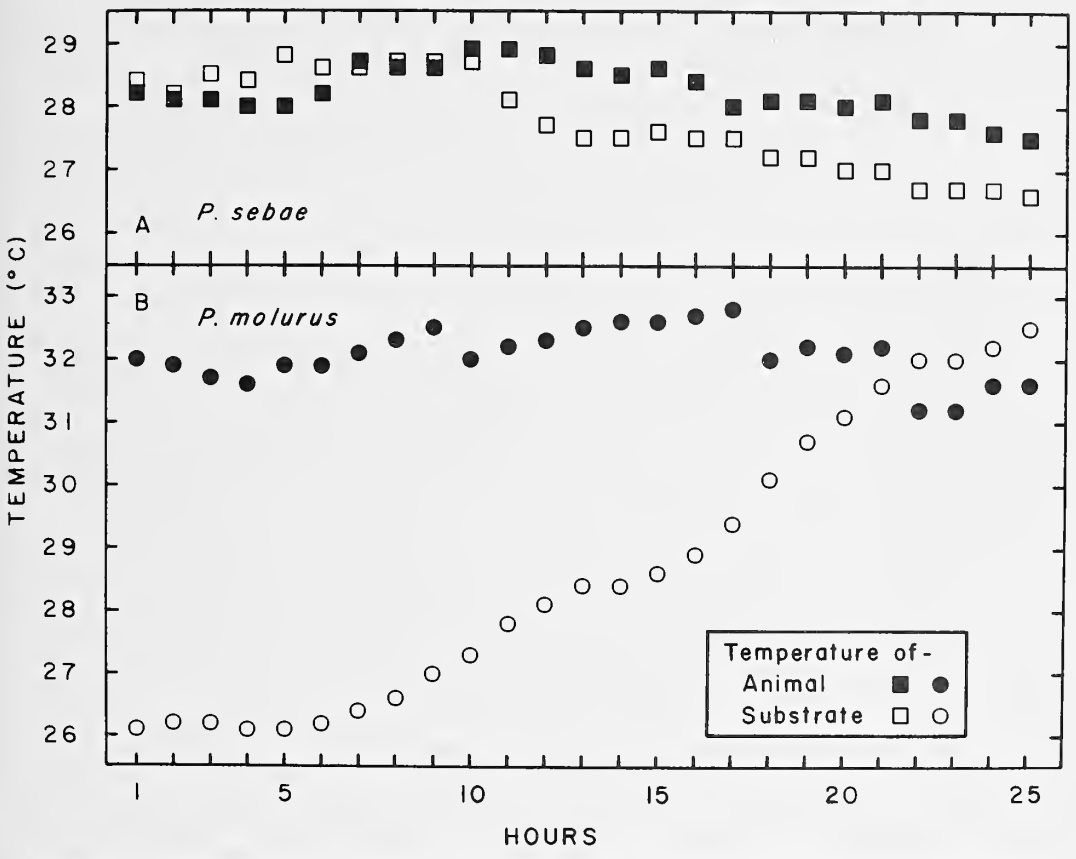
In 1960, Dowling (1960 and unpublished data) observed two brooding female pythons. One was a 53.07-kilogram (including eggs) specimen of *Python molurus* (NYZP No. 540616) and the other a 20.86 kilogram (including eggs) specimen of *P. sebae*. The former laid 53 eggs on April 6 and the latter laid 45 eggs on April 11. Temperatures of the animals, substrate, and air were taken at various times during the brooding period. Hourly readings for both animals were made from 0745 on April 29 until 0745 on April 30. The temperatures recorded for the animals and the adjacent substrate are shown in text-fig. 18. Text-fig. 18A shows that the body temperature of the *P. sebae* followed that of the substrate when the substrate temperature was lowered. Text-fig. 18B shows that the body temperature of *P. molurus* while brooding is relatively independent of the substrate temperature. Dowling noted that the Indian python contracted its musculature at a rate that was inversely proportional to temperature. No muscular contractions were seen in the African python. Text-fig. 19 shows the relationship between contraction rate and temperature differential (animal temperature minus substrate temperature). These data are presented for the above Indian python and for another individual (NYZP No. 510720) weighing 43.41 kilograms, including eggs laid on April 5, 1961. The African python was seen to leave its eggs during brooding and to go into the heated pool in the cage and later return to the eggs. It seems probable that the African rock python also lacks the thermoregulatory ability exhibited by the Indian python.

Morelia spilotes variegata

Cogger and Holmes (1960) produced good evidence to show that the carpet python *(Morelia spilotes)* regulates its temperature behaviorally. The animal basks in the sun and then forms a tight coil while resting. Heat was retained even through the night. However, if the following day was cloudy, the animal eventually came into equilibrium with the surrounding air. The data would have been more convincing if substrate temperatures had been given. The warmest temperature recorded for the snake on a sunny day was about 90°F (32.2°C), which is the lower critical temperature demonstrated for brooding Indian pythons. Cogger and Holmes suggested that *Morelia* may regulate its temperature similarly while brooding its eggs.

Egg Brooding in Various Reptiles

Reptile eggs get various degrees of care by the parent after being laid. Many eggs are merely laid in a hole in the ground or under the bark of a fallen log and left to the elements. Some animals lay their eggs in places that do not fluctuate much in temperature or humidity. One such



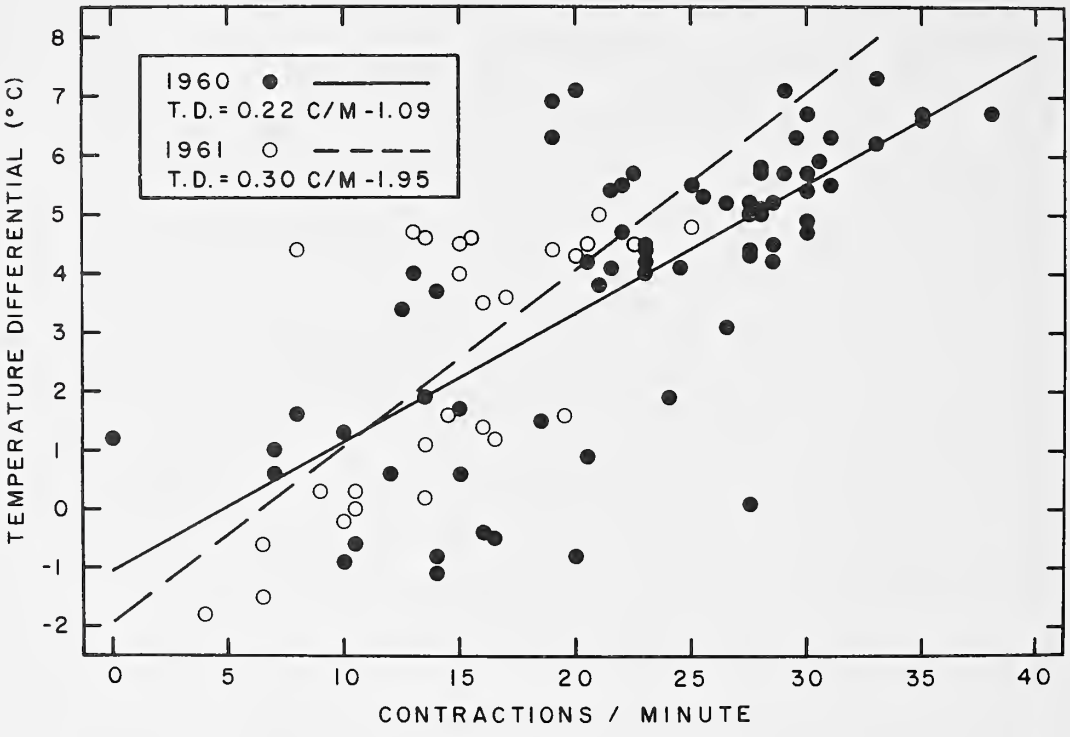
TEXT-FIG. 18. Body and substrate temperatures over a one day period for two brooding pythons *(Python molurus* and *P. sebae).* Key to symbols in lower right of figure.

place is in termite nests. An account of temperature regulation in termite nests was given by Luscher (1961). The South American teid, *Tupinambis nigropunctatus,* lays its eggs in termite nests (Hagmann, 1906). *Gehyra pilbara,* an Australian gecko, not only lays its eggs in mounds of the termite, *Eutermes triodiae,* but also lives in the mounds, thus escaping the rigors of the surrounding desert (Mitchell, 1965). A small python, *Liasis childreni perthensis,* which reaches an adult length of 30 centimeters, is found also in the nest with *Gehyra.* Although Mitchell did mention that *Gehyra* is a food item of the python, no information was given as to whether *Liasis* also lays its eggs in the termite mound. Noble and Mason (1933) summarized the literature up to that time on various snakes and lizards that actually stay with or brood their eggs. While some of the animals may sun themselves between periods of brooding, their size makes it unlikely that they contribute any appreciable heat for the development of their eggs. Most cases presented seem to be examples of egg protection rather than egg incubation. Noble and Mason (1933) provided some of their own data on brooding in *Eumeces fasciatus, E. laticeps,* and *Ophisaurus ventralis.* Body temperatures of *Eumeces* were reported to be 1.6°C to 3.2°C higher than that of the eggs. However, mention was made also of how quickly the body temperatures could change. This, along with the infrequency of temperature readings, casts doubt on any degree of thermoregulation existing for *Eumeces.* The authors concluded from similar infrequent data recordings that *Ophisaurus* probably does not have any thermoregulatory ability. A more detailed account of brooding in *Ophisaurus* was given by Vinegar (1968).

A detailed account of the manner in which the Nile monitor, *Varanus niloticus,* makes use of the nests of the termite, *Nasutitermes trinerviformis,* for incubation of its eggs in Natal, was given by Cowles (1930). Kopstein (1938) summarized several papers concerning the snake *Boiga drapiezii* and its laying of eggs in the nest of the termite, *Lacessititermes batavus.*

Energetics of *Python curtus*

No previous attempt has been made to consider a complete energy budget for a snake. With

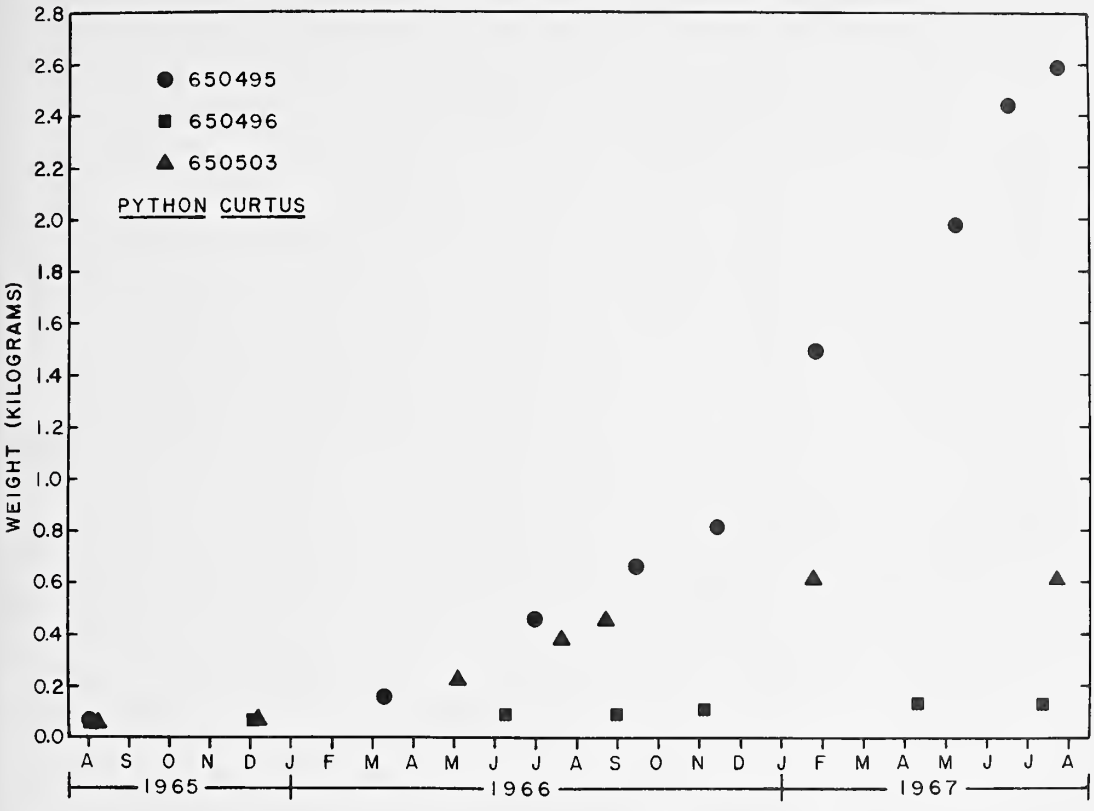


TEXT-FIG. 19. Correlation of body contractions with temperature differential (body temperature — substrate temperature) for two different *Python molurus bivittatus*. Lines and regression equations calculated by method of least squares. Key to symbols in upper left of figure.

hope of accomplishing this, in August 1965 three hatchlings of *Python curtus* were placed in individual cages in a temperature-controlled room (27°C). The animals were kept at this temperature except for periods of several weeks when they were acclimated to different temperatures for measurement of gas exchange. A water bowl was kept in each cage. Food consisted of albino mice except for one snake which was fed rats after 14 months. The food was weighed before being fed to the snakes. After each defecation the pythons were weighed. Lengths were not taken because of the difficulty in getting the animals stretched out. The severe struggling of the animals would have injured them and probably made them stop feeding. The defecations and renal waste were frozen and later oven-dried, weighed, and the caloric content determined. Caloric content was also determined for mice and for a python (a young individual of *P. sebae*). The caloric value obtained from this animal was used to calculate the energy budget of *Python curtus*, but is a tentative value, to be replaced as soon as caloric determinations of a series of *P. curtus* can be made.

Caloric values were determined with a Parr Adiabatic Oxygen Bomb Calorimeter. Six determinations were made on each sample with the mean value being used as the caloric value of the sample.

Text-fig. 20 shows the growth of the three *P. curtus* over a period of two years (August 1965 to July 1967). A marked difference in growth rate is evident. Photographs taken on February 14, 1967, emphasize this difference (Plate II). Some explanation of this divergence in growth was sought and found partially in the behavioral history of the animals. Individual No. 650495 showed aggressive behavior from the date it hatched. It is the only one of the three pythons that has attempted to bite. All three animals initially had to have food placed in their mouths to induce them to eat, but python No. 650495 was the first one to start feeding by itself. Number 650503, which showed signs of aggressiveness only when forcedly excited, was the second individual to start feeding by itself. Python No. 650496 could not be induced to bite and never took food voluntarily.



TEXT-FIG. 20. Growth of three hatchling *Python curtus* over a two-year period.

Table 3 summarizes the feeding and growth data of the three pythons for their first two years of life. The data for No. 650496 show the very slow rate of growth for each weight period. A slight loss of weight occurred for two periods, indicating that the food consumed since the previous weighing was enough to sustain life but not enough for additional growth. Individual No. 650495 shows a much higher growth rate; the maximum for one period was 9.18 grams gained per day for a 74-day period compared with a maximum of 0.27 grams gained per day for a 65-day period for No. 650496. The rates of growth for No. 650503 are intermediate between the other two animals. Another factor contributing to the difference of size attained by these animals is shown in Table 3. Data are shown which express the amount of food consumed which goes into producing new python protoplasm. These data are expressed as weight gain divided by food consumed times 100. The values based on total weight gain and total food consumed are for No. 650495, 52 percent; No. 650503, 45 percent; No. 650496, 29 percent. Not only was No. 650496 not consuming as much food as its siblings, but the low value of 29 percent indicates it was not using food as efficiently as the others.

Caloric values of the intestinal and renal wastes of the pythons are summarized in Table 4. Laboratory mice under 10 grams (wet) had a caloric content of 5204.39 $\pm$ 92.25 calories per gram (dry), while those over 10 grams (wet) had a caloric content of 5460.15 $\pm$ 48.34 calories per gram (dry). The specimen of *Python sebae* weighing 173.69 grams (wet) had a content of 4136.22 $\pm$ 67.51 calories per gram (dry). The ratio of dry weight to wet weight for the mice was 0.27, for the python, 0.21. Oxygen consumption of *Python curtus* is summarized in text-fig. 2. The caloric values, oxygen consumption data, and growth figures can be used to calculate an energy budget for the pythons over any growth interval for which there is complete data. Table 5 shows such an energy budget for each of the three pythons.

Discussion of Reptile Energetics

Pope (1965) gave an account of an Indian python, *Python molurus,* eating 123 laboratory rats (61 pounds) during its second year and most of its third year. The snake increased its

TABLE 3.

FOOD CONSUMPTION AND GROWTH OF THREE YOUNG *Python curtus* HATCHED FROM SAME CLUTCH OF EGGS.

Date	*Days Since Last Weight*	*Weight g*	*Gain/ Day g*	*Weight Gain g*	*Gain X 100/ Food*	*Food Consumed (wet) g*	*Food Size g*	*Food Accumul.*	*Number of Food Animals*
				Animal No. 650495					
1 VIII 65		69.7							
9 III 66	220	159.75	0.41	90.05	52	171.89	12.28(2.01-33.81)	171.89	14 mice
30 VI 66	113	455.00	2.61	295.25	60	494.81	30.93(23.06-35.32)	666.70	16 "
14 IX 66	76	656.09	2.65	201.09	52	388.28	29.87(22.70-36.72)	1054.98	13 "
14 XI 66	61	810.56	2.53	154.47	41	381.06	127.02(81.68-184.60)	1436.04	3 rats
27 I 67	74	1490.00	9.18	679.44	51	1319.63	164.95(98.50-244.72)	2755.67	8 "
8 V 67	101	1980.00	4.85	490.00	45	1084.51	216.90(184.33-240.50)	3840.18	5 "
18 VI 67	41	2440.00	11.22	460.00	64	722.92	180.73(101.90-260.02)	4563.10	4 "
25 VII 67	37	2590.00	4.05	150.00	26	570.57	285.29(257.05-313.52)	5133.67	2 "
				Animal No. 650496					
1 VIII 65		56.50							
2 XII 65	123	69.81	0.11	13.31	64	20.81	3.47(1.80-5.00)	20.81	6 mice
9 VI 66	189	92.75	0.12	22.94	33	68.65	3.43(2.00-9.17)	89.46	20 "
31 VIII 66	83	92.30	−0.0054	−0.45		27.36	3.04(2.16-4.58)	116.82	9 "
4 XI 66	65	109.86	0.27	17.56	40	44.34	2.61(1.24-4.95)	161.16	17 "
10 IV 67	157	130.23	0.13	20.37	22	92.50	2.50(1.32-4.07)	253.66	37 "
12 VII 67	93	129.76	−0.0051	−0.47		39.43	3.94(1.61-11.50)	293.09	10 "
				Animal No. 650503					
7 VIII 65		58.30							
6 XII 65	121	73.36	0.12	15.06	70	21.59	3.08(2.18-4.32)	21.59	7 mice
3 V 66	148	232.40	1.07	159.04	53	299.19	21.37(9.30-29.58)	320.78	14 "
20 VII 66	78	383.53	1.94	151.13	55	273.75	27.38(17.67-31.94)	594.53	10 "
23 VIII 66	34	460.09	2.25	76.56	50	151.90	30.38(29.13-32.62)	746.43	5 "
25 I 67	155	614.78	1.00	154.69	50	309.83	30.98(22.58-34.78)	1056.26	10 "
23 VII 67	179	608.99	−0.0323	−5.79		175.29	29.22(16.78-43.60)	1231.55	6 "

TABLE 4.
WEIGHT AND CALORIC VALUES OF WASTE PRODUCTS FROM THREE SIBLING *Python Curtus* OVER A TWO-YEAR PERIOD.

Date	Sample	Dry Weight g	% Ash	Ash-free Dry Wt. g	Calories/ g	Calories/ (Ash-free) g	Total Calories in Sample
			Animal No. 650495				
15 XII 65	Defec.	0.46	10.95	0.41	5094.06	5720.76	2343.27
9 III 66	Defec.	4.64	31.12	3.20	3483.59	5056.61	16163.86
26 VI 66	Defec.	15.11	29.32	10.68	3653.99	5170.92	55211.79
14 IX 66	Defec.	12.71	29.53	8.96	3517.79	4991.12	44711.11
14 IX 66	Uric A	11.03	5.62	10.41	2645.04	2802.41	29174.79
14 XI 66	Defec.	14.60	35.30	9.45	3084.18	4765.49	45029.03
14 XI 66	Uric A	16.02	2.57	15.61	2734.80	2807.03	43811.50
27 I 67	Defec.	49.12	36.59	31.15	3057.01	4820.63	150162.62
27 I 67	Uric A	54.35	4.85	51.71	2664.96	2800.73	144840.58
23 IV 67	Defec.	42.14	35.07	27.36	3134.35	4828.73	132081.51
23 IV 67	Uric A	43.58	5.54	41.17	2631.54	2785.85	114682.51
18 VI 67	Defec.	19.95					
18 VI 67	Uric A	23.55					
13 VII 67	Uric A	2.16					
25 VII 67	Defec.	34.09					
25 VII 67	Uric A	27.30					
			Animal No. 650496				
2 XII 65	Defec.	0.29	10.08	0.26	5324.24	5920.97	1544.03
21 III 66	Defec.	0.76	27.92	0.55	4183.93	5804.62	3179.79
8 VI 66	Defec.	0.67	23.03	0.52	4201.13	5457.92	2814.76
19 VIII 66	Defec.	0.75	30.03	0.52	3660.76	5232.05	2745.57
4 XI 66	Defec.	0.63	25.89	0.47	4020.47	5424.84	2532.90
21 III 67	Uric A	1.37	2.55	1.34	2694.78	2765.23	3691.85
10 IV 67	Defec.	1.85	31.43	1.27	3643.02	5312.53	6739.59
10 IV 67	Uric A	0.41	5.19	0.39	2453.23	2587.39	1005.82
5 VI 67	Uric A	1.55	2.08	1.52	2699.87	2757.19	4184.80
28 VI 67	Uric A	0.70					
12 VII 67	Defec.	1.76					
12 VII 67	Uric A	0.42					
			Animal No. 650503				
6 XII 65	Defec.	0.41	12.28	0.36	5028.39	5732.27	2061.64
3 V 66	Defec.	8.40	35.34	5.43	3403.81	5264.04	28592.00
3 V 66	Uric A	7.73	3.04	7.50	2709.68	2794.76	20945.83
20 VII 66	Defec.	9.27	28.17	6.66	3724.23	5184.51	34523.61
20 VII 66	Uric A	8.65	4.77	8.24	2668.64	2802.23	23083.74
23 VIII 66	Defec.	6.13	32.88	4.11	3259.58	4855.59	19981.23
23 VIII 66	Uric A	4.35	6.88	4.05	2549.74	2738.03	11091.37
25 I 67	Defec.	10.96	31.57	7.50	3417.19	4993.99	37452.40
25 I 67	Uric A	13.32	2.97	12.92	2735.01	2818.77	36430.33
23 VII 67	Defec.	5.79					
23 VII 67	Uric A	8.94					

weight by 34.5 pounds which is a gain of one pound for every 1.77 pounds of food or an efficiency of 56 percent. Barton and Allen (1961) gave data for an anaconda, *Eunectes murinus,* which when received was 16 feet 4 inches and 108 pounds. Over the next 81 months the snake ate 539 pounds of ducks and gained 92 pounds (one pound gain per 5.86 pounds food). This represents an efficiency of 17 percent. An African python, *P. sebae,* received at slightly over two feet consumed 148 pounds of food during its seventh, eighth, and ninth years of captivity (37 months) and gained 19 pounds, or 7.8 pounds of food for each pound of body weight gain for an efficiency of 13 percent. Brown (1958) found food efficiencies of 28 percent after one year, 34 percent after two years, and 20 percent after five and six years for *Natrix sipedon sipedon.* Food efficiencies for *Spalerosophis cliffordi* were given by Dmi'el (1967). Data are broken down by age groups and sex. Animals under one year had efficiencies

TABLE 5.
ENERGY BUDGET FOR THREE SIBLING *Python curtus* OVER A TWO-YEAR PERIOD

Animal No.	*650495*	*650496*	*650503*	*650503*	*650503*	*650503*
Dates	*30 June 66*	*4 Nov. 66*	*6 Dec. 65*	*3 May 66*	*20 July 66*	*23 Aug. 66*
	14 Sept. 66	*10 Apr. 67*	*3 May 66*	*20 July 66*	*23 Aug. 66*	*25 Jan. 67*
Days	*76*	*157*	*148*	*78*	*34*	*155*
Budget (in % of total energy available)						
Growth	27.9	6.1	24.7	28.3	28.6	13.7
Maintenance and Movement	60.3	91.2	66.4	59.2	58.1	78.8
Intestinal Waste	7.1	2.3	5.1	7.4	8.6	3.8
Renal Waste	4.7	0.3	3.7	5.0	4.8	3.7
Total Energy	100.0	99.9	99.9	99.9	100.1	100.0

for males and females of 21 percent and 22 percent; one- to three-year animals, 26 percent and 33 percent; four- to eight-year animals, 11 percent and 23 percent; nine- to 13-year animals, 7 percent and 12 percent. The above data show that, after animals reach a certain age (size?), less energy is expended in growth and more in maintenance.

Pope (1962), in discussing the natural superiority of efficiency in pigs, mentioned that "the domesticated pig attains its size more rapidly than does any other barnyard animal, and on less food, too." Vanschoubroek et al. (1967) summarized the literature containing data for fully-fed pigs. The values are given as kilogram feed per kilogram gain and range from 3.17 to 4.09. These represent efficiencies of 31.5 percent to 24.4 percent, but the food provided was of optimal quality to promote growth with little non-digestable material. Data cited by Broady (1945) for cattle and sheep when calculated as efficiencies give a value of 9.6 percent. The pig with its high food conversion efficiency still is not as efficient as the pythons. There are two probable reasons for the high efficiency of pythons. First, being ectotherms they do not have to expend as much energy in maintaining high metabolic levels (brooding excepted). Second, because they are rather sluggish except when looking for food they expend less energy in activity. A python in the wild would probably expend more energy in activity since food is not as readily available as under captive conditions.

Various aspects of lizard energetics have been considered by some authors. The effects of prolactin on growth of adult male *Anolis carolinensis* were studied by Licht and Jones (1967). The energetics of food intake and growth were evaluated. Caloric contents of food items were calculated and corrected for fecal losses. These figures were used to calculate average calories assimilated per animal per day. The data recalculated for control animals show that those weighing about 5.2 grams at 32°C on a 14-hour photoperiod in the spring assimilated 54 to 63 calories per gram-day; 5.7-gram animals at 32°C on a six-hour photoperiod in the winter assimilated 26 to 42 calories per gram-day; and 5.4 gram animals at 32°C on a 14-hour photoperiod in the winter assimilated 12 calories to 17 calories per gram-day. Animals in the spring, having normal appetities, show caloric intakes which agree well with the results of Johnson (1966) and McNab (1963).

Johnson (1966) dealt with one aspect of the energetics of three species of lizards *(Sceloporus undulatus, S. magister,* and *Cnemidophorus tigris),* namely, assimilation. His data are based on analyses of stomach contents. The weight of food eaten is estimated from these analyses. The caloric value of grasshoppers (5363 calories per gram) as determined by Golley (1961) is taken as representative of all food items. The energy assimilated by a 15-gram *S. undulatus,* a 22-gram *C. tigris,* and a 30-gram *S. magister* is estimated at 0.83 kilocalories, 1.57 kilocalories, and 2.17 kilocalories per day (55 calories, 71 calories, and 72 calories per gram-day), respectively.

McNab (1963) estimated an energy budget for a 19-gram *Sceloporus undulatus* and made some comparisons with a *Peromyscus maniculatus* of the same weight. The estimate involves these assumptions: the body temperature drops at night; the body temperature is regulated at a mean of 35°C by behavioral means during the day; the lizard is active about one-fifth of the daylight hours; and its active metabolism is about 2.5 times its resting metabolism. Recalculated data of Bartholomew and Dawson (1956) show that a 19-gram lizard uses about 1.12 kilocalories per day, of which 23 percent (0.26 kilocalories per day) is used in activity. The estimate of active metabolism being 2.5 times

standard metabolism may be low (Dawson and Bartholomew, 1958). McNab pointed out that a 19-gram *S. occidentalis* uses about 0.26 kilocalories per day for activity compared with a 19-gram *Peromyscus maniculatus* which uses about 1.70 kilocalories per day; the mouse thus uses 6.5 times more energy. In spite of the greater energy collected by the mouse, it is unlikely that 6.5 times more energy is needed for food gathering.

Surface Area-Weight Relationships

Benedict (1932) attempted to relate surface area to weight for a series of snakes. His determinations of surface area were obtained by measuring distances from the nose and corresponding girths and then taking mean values of these measurements to calculate area. Then, assuming that surface area $= K$ weight$^{0.67}$, Benedict proceeded to determine K for each snake. The mean value of K for a series of eight snakes of several species weighing 3.49 kilograms to 13.21 kilograms was 12.5 and ranged from 12.0 to 13.2. Values of 14.3 and 14.4 obtained for a 31.80-kilogram python on two separate occasions were discarded by Benedict because they were "probably a little too large." A value of 17 was obtained for the 5.58-kilogram 1931 python which was the only surface area determined directly from the skin. Benedict compared his K values with those of Inaba (1911) which were obtained from five snakes ranging in weight from 48 grams to 109 grams. The values were 19.1, 17.0, 17.5, 18.7, and 19.9, with a mean of 18.6. Benedict attributed the difference between his values and those of Inaba to the fact that Inaba skinned his animals, thereby stretching the skin. However, values of K from the present study range from 23.1 and 24.7 for animals weighing 132.6 grams and 166.1 grams to 10.8 for a 99-kilogram animal. From these data it would seem that K varies inversely with weight and is a constant only within limited ranges of weight. These observations are consistent with those of Meeh (1879). He stated that K is a constant only for a group of similarly shaped animals.

Benedict (1932) and Inaba (1911) assumed that area is proportional to weight to the 0.67 power. However, the actual relationship for a series of seven pythons was determined by calculating a regression of surface area on weight (text-fig. 21). This resulted in the equation $A = 43.16\ W^{0.549}$, where 0.549 is significantly different from 0.67 at the 95 percent level. Thus, the actual proportionality between surface area and weight must be determined by calculation rather than by assuming that the coefficient of weight is 0.67.

Surface Area-Length and Weight-Length Relationships

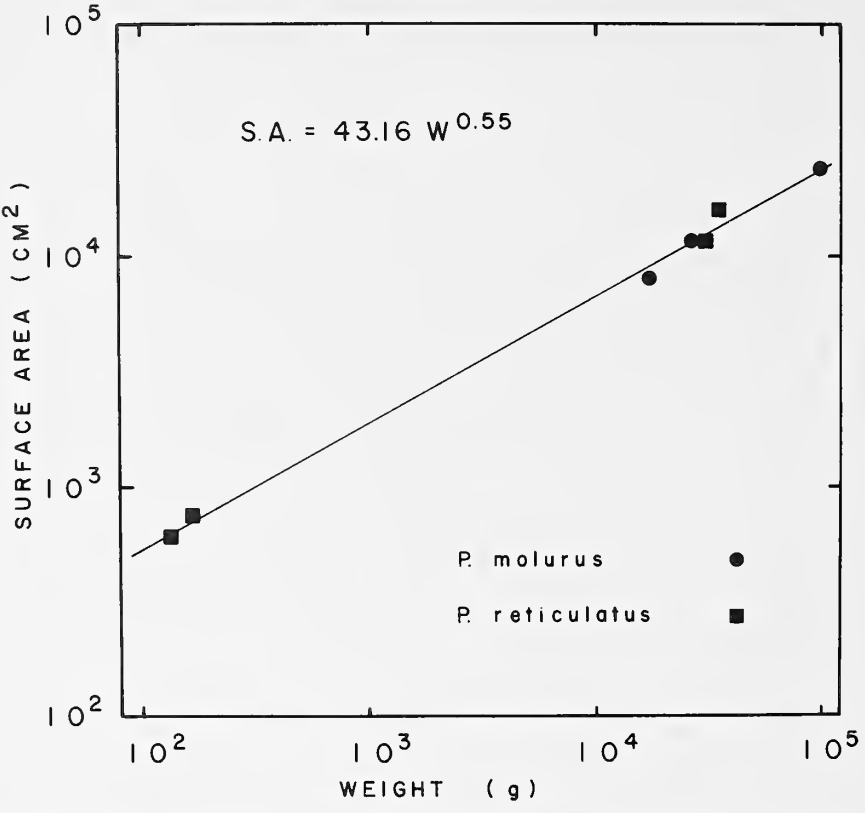
For a given length, *Python molurus* is a thicker snake than *P. reticulatus*. Plots of surface area against length (text-fig. 22) and weight against length (text-fig. 23) show this relationship. Data for *P. sebae* are included in the latter figure which show that its shape is closer to *P. molurus* than to *P. reticulatus*.

Physiological and Ecological Implications of the Geographic Distribution of *Python molurus* and *Python reticulatus*

Licht and Moberly (1965) found that a temperature near 30°C is required for development of the eggs of the green iguana, *Iguana iguana*. Temperatures a few degrees above and below 30°C resulted in death of the embryos. Their concluding statement that ". . . these results illustrate the need for careful attention to the thermal requirements of the eggs in consideration of the ecology and distribution of lizards," can apply equally to snakes. It becomes of particular significance when applied to the distribution of pythons. For the Indian python, *Python molurus*, to have a well developed system of physiological thermoregulation while brooding its eggs suggests that the additional heat supplied to the eggs may be needed to prevent them from reach a critically low temperature.

The mainland distribution of *Python molurus molurus* includes peninsular India from Sind, West Pakistan, and Punjab in the northwest to Bengal in the northeast. *Python m. bivittatus* is found over the whole Indo-Chinese subregion. It is recorded as far south as Zinba Chanun, Tavoy district, Burma. It has been collected to the north in the region of Yenping, Fukien, China, in the east and in Yuankiang, Yunnan, China, and Myitkyina, Burma, in the west. *Python reticulatus* is found on the mainland in southern Burma and Thailand as far north as latitude 18°; to the east as far north as Yen-Bai, North Viet Nam; to the south, throughout Malaysia (Pope, 1935; Smith, 1943). These distributions are shown in text-fig. 24 with some of the specific localities mentioned above plotted on the map. The distribution of these pythons conforms closely with the zoogeographical areas set up by Smith (1931). The area of sympatry corresponds to Smith's Annam area in the east and Indo-Chinese Great Plain area in the west. North of the area of sympatry is Smith's Trans-Himalayan Mountainous area.

The factor limiting the northern distribution of *P. reticulatus* may be the critical minimum temperature for the development of its eggs. Unfortunately, data on minimum temperature



TEXT-FIG. 21. Correlation of surface area with weight of two species of pythons *(Python molurus* and *P. reticulatus).* Line and regression equation calculated by method of least squares.

requirements of python egg development are scarce. Some indication of the requirements for the hatching of *Python sebae* eggs was given by Joshi (1967). He separated a clutch of 28 eggs into four batches. Five of seven of the eggs kept at 72°F to 84°F (22.2°C to 28.9°C) and 65 percent to 80 percent relative humidity hatched in 52 days. Four eggs kept at 86°F to 90°F (30.0°C to 32.2°C) and 80 percent to 90 percent relative humidity hatched in 49 days. Eggs kept at 70°F to 90°F (21.1°C to 32.2°C) and less than 40 percent relative humidity did not hatch. The last batch kept surrounded by moist soil in a dry sunny place did not hatch, but no temperature or humidity data were given. Although the times that the eggs were at the lower temperatures are not provided, it appears that temperatures as low as 72°F (22.2°C) are not deleterious to the eggs as long as the humidity is fairly high.

In addition to better information on temperature requirements of egg development, information is also needed on climate for the regions where these snakes are found. The distributions of *P. molurus* and *P. reticulatus* correspond roughly with the surface temperature regions of Parkins (Espenshade, 1964). *Python reticulatus* ranges through the area of hot summers and winters, while *P. molurus* is found also in the regions of hot summer and mild or cool winters (hot = above 20°C; mild = 10° to 20°C; cool = 0°C to 10°C).

These data support the hypothesis that distribution of these two pythons is limited by egg development temperature. However, additional data are needed to substantiate this hypothesis.

General Discussion

Colbert, Cowles, and Bogert (1946) determined heating rates of alligators by tethering them in the sun and recording their cloacal temperatures. They demonstrated a 1°C/1.5 minute (6.6×10^{-1} °/minute) increase in temperature for a 50-gram alligator and a 1°C/7.5 minute (1.3×10^{-1} °/minute) increase for a 13,000-gram alligator with 260 times the body mass. They interpolated to find a 1°C/86 hour (1.9×10^{-4} °/minute) temperature rise for a nine-million-gram dinosaur, having 700 times the body mass of the large alligator. In a later paper (Colbert, Cowles, and Bogert, 1947) they de-

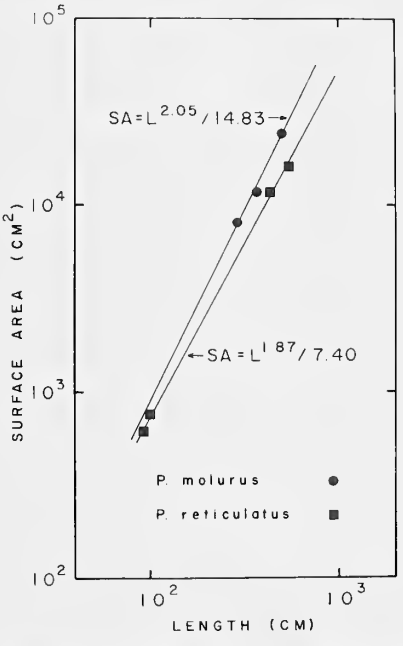
fended objections made to their interpolation. The objectors had pointed out that surface area, mass, and heat capacity must be taken into consideration. The recalculated times for a 1°C rise were 67 minutes (1.5×10^{-2} °/minute) from one person and 66 minutes to 81.5 minutes ($1.5\text{-}1.2 \times 10^{-2}$ °/minute) from the other. The authors admitted their original figure was derived incorrectly but insisted that the time would still be as long as several hours rather than the low figures submitted. They claimed that the alligator cannot be treated as a cylindrical, inanimate mass but that various physiological processes must be considered. Bartholomew and Tucker (1963) gave an example of what effect physiological processes have on heating and cooling in the agamid lizard, *Amphibolurus barbatus*. They compared the heating and cooling rates of live and dead lizards. The live lizards heated more rapidly than they cooled, thus showing some physiological control. However, the cooling rate of the live lizards was still more rapid than the heating and cooling rates of the dead lizard. This would tend to support the objections raised against the conclusions of Colbert, Cowles, and Bogert. A live animal with physiological control of its heating and cooling rates heats up more rapidly than a dead animal. Therefore, a live animal should also heat up more rapidly than an inanimate model having the same thermal conductivity.

The demonstration of physiological thermoregulation in some lizards (Bartholomew and Tucker, 1963, 1964; Bartholomew, Tucker, and Lee, 1965) and of physiological thermoregulation and thermogenesis in pythons (Hutchison, Dowling, and Vinegar, 1966) suggests that mechanisms of physiological thermoregulation occurred in some of the large primitive reptiles and did not originate *de novo* in mammals and birds.

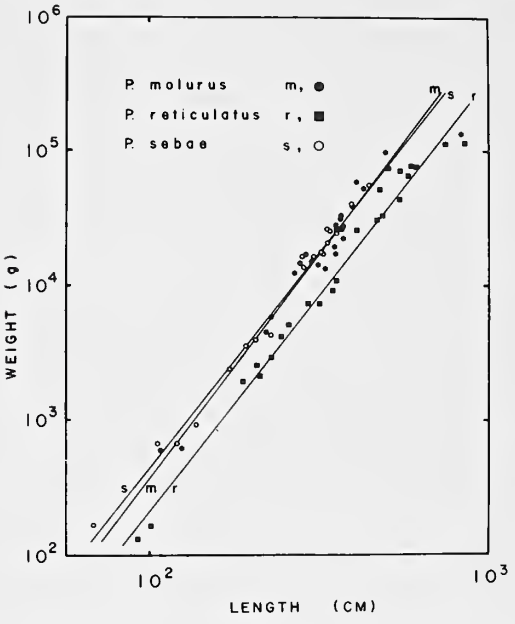
Rodbard (1949) discussed the possibility that the large membranous sail-like structures of the Permian reptiles *Dimetrodon* and *Edaphosaurus* were used as absorbers of solar radiant energy. An argument for dinosaurs having had some sort of physiological thermoregulation was presented by Russell (1965). He first established that the dinosaurs were intermediate between birds and crocodilians and in many ways closer to birds in their skeletal anatomy. Then assuming that the similarities are carried through to their soft anatomy, he suggested that dinosaurs had separate arterial and venous circulations and therefore, some degree of homoiothermy.

Cys (1967) refuted Russell's hypothesis of endothermy in the dinosaurs, stating that its presence is not necessary to explain dinosaur extinction. Instead, Cys maintained that the large size of dinosaurs prevented them from finding hibernation sites. Russell (in a comment at the end of Cys, 1967, p. 267) pointed out that several dinosaurs were certainly small enough to find hibernation sites. He summarized his comments by stating that there appears to be some "flaw" in dinosaur make-up, relating to their extinction, that is independent of size, shape, feeding habits, or systematic position. Proving that the "flaw" is homoiothermy may not be possible, but the hypothesis fits much of the available evidence.

The ability of large ectotherms to maintain body temperatures above ambient is not confined to reptiles. Carey and Teal (1966) demonstrated that tuna (big eye, *Thunnus obesus*, and yellowfin, *T. albacares*) can maintain elevated body temperatures with a countercurrent heat exchange system located in the vascular system of the red muscle masses. The heat exchanger provides a thermal barrier which prevents heat from being carried off by the blood and lost through the gills. Maximum temperatures found were 32°C in a 70 kilogram *T. obesus* from 20°C water and 26°C in a 12 kilogram *T. albacares* from 19°C water. The objection that the high internal temperature results from the fish struggling on deck was disproved by experiments with curarized fish and measurements of free-swimming fish.



TEXT-FIG. 22. Correlation of surface area with length of two species of pythons *(Python molurus* and *P. reticulatus)*. Lines and regression equations calculated by method of least squares.



TEXT-FIG. 23. Correlation of weight with length of three species of pythons *(Python molurus, P. reticulatus* and *P. sebae).* Lines determined by method of least squares.

P. molurus – $W = (L^{3.358})/14180$
P. sebae – $W = (L^{3.217})/6155$
P. reticulatus – $W = (L^{3.231})/13750$

Tied in with the ability to regulate body temperature is the ability to sense the temperature of the environment. A temperature sensitive center, with maximum sensitivity at the level of the third ventricle of the brain, was demonstrated in *Pseudemys elegans* by Rodbard, Samson, and Ferguson (1950). Warming of the area resulted in a rise in blood pressure and cooling resulted in a fall in pressure. Evidence for a temperature control center located in the head was given by Heath (1964); he demonstrated head-body temperature differences in *Phrynosoma coronatum* with the head 3°C to 5°C higher in partially buried animals and 2°C to 4°C higher in active animals. Emergence of these lizards possibly is dependent on head temperature and independent of body temperature. Hammel, Caldwell, and Abrams (1967) demonstrated that the behavioral regulation of body temperature in the blue-tongued skink, *Tiliqua scincoides,* is dependent on a combination of hypothalamic and other body temperatures. This demonstration was made by implanting thermodes across the preoptic region of the brainstem and heating and cooling this area while the lizard was at environmental temperatures of 15°C or 45°C. Further experiments on *Tiliqua scincoides* (Cabanac, Hammel, and Hardy, 1967) demonstrated five "warm neurons" which increase their activity with rising brain temperature and three "cold neurons" which increase activity with falling brain temperature. These neurons are located in the preoptic region of the brain.

The similarities in temperature sensitivity between the brains of mammals and reptiles point to the possibility of reptilian brains having contained the progenitor of the more finely developed hypothalamic thermostat of mammals. Rodbard (1948) discussed some of the evolutionary implications of blood pressure changes in response to body temperature changes. These responses were noted in chickens, rabbits, turtles, and frogs. Rodbard suggested that the first amphibians coming onto land encountered greater diurnal changes in temperature than did the ancestral fish. Thus, the function of hypothalamic sensitivity was to adjust metabolic activity to body temperature changes. This hypothesis is supported by the work of Cabanac et al. (1967), Hammel et al. (1967), and Heath (1964). The function of the hypothalamus for the fine control of body temperature probably came with the development of homeothermy in mammals and birds. The thermoregulation of brooding pythons may represent an intermediate step in the development of the latter function for the hypothalamus. Bligh (1966) speculated on the significance of the earlier function of the hypothalamus as a "broad-band" control in modern mammals. He suggests that the remnant of the "broad-band" control, dependent only on thermal sensitivity of the hypothalamus, may act in the case of emergencies as during fever, heat and cold stress, and intense activity. The 24-hour shift in deep body temperature demonstrated in water-deprived camels (Schmidt-Nielsen et al., 1957) may be an example of "broad-band" dominance. Similarly, the diurnal or seasonal heterothermy of various mammals may be derived from the early "broad-band" control (Twente and Twente, 1964).

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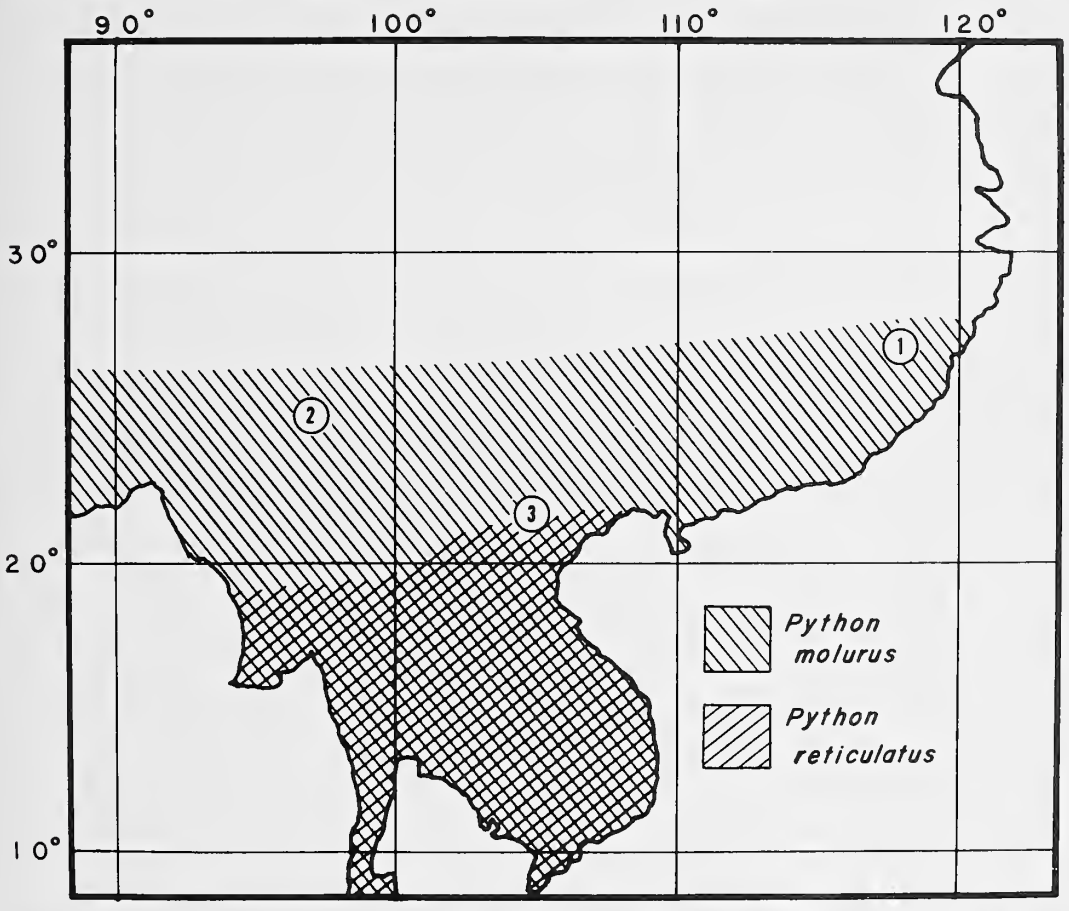

TEXT-FIG. 24. Distribution of *Python molurus* and *P. reticulatus*. Western part of range of *P. molurus* and island distribution of both species not indicated. 1 – Yenping, China; 2 – Myitkyina, Burma; 3 – Yen-Bai, North Viet-Nam.

gree in zoology at the University of Rhode Island.

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EXPLANATION OF THE PLATES

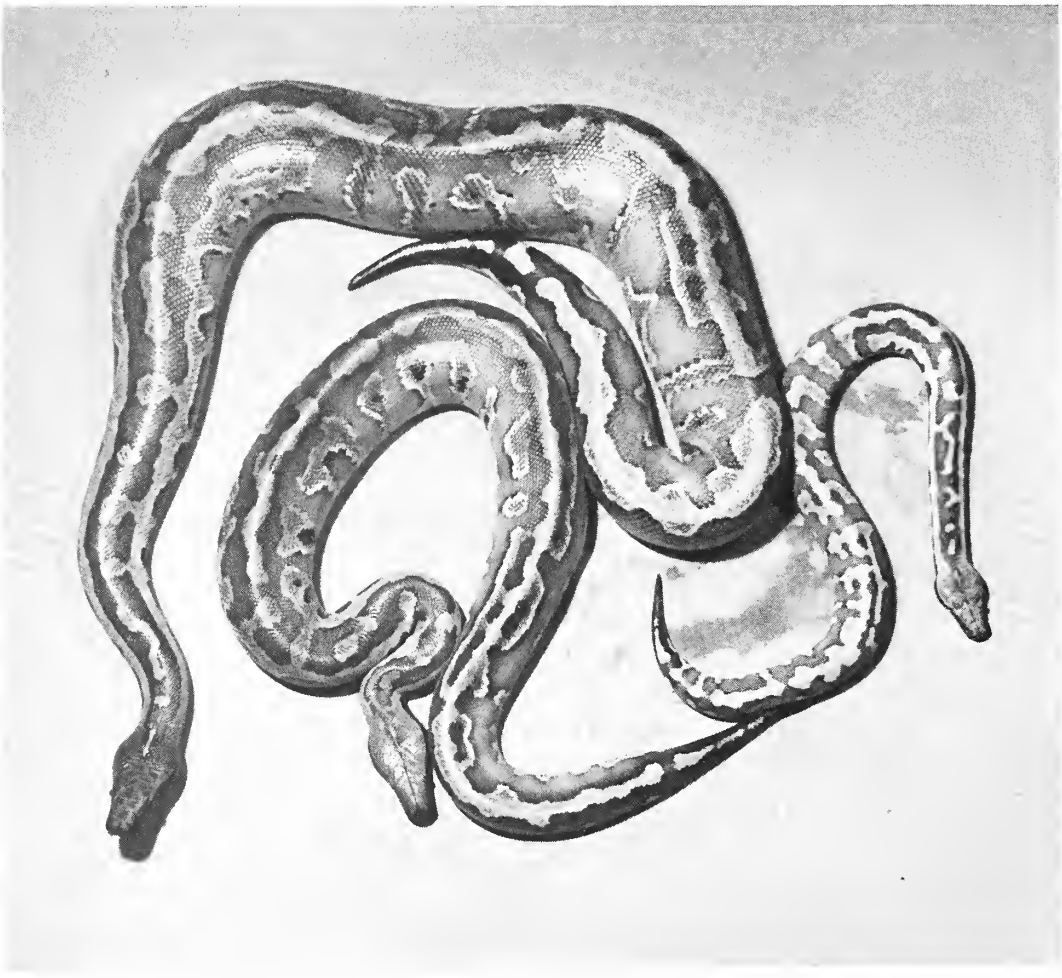
Plate I

Brooding *Python molurus bivittatus* (NYZS Photo). (Vinegar, Hutchison, Dowling).

Plate II

Three members of a single brood of *Python curtus* (William Meng Photo). (Vinegar, Hutchison, Dowling).

METABOLISM, ENERGETICS, AND THERMOREGULATION DURING BROODING OF THE GENUS PYTHON (REPTILIA, BOIDAE).



METABOLISM, ENERGETICS, AND THERMOREGULATION DURING BROODING OF THE GENUS PYTHON (REPTILIA, BOIDAE).

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3

A Preliminary Study on the Immobilization of the Asiatic Elephant *(Elephas maximus)* Utilizing Etorphine (M-99)

C. W. Gray[1]
AND
A. P. W. Nettashinghe[2]

(Plates I-II)

A preliminary study of M-99 for the immobilization of the Ceylonese elephant indicates the effective dosage is approximately twice that used in the African elephant, based on comparative body weights. A dosage rate of 7 to 8 mgs of M-99 was necessary to immobilize the Ceylonese elephant as compared to 5 or 6 mgs of M-99 for African elephants of almost double the weight.

Introduction

In 1966 a survey of Ceylon's elephant population was formally launched by the Office of Ecology and the National Zoological Park of the Smithsonian Institution. Grants for support of this project were awarded to Dr. H. K. Buechner and Dr. J. F. Eisenberg from the Smithsonian Foreign Currency Program and the World Wildlife Fund. As part of the project mission, we were charged with the responsibility of introducing the staff of the Wildlife Department of Ceylon to the use of M-99 as a possible method of tranquilizing elephants involved in crop damage. Utilization of M-99 would allow troublesome animals to be immobilized, tied, and transported to areas for either release in the wild or recruitment into the domestic elephant population which are still employed in lumbering operations in parts of Ceylon.

Since virtually no work had been undertaken previously to test the immobilizing effects of M-99 on the Asiatic elephant, Dr. C. W. Gray went to Ceylon in October 1967 in order to test the effectiveness of the drug, to determine a correct dosage for the Asiatic elephant, and to instruct personnel of the Wildlife Department of Ceylon in the use of pertinent equipment.

The following report is a summary of six attempted immobilizations on the Ceylon elephant. Our operation commenced on October 13, 1967, at Inginiyagala and terminated on October 22, 1967.

Study Area

The elephant immobilization studies were conducted in the Gal Oya region of eastern Ceylon on the shores of a large tank, or reservoir, formed by an earthen dam. The dam was constructed to provide water power for the generation of electricity and irrigation. The area surrounding the tank (Senanayake Samudra) is heavily wooded and extremely bushy. Every effort was made to locate an elephant in a situation where observation could be continuous from the time of injection until immobilization occurred.

Methods

All personnel were instructed in the assembly of the projectile dart and the handling of the CapChur rifle. Prior to departure from camp, two projectile syringes were prepared, each containing M-99 at a 4 mg dose. The unused darts were emptied at the end of each day in order to eliminate the possibility of product deterioration. Another change in technique was the reconstitution of immobilizing drugs in the field in order to eliminate completely the possibility of product potency loss. This also allowed dosage regulation, depending on the estimated height and weight of the elephant (unpublished data

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obtained through a cooperative study of captive elephants, made in 1967 with the staff and students of the University of Ceylon School of Veterinary Medicine, Kandy, permitted us to determine weight based on the estimated height).

ELEPHANT NO. 1

The animal was sighted in the afternoon moving southwest from the lake toward the forest. After a ten-minute stalk, the animal was shot in the left gluteal with a projectile dart containing 4 mg of M-99 in a 1-cc syringe from a distance of 20 yards, using a medium-charge cartridge. This dosage was selected due to its effectiveness in the immobilization of the African elephant as reported by Horthoorn and Bligh in their paper "The Use of a New Oripavine Derivative with Potent Morphine-like Activity for the Restraint of Hoofed Wild Animals" (*Research in Veterinary Science,* vol. 6, no. 3, July 1965). The dart did not stick in the animal and when it was recovered, the needle was bent at a 45° angle. When the elephant was shot, it wheeled, touched the ground with its trunk, picked up a stick, threw it into the air, and entered the brush, where visual contact was lost. The elephant continued moving for 15 minutes, and it was apparent that immobilization was unsuccessful.

ELEPHANT NO. 2

This eight-and-one-half-foot male Ceylonese elephant was seen on the lake shore bathing. It left the water and was shot inland with 5 mg of M-99, using a high-powered cartridge from a distance of 30 yards. The syringe was equipped with a heavy-duty needle with a barb. The animal was hit in the gluteal region, turned and entered a small, bushy area 150 yards distant. It made no effort to remove the dart. At 15 minutes after injection, a search was instituted, and the elephant was found leaning with its head against a tree, making paddling movements with the left front foot. There was no response to calling or breaking of small branches. When a larger branch was broken, the elephant turned toward the source of the noise, showing obvious narcosis. At 30 minutes after injection, it had returned to its original position, leaning against the same tree, and again responded to the noise stimulant. At 45 minutes after injection, it did not respond to the breaking of larger branches, but when struck on the trunk with a branch, it wheeled and charged; the tail was lifted but its trunk was limp. When approached at 70 minutes after injection, the elephant moved about ten steps toward the group. It picked up dirt, threw it over its back, indicating a functional trunk; and at this point, observation ceased.

On the following day we tracked the elephant from the same bushy area, over a log and down into the forest about a quarter-mile distant. There was no indication of any impairment of gait.

It should be noted that dosage rates of M-99 were being increased by 1 mg per elephant, in an effort to reach an immobilizing dosage.

ELEPHANT NO. 3

A lone male was sighted and stalk was started (Pl. I, fig. 1); the elephant was shot 30 minutes after being sighted. A 7 mg dose of M-99 was loaded into a 5cc projectile syringe, and a high-powered load was used from a distance of 35 yards. The dart bounced off the animal and the needle was bent in the shape of an S. Apparently the dart struck the pelvis rather than a muscle mass. The elephant went into the jungle about one-half mile distant, turned, and crossed an open space one-quarter mile further, and went into dense jungle. No further attempt was made to follow the elephant, and the last observation was made 25 minutes after the attempted injection.

ELEPHANT NO. 4

A male elephant, with an estimated height of six and one-half feet and weight of 6,000 pounds, was sighted. By means of a projectile syringe equipped with a heavy-duty needle, a 6 mg dose of M-99 was injected, using a low-powered load from a distance of 15 yards.

The elephant was under constant observation; at seven minutes post injection it sat down in a dog-like attitude, and as it turned, fell recumbent. At ten minutes post injection, the ear moved slowly but the trunk was flaccid. This animal was marked with paint on the right hip and measured. Since it was in direct sun, 12 mg of M-285 was injected intramuscularly into the right gluteal region (Pl. I, fig. 2).

At 20 minutes post injection, 4 mg of M-285 was given intravenously into the ear vein (Pl. II, fig. 3). Initial response was movement of the trunk tip, then ear, and front foot; one minute after the intravenous injection, the ear started to fan and foot movement increased; at four minutes, the head was raised. Activity continued, and at four and one-half minutes, the animal made its first attempt to rise; at five minutes, the hind foot was swinging as a prelude to rising (Pl. II, fig. 4); and at ten minutes, the animal was on all four feet and moving off with no impairment of gait. It was using the tip of its trunk in searching movements until it entered the jungle (Pl. II, fig. 5). No further observation was possible.

ELEPHANT NO. 5

A full-grown elephant, with an estimated height of seven and one-half feet and weight of

6,000 pounds, was injected with a 7 mg dose of M-99, using a low-powered charge from a distance of 20 yards. The animal moved about one and one-half miles, collapsed, and became immobile at 16 minutes post injection (the first pedal impediment was noticed at 12 minutes post injection). The head was raised one-half minute following its recumbency but there was no attempt to rise. This elephant was measured and found to be eight and one-half feet tall, making the weight approximately 7,000 pounds (data cited on page 52). It was marked on the right hip and the projectile syringe was removed. At 35 minutes after immobilization, the elephant received 14 mg of M-285 intramuscularly. At 48 minutes after injection, ear movement was noted; at 56 minutes, the elephant got up; and at 60 minutes, it took several steps and sat back down on the hind quarters. At this point it was dark, rain had started, and further observations were not made. Early the following morning, the elephant was seen near the same spot with a normal gait and unimpaired movement and activity.

Elephant No. 6

An eight-and-one-half-foot elephant was injected with an 8 mg dose of M-99, using a heavy-duty needle in the projectile syringe and a medium load, from a distance of 20 yards. Under continuous observation from time of injection, the elephant walked 300 yards and entered the jungle, turned to the left for approximately 25 yards, entered a small open clearing, and went down seven and one-half minutes after injection. Measurement of the animal confirmed the height estimate; it was then marked and the dart was removed. At 23 minutes after injection, the elephant received 16 mg of M-285 intravenously; at 43 minutes, it received 5 mg of M-285. Later it moved by pushing itself in a half-circle, and when it stopped, the elephant had a tree between the front and hind legs. It was able to move its ear, its trunk made searching movements, and it made an effort to rise. A 10 mg dose of M-285 was administered intramuscularly, and the elephant was left lying in the shade. There was a breeze blowing, the elephant's respirations were 9 to 10 per minute and no evidence of cyanosis was seen. Close examination revealed the presence of a perforation in the gluteal region, leading us to believe that this was the same elephant described as No. 2. Darkness prevented further observation, but the animal was seen two miles distant the next morning on the edge of the tank.

Observation

It seems from this limited study that to immobilize the Ceylonese elephant, the requirement of M-99 is approximately twice that for the African elephant, based on comparative body weight. Pienaar, Niekerk, and Young, in their report on the use of oripavine hydrochloride in the drug immobilization and marking of wild elephants in the Kruger National Park in describing the effect of M-99 on 31 bull elephants (*Journal for Scientific Research in the National Parks of South Africa,* No. 9, 1966), indicate an effective dosage level of M-99 combined with acetyl promazine totaling 7 mg or 8 mg for African elephants weighing close to 15,000 pounds, and of 5 mg or 6 mg for elephants weighing from 6,000 to 12,000 pounds.

Acknowledgments

The authors gratefully acknowledge the efforts of Warden Lyn DeAlwis, Wildlife Department of Ceylon, who furnished the necessary guidance and permission to execute the immobilization attempts. We were further assisted by Dr. S. Attapattu, Veterinarian, Zoological Gardens at Dehiwala. The immobilization team was composed of Ranger B. Ekanayaka and Mr. Melvin Lockhart. Mr. G. McKay and Mr. Anil Jayasuriya assisted in the photography. Field operations were directed by Dr. John F. Eisenberg.

EXPLANATION OF PLATES

PLATE I

FIG. 1. Elephant stalk in progress. (All photographs by Dr. F. Kurt)

FIG. 2. Intramuscular administration of M-285.

PLATE II

FIG. 3. Intravenous injection of M-285 using the ear vein.

FIG. 4. Sitting position immediately prior to regaining feet.

FIG. 5. Elephant now ambulatory showing no evidence of narcosis.

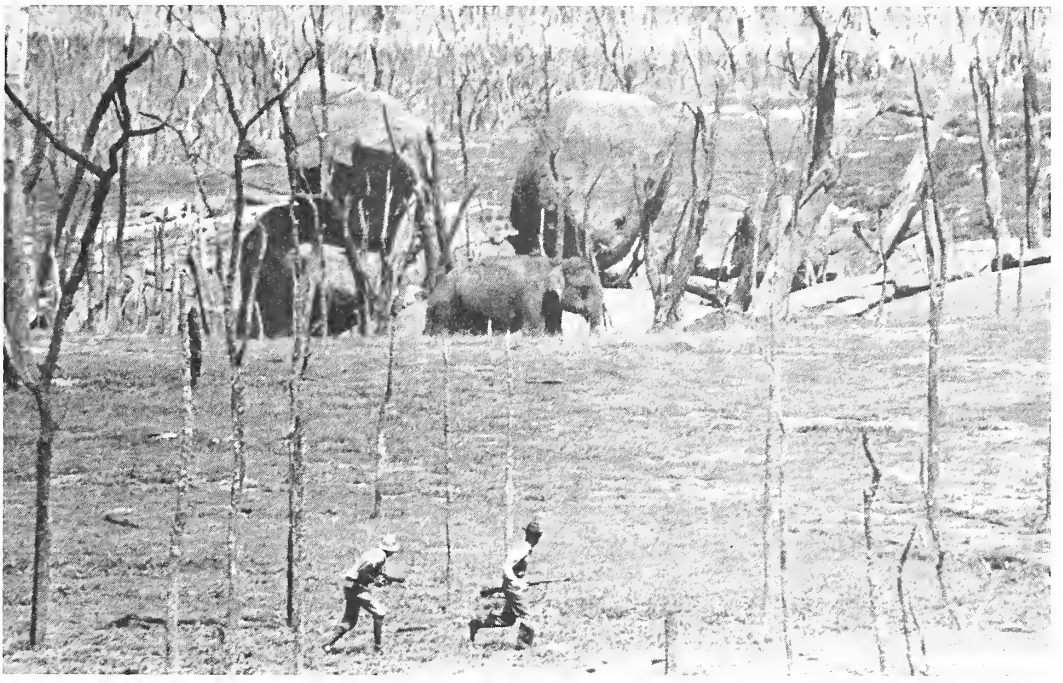

FIG. 1

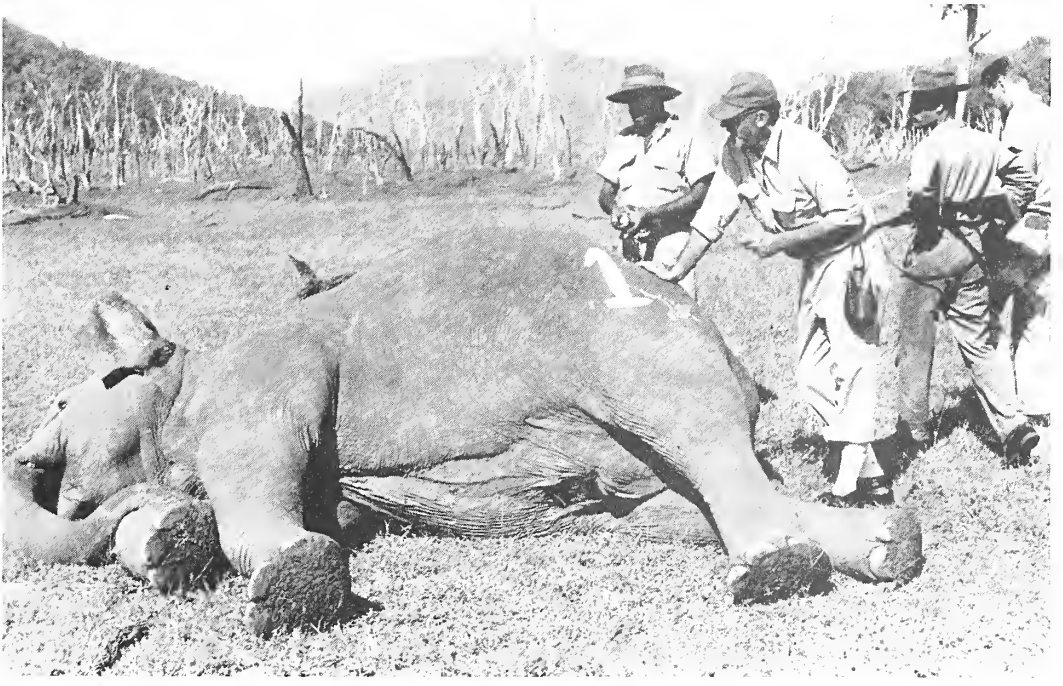

FIG. 2

A PRELIMINARY STUDY ON THE IMMOBILIZATION OF THE ASIATIC ELEPHANT (ELEPHAS MAXIMUS) UTILIZING ETORPHINE (M-99)

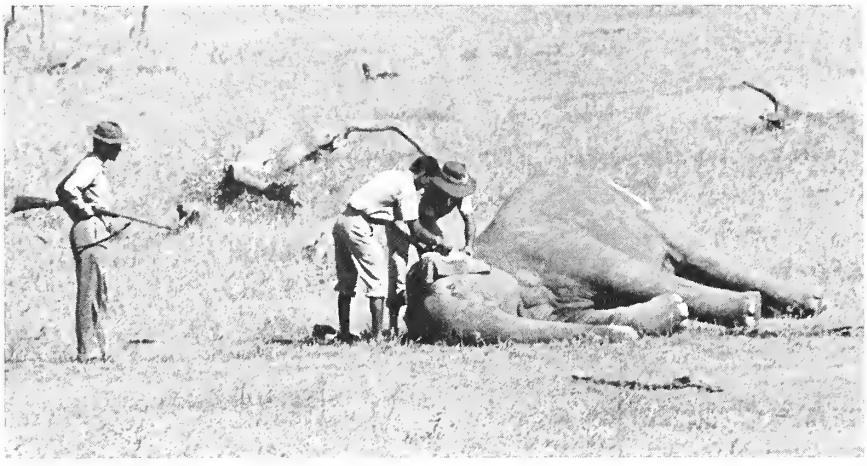

FIG. 3

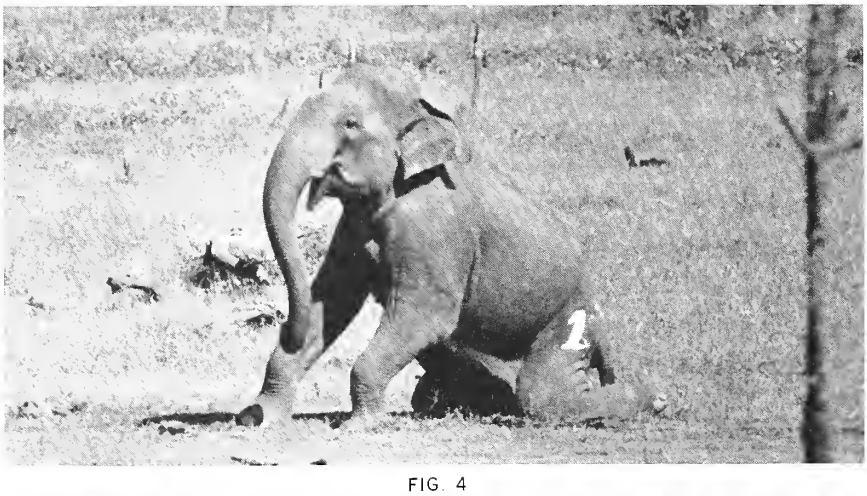

FIG. 4

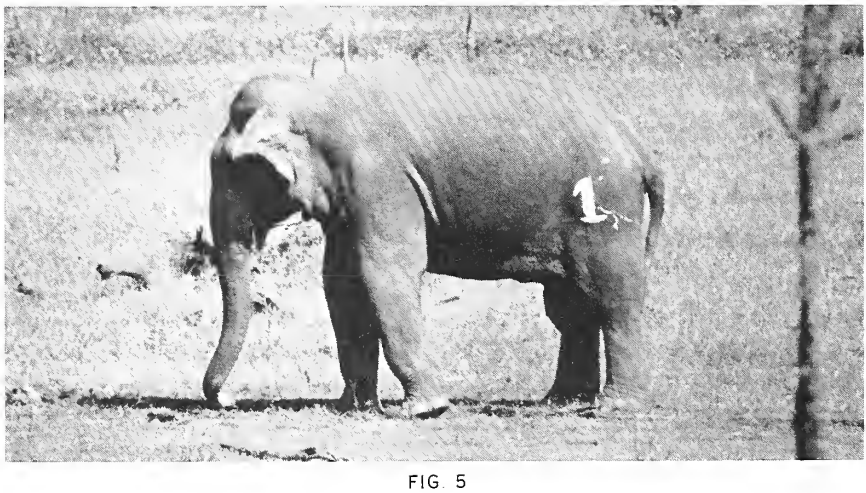

FIG. 5

A PRELIMINARY STUDY ON THE IMMOBILIZATION OF THE ASIATIC ELEPHANT (ELEPHAS MAXIMUS) UTILIZING ETORPHINE (M-99)

4

Epizootics in Yellowtail Flounder, *Limanda ferruginea* Storer, in the Western North Atlantic Caused by *Ichthyophonus*, an Ubiquitous Parasitic Fungus

George D. Ruggieri, S.J.,[1] Ross F. Nigrelli,[1] P. M. Powles,[2] and D. G. Garnett[3]

(Plates I-X; Text-figure 1)

Yellowtail flounders (*Limanda ferruginea*) were collected from several areas off Nova Scotia and analyzed for *Ichthyophonus*. The infection was confined to flounders from the Sable Island Bank and Western Bank. Extensive lesions caused by the fungus were present in the heart, liver, kidney, spleen, gastrointestinal tract, and body musculature. The gills, gall bladder, brain, and testes were mildly infected. All developmental stages of the fungus were observed, although in most histological sections the fungi appear as "resting" or as stages in germination and hyphal development. An analysis of infected fish indicates no relationship to sex and that yellowtails in the size range from 24 cm to 40 cm are more heavily infected. The pathological manifestations of the fungus in the various organs and their significance are discussed.

Introduction

Ichthyophonus, the cause of systemic mycosis in fishes, is characterized macroscopically by the presence of single, multiple, or confluent whitish cyst-like lesions in the viscera and in other parts of the body. The disease has been reported in a wide variety of feral and captive fishes of fresh water, brackish water, and marine habitats in cold, temperate, and tropical parts of the world. In every case the causative organism has been identified as a single, pleomorphic species, *Ichthyophonus hoferi* Plehn and Mulsow, 1911. Whether or not a single species of fungus is indeed responsible for the disease in all host species remains to be established. This fungus had also been referred to as *Ichthyosporidium hoferi* (Plehn and Mulsow) Pettit, 1911. The generic name *Ichthyosporidium* Caullery and Mesnil, 1905, is now restricted to certain protozoan parasites of fishes and marine invertebrates in the order Haplosporidia, class Sporozoa (Sprague, 1965).

Most of the literature on *Ichthyophonus* deals with sporadic cases. However, recurring incidences in epizootic proportions have been reported in: the sea herring (*Clupea harengus*) in the western North Atlantic (see Sindermann, 1961, 1966, and 1970 for reviews); the mackerel (*Scomber scomber*) in the eastern North Atlantic (Sproston, 1944); rainbow trout (*Salmo gairdneri*) in hatcheries in the western United States (Rucker and Gustafson, 1953); and, more recently, yellowtail flounder (*Limanda ferruginea*) in the western North Atlantic, especially from western Sable Island Bank (Powles, Garnett, Ruggieri and Nigrelli, 1968).

The present investigation deals with the pathology of the disease in the yellowtails. It also includes a further analysis of the distribution of the infection in fish collected in 1967 from several areas off Nova Scotia, including the site where the epizootic first occurred in 1966.

Material and Methods

The yellowtail flounders were collected from the Gulf of St. Lawrence, Banquereau, Middle Ground, Western Bank, and Sable Island Bank (text-fig. 1.) The sampling routine was as follows: 200 flounders were collected from each area; from each group a sub-sample of 50 specimens preserved in formalin was shipped to the Pathology Laboratory of the Osborn Laboratories of Marine Sciences, New York Aquarium, for detailed microscopic examination of the infection. All fish were examined and the organs of those showing macroscopic evidence of the lesions

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were embedded in paraffin, sectioned between 3 and 5 microns, and stained with Harris' hematoxylin-eosin, Periodic acid Schiff, Bauer's chromic acid Schiff, Gram Weigert, Mallory phosphotungstic acid hematoxylin, Masson's trichrome stain, and Mallory's modification of the Azan staining method.

Observations

1. *Incidence and distribution.* Text-figure 1 is a map showing the localities from which the samples were taken. The infection was confined to the Sable Island Bank and the Western Bank, with incidences of 25 percent and 57.4 percent, respectively. The incidence seems to decrease eastward; e.g. yellowtails from south of Sable Island show only a 2.8 percent incidence. Fish from all other areas, including the Gulf of St. Lawrence, appeared to be free of the infection, at least macroscopically. The evidence indicates that the stocks from Sable Island, Banquereau, and St. Lawrence do not mix. It is suggested that the isolation of the disease to the Sable Island area may be related to higher bottom temperatures. Whether or not this is a significant factor remains to be determined.

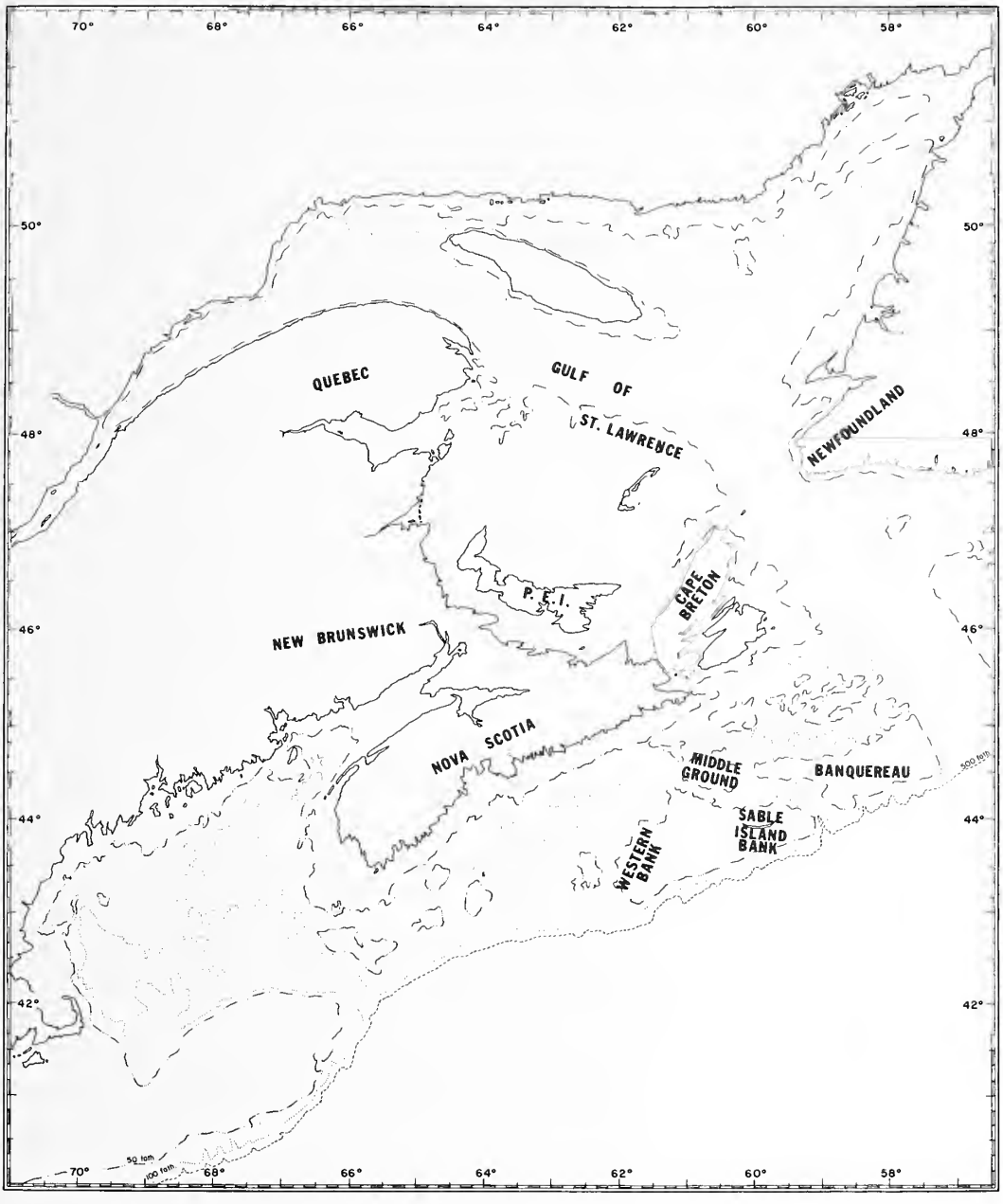
An analysis (Table 1) of the fish collected from Western Bank and Sable Island Bank shows the incidence of the disease in relation to size and to the intensity of the infection as seen macroscopically in the liver, heart, kidney, and gastro-intestinal tract.

There is no apparent relationship as to sex, although there is some evidence that males appear to be more susceptible. Yellowtails in the size range from 24 cm to 40 cm are more susceptible; the disease appears to be present in proportion to the most abundant sizes, which fits in with the observation that there is little or no size segregation in this species. Further, a preliminary analysis (Garnett and Powles) suggests that there is no significant length-weight (coefficient of condition) relationship between diseased and normal fish. A growth and mortality study should be done to make such observations significant. It should be emphasized that the absence of obvious lesions in both younger and older yellowtails as shown in Table 1 does not indicate that they are entirely free of the fungi, since it is possible that deep-seated and isolated "cysts" may be present, e.g. in the brain.

2. *Parasite.* Because of certain morphological and pathological characteristics, the causative organism was identified as a phycomycete of the genus *Ichthyophonus.* Whether or not *Ichthyophonus hoferi* is the specific agent responsible for the mycosis in the yellowtails can only be determined after a more thorough study of the organism under cultural conditions, by experimental infections, and by comparative analysis with isolates from other freshwater and marine hosts. The use as a diagnostic procedure of measurements and descriptive terms for the various stages seen in tissue sections is at present meaningless. All stages in the development are identifiable, but in most sections the fungi appear as "resting," often thick-walled cysts (e.g., Pl. III, fig. 6), or as stages in germination and hyphal development (Pl. III, fig. 5, and Pl. VII, fig. 13) generally characteristic of this type of mycotic infection in fishes. The presence of condia-like bodies in kidney tissue (Pl. V, fig. 10) is interesting; similar spores have been reported by Sproston (1944) in fungal cultures made from mackerel and their development was also referred to by Reichenbach-Klinke and Elkan (1965) for *Ichthyophonus* infection in other marine fishes, but not the herring.

3. *Pathology.* Extensive lesions caused by the fungus were present in the liver (Pl. I-III, figs. 2-6), kidney (Pl. IV-V, figs. 7-10), spleen (Pl. VI, fig. 11), body musculature (Pl. VI, fig. 12; Pl. VII, fig. 13), heart Pl. I, fig. 1; Pl. VII, fig. 14; and Pl. VIII, fig. 15), gastro-intestinal tract (Pl. VIII-X, figs. 16-19) and to a lesser degree in the gills, gall bladder, brain, and testes (Pl. X, fig. 20). Lesions were not present in the ovary. The absence of the infection in the ovary and the relatively mild pathology in the testes indicates that the disease may have very little effect on potential reproductive ability.

As is well known for mycotic diseases, no one tissue change is entirely pathognomic of the fungus infection in fish. In the yellowtails, the lesions are generally characterized by the absence of classical inflammatory responses. However, the lesions involve a great deal of necrosis, especially in those areas showing activities associated with germination and hyphal growth. In areas where numerous "resting cysts" are present, the fungi are relatively inert and behave as foreign bodies, i.e. they become surrounded by histiocytes (epithelioid elements), typical of many granulomas (Pl. V, fig. 9; Pl. VI, fig. 11; and Pl. VIII, fig. 16), or by connective tissue fibers (Pl. II, figs. 3, 4; Pl. IV, fig. 7; Pl. VII, fig. 14; Pl. VIII, fig. 15; Pl. X, fig. 20). This is not surprising since similar reactions have been reported for certain mycoses in humans and other mammals. In relatively heavy infections, atrophy effects due to pressure with concomitant necrosis are quite evident in the parenchymal tissue, e.g. liver (Pl. II, figs. 3, 4) and kidney (Pl. IV, figs. 7, 8, and Pl. V, fig. 9), and in the heart (Pl. VII, fig. 14, and Pl. VIII, fig. 15) and body musculature (Pl. VI, fig. 12).



TEXT-FIGURE 1. Map of Western North Atlantic. Yellowtail flounders were collected from the Gulf of St. Lawrence, Banquereau, Middle Ground, Western Bank, and Sable Island Bank.

TABLE 1. MACROSCOPIC ANALYSIS OF FISH FROM WESTERN BANK AND SABLE ISLAND BANK.

Total length in cm	*Total number fish*	*Number Infected*	*Intensity of Infection*			
			Liver	*Heart*	*Stomach Intestine*	*Kidney*
16	4	0				
17	3	0				
18	2	0				
19	2	0				
20	5	0				
21	3	0				
22	5	1	+			
23	2	0				
24	2	1	++++		+	
25	2	1	+++	+++	+	
26	7	3	++++	++	+	++++
			+++	+++	+++	+++
			+++	++	+	++
27	7	1	++++	+++		
28	6	2	++++		+++	+++
			++	+++		+++
29	4	3	+++	+++	+	+
			+++	+++		++
			++	+++	+	++
30	8	5	+	+		
			++++	+++	+	+++
			+	++		
			++++	++++	++++	++++
			++++		+++	++++
31	6	1	+		+	+
32	3	2	+++		+	+
			++	++		
33	8	5	++++	++++	++++	++++
			++++	++++		
			+++	++	+	+++
			++	+	+	+
			+		+	
34	3	1	++	+++		
35	5	4	+++	+++	+++	+++
			+++	+++	+	++
			+++	+++	+	+
			+			
36	4	2	++++	++++	+	++
			+			+
37	5	3	++++	+++	++	+++
			+++	+++	+++	+++
			++	+++	+	+
38	1	0				
39	1	1	++			
40	2	2	+++	+++		+++
			+			
41	1	0				
43	1	1	+++			
44	2	0				
46	2	1	+	+++		

\+ = less than 5 small cyst-like lesions
++ = 6-10 small cyst-like lesions
+++ = 10-20 small cyst-like lesions or 1 large confluent patch
++++ = over 20 small cyst-like lesions or several large confluent patches

Discussion and Summary

The yellowtail flounder *(Limanda ferruginea)* is the third major species of North Atlantic food fish to be affected by recurring epizootics caused by the fungal parasite *Ichthyophonus,* with incidences ranging from 2.8 percent to 57.4 percent. The other two species, referred to above, are the Atlantic herring and mackerel, with incidences ranging from 2-80 percent (average 25 percent) and 38-70 percent respectively (Walford, 1958). The disease in the yellowtails appears to be limited to populations in the area of Sable Island off the coast of Nova Scotia but the effects of the infection on growth and on mortality rate are at present unknown. The absence of striking evidence of mass mortalities or fluctuations in the populations of this species in the epizootic regions is surprising. The damage to such vital organs as the heart, liver and kidney is so extensive that there can be no question that homeostasis is affected to the extent that many must succumb directly to the infection, or are made so weak that they become easy prey, or are readily killed off by any drastic change in the physical and chemical characteristics of the environment.

The absence of the disease in yellowtails in the Gulf of St. Lawrence is puzzling since this is one of the areas in which epizootics in the herring have been reported almost in a cyclic fashion since 1900 (Sindermann, 1970).

Acknowledgments

The authors acknowledge the help of the Fisheries Research Board of Canada and especially the crew of the "A. T. Cameron."

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EXPLANATION OF PLATES

Plate I

Fig. 1. Gross manifestation of lesions on the heart of a yellowtail caused by the phycomycete *Ichthyophonus.*

Fig. 2. Numerous cysts of the parasite in the liver.

Plate II

Fig. 3. Liver showing numerous "resting cysts." Note extensive damage and development of connective tissue. Azan.

Fig. 4. Area of the liver showing necrosis and distortion of the parenchymal architecture. Azan.

Plate III

Fig. 5. Germination and hyphal development in liver with extensive necrosis of the parenchyma. Note absence of typical inflammatory reaction. Resting cysts strongly PAS positive.

Fig. 6. A typical resting cyst with multi-nucleated plasmodium in the liver. Hematoxylin-eosin.

Plate IV

Fig. 7. Extensive infection in the kidney with damaged tubular elements. Bauer's.

Fig. 8. Area of infected kidney with extensive necrosis. Gram Weigert.

Plate V

Fig. 9. Granuloma-like reaction around a "resting cyst" in the kidney. Note the massing of histiocytes and degenerative changes of tubules due to pressure effects. Azan.

Fig. 10. Conidial elements of *Ichthyophonus* seen in the kidney. Cysts surrounded by histiocytes. Hematoxylin-eosin.

Plate VI

Fig. 11. Granulomatous reaction around "resting cysts" in the spleen. Masson's.

Fig. 12. Infection in the body musculature showing damage characteristic of Zenker's degeneration. Mallory's.

Plate VII

Fig. 13. Details of stages of germination in the muscle pathway. Hematoxylin-eosin.

Fig. 14. Myocardial degeneration caused by the fungal infection. Note extensive connective tissue development resulting in fibroid swelling. Azan.

Plate VIII

Fig. 15. Germination of fungus in myocardium causing necrosis of the heart muscle fibers; granulomatous lesions were also seen in the pericardium. Hematoxylin-eosin.

Fig. 16. Submucosa of the stomach showing the pressure effects of the parasite and the massing histiocytes on the mucosa. Masson.

Plate IX

Fig. 17. Nest of fungal elements in the submucosa of the large intestine; the small intestine was equally infected. Hematoxylin-eosin.

Fig. 18. Cysts in the mucosa of the large intestine. The basement membrane has been penetrated. PAS.

Plate X

Fig. 19. Multi-nucleated cysts in a capillary of the submucosa of the stomach. Hematoxylin-eosin.

Fig. 20. A fungal cyst in the testis. No pathological changes were noted in spermatogonia or spermatids. Hematoxylin-eosin.

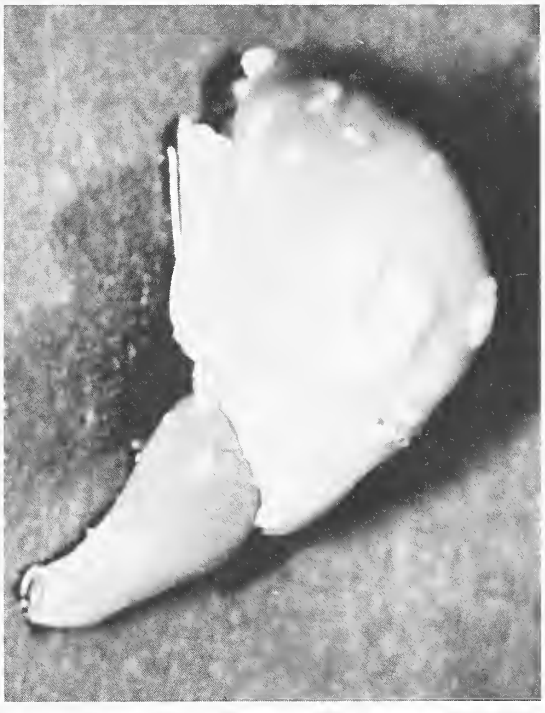


FIG. 1

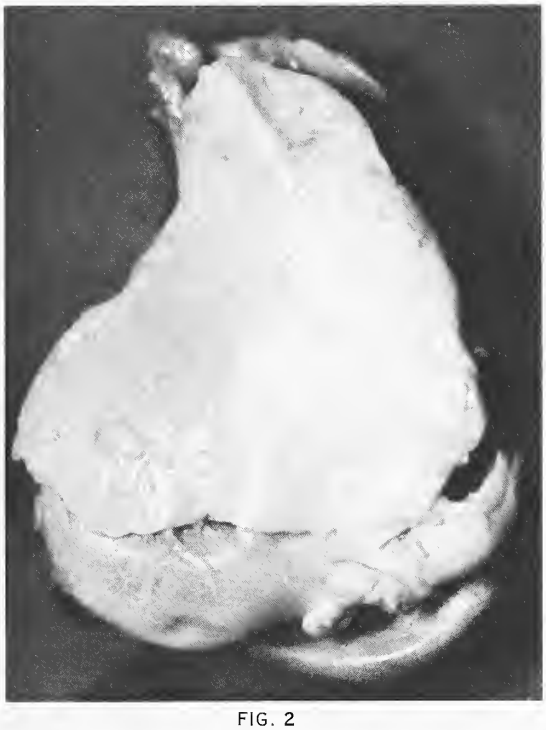


FIG. 2

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

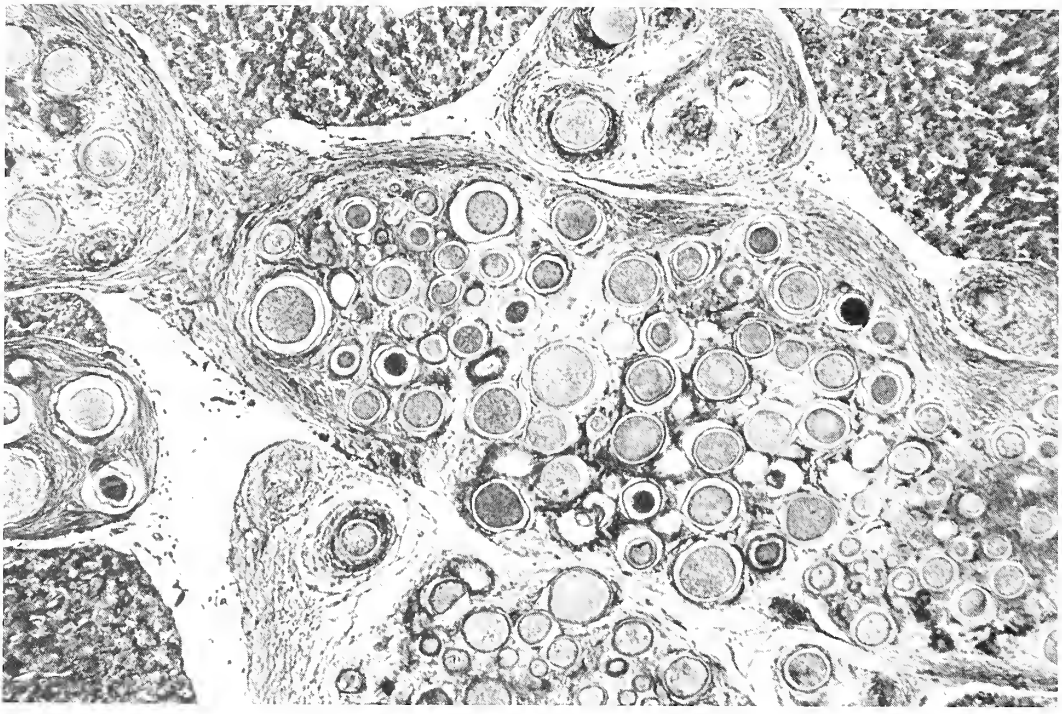


FIG. 3

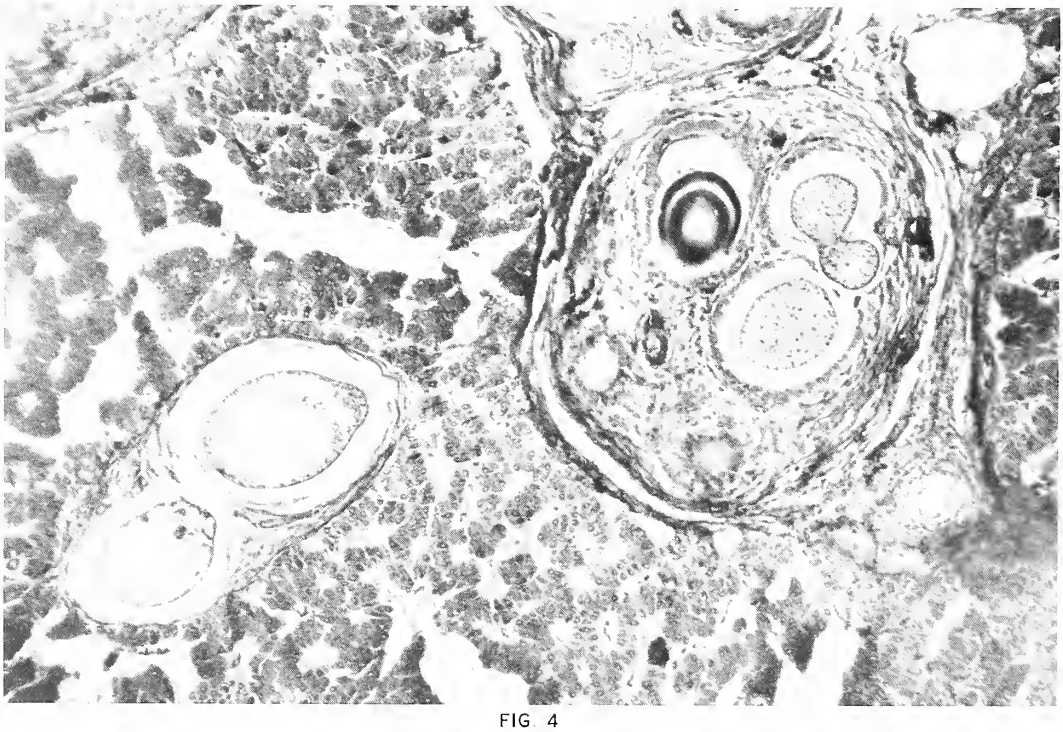


FIG. 4

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

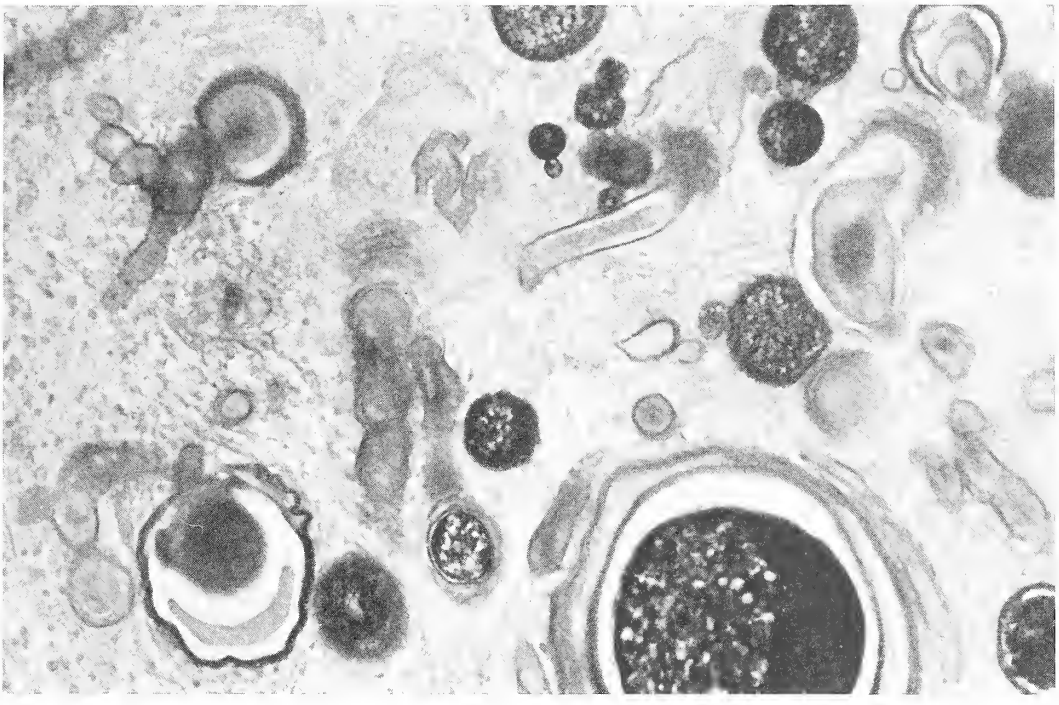


FIG. 5

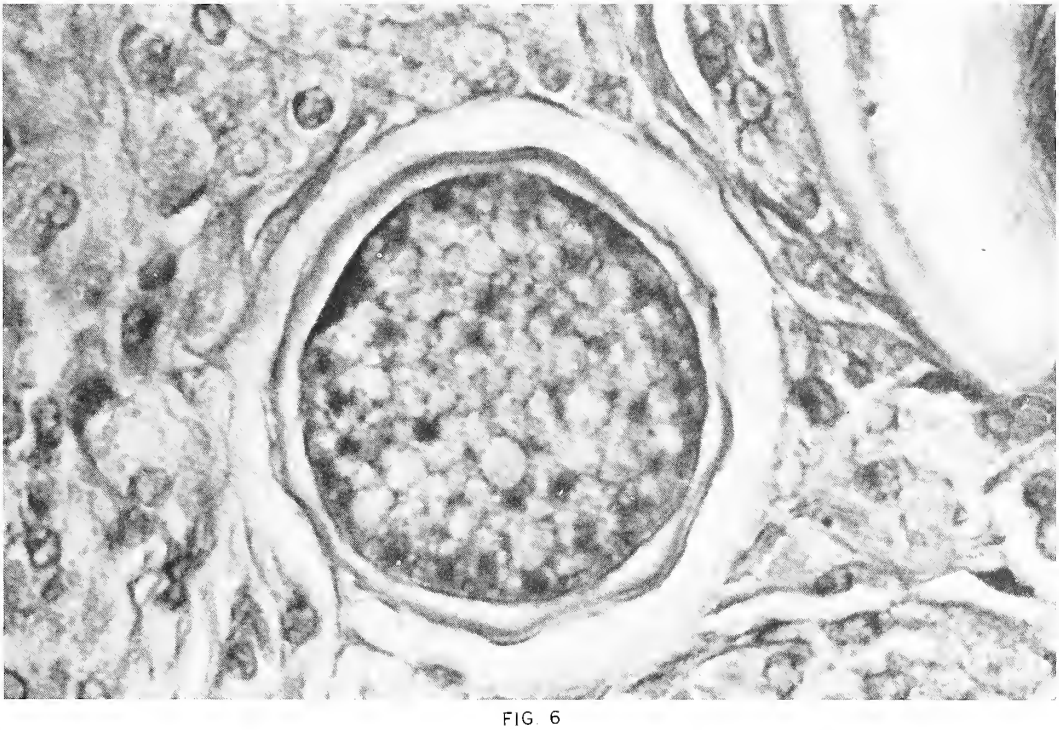
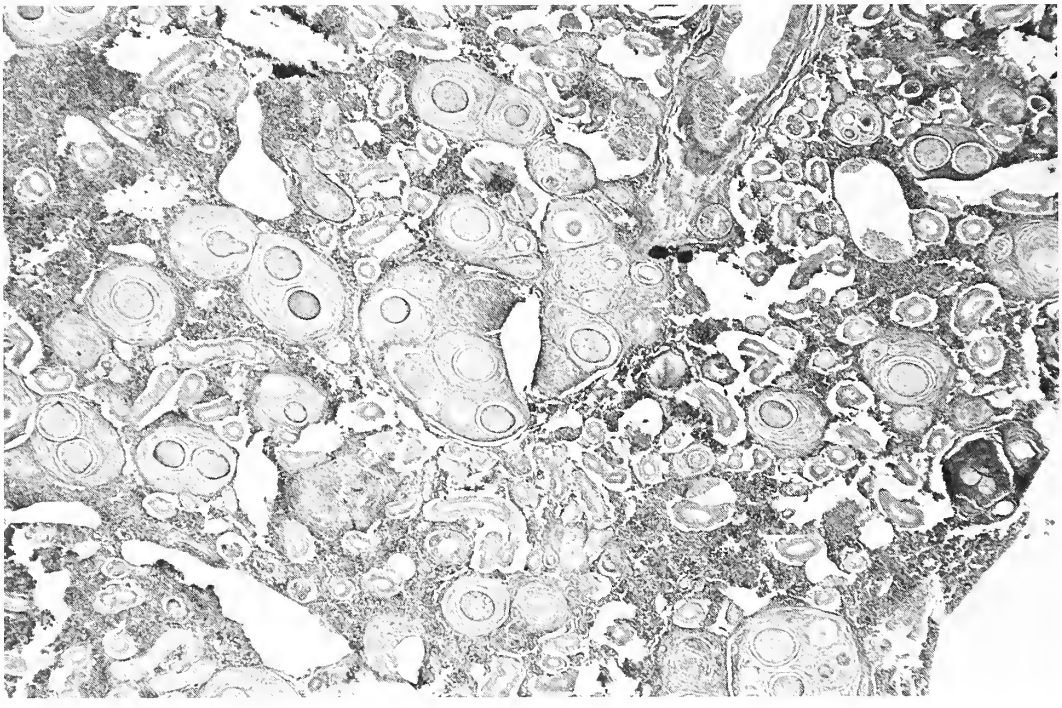


FIG. 6

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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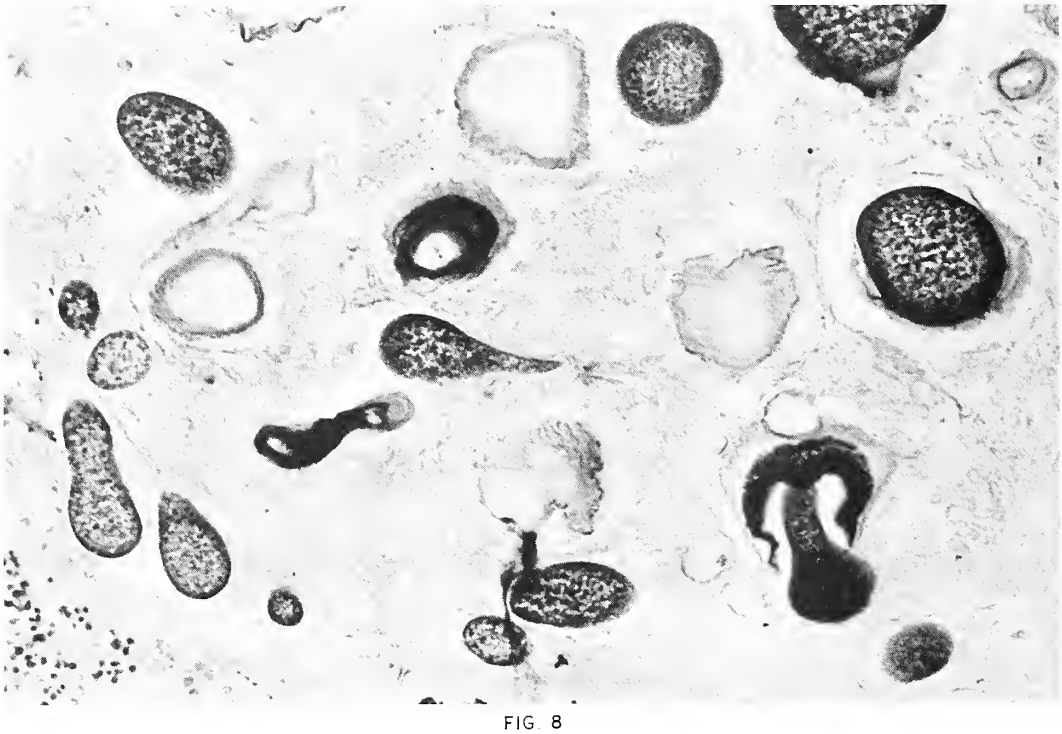


FIG. 8

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

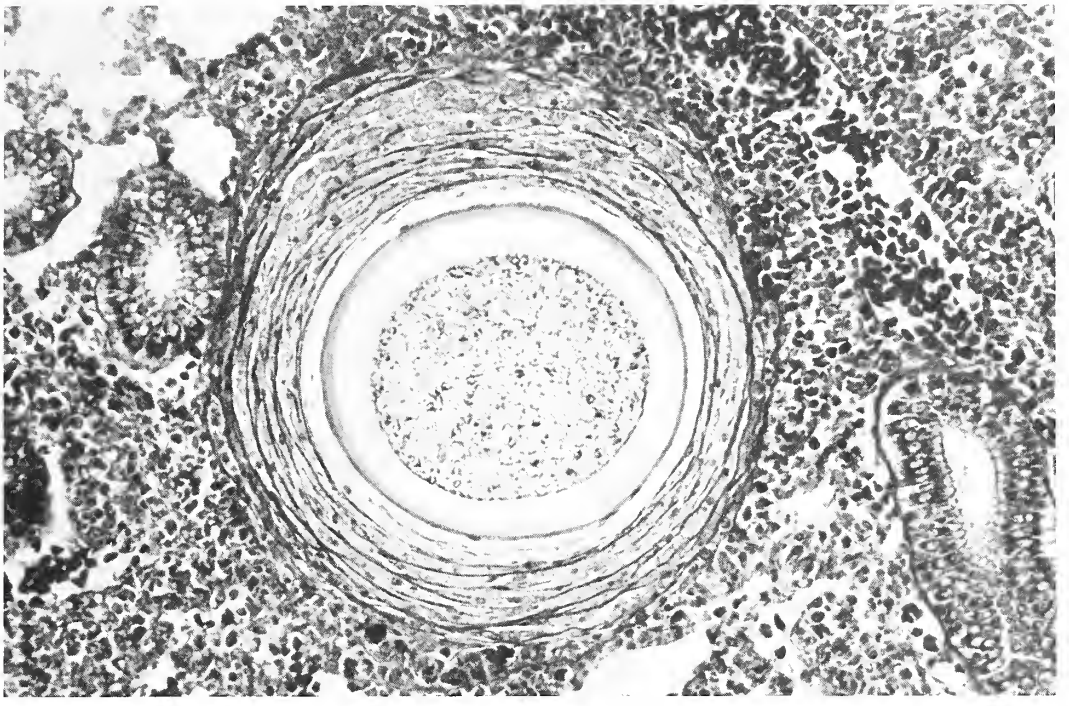


FIG. 9

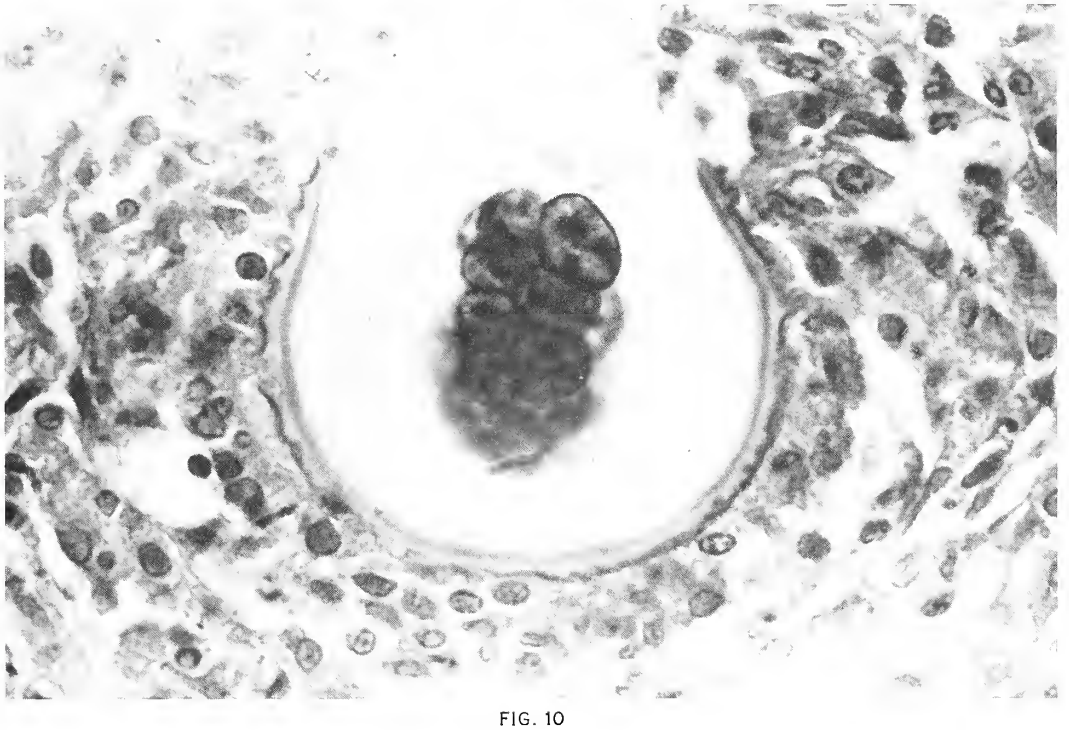
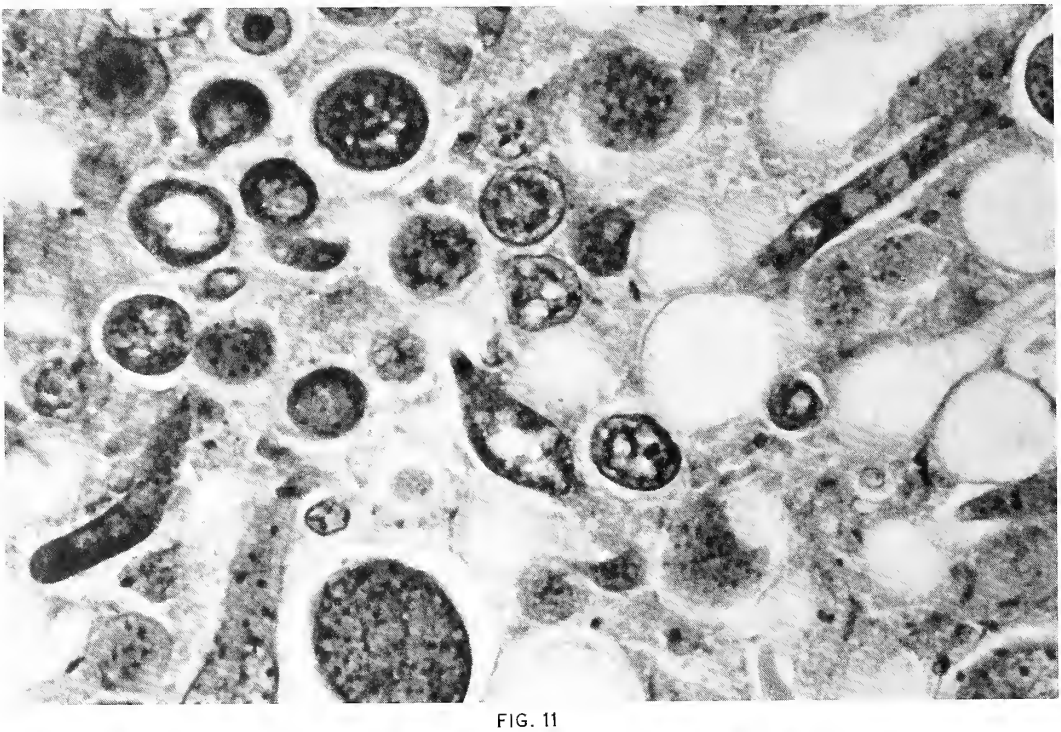


FIG. 10

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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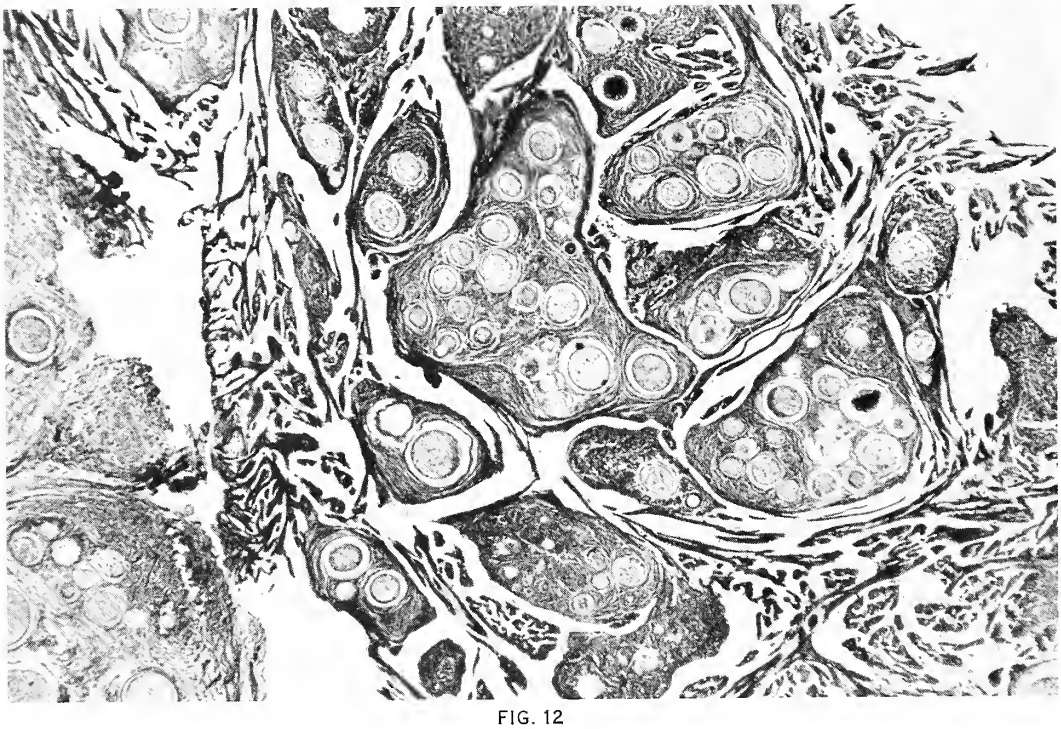
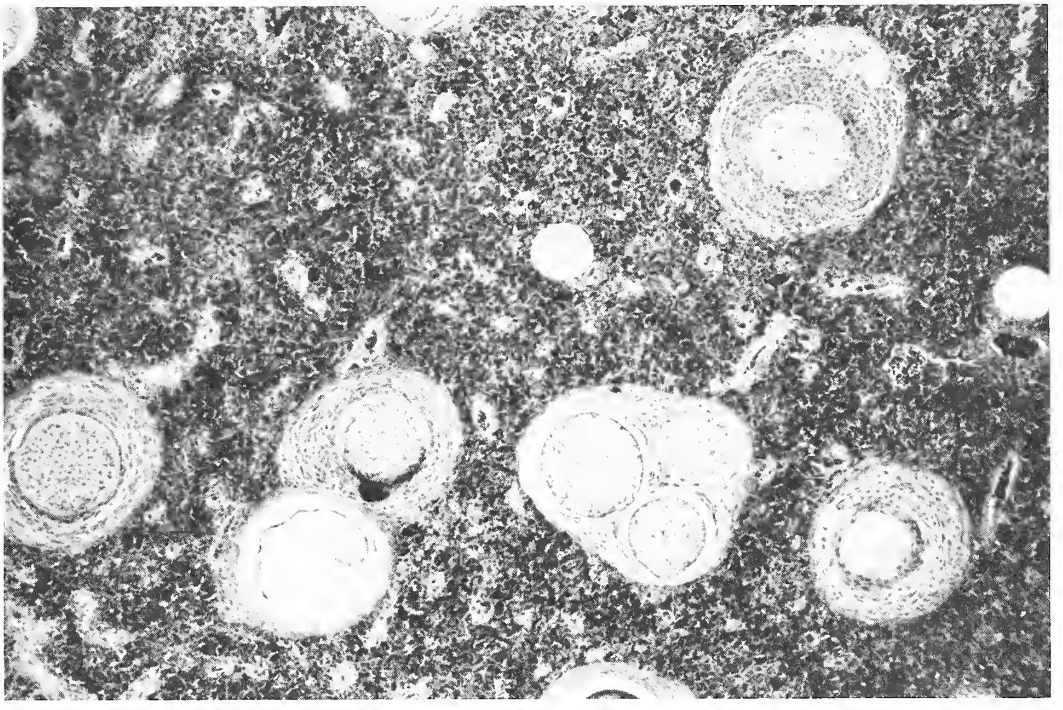


FIG. 12

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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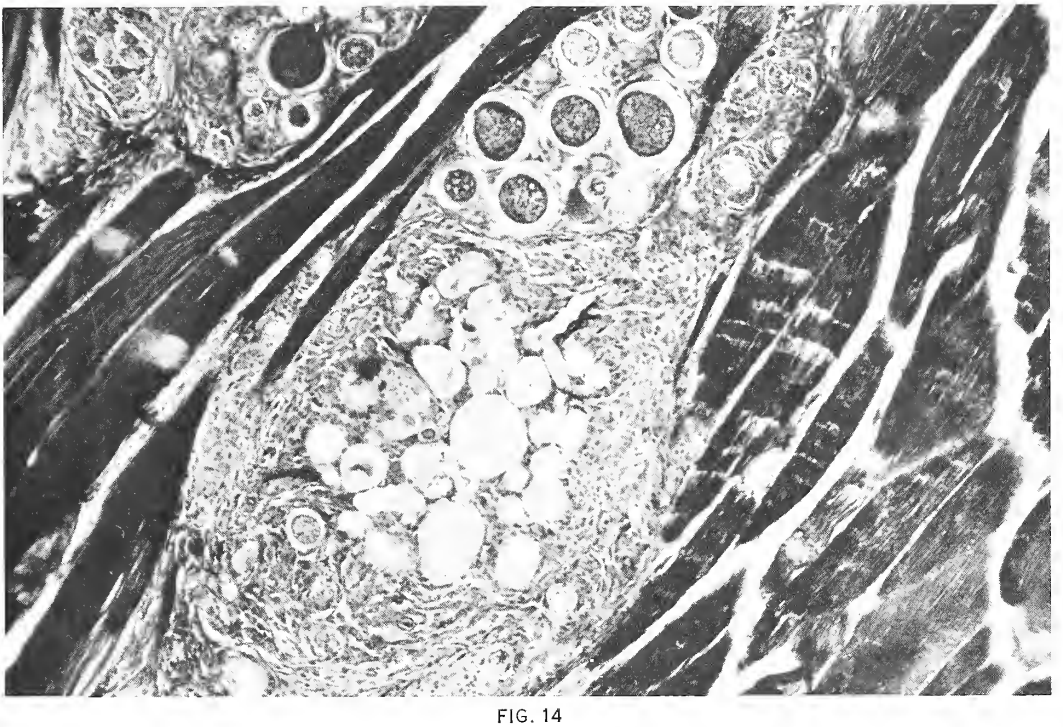
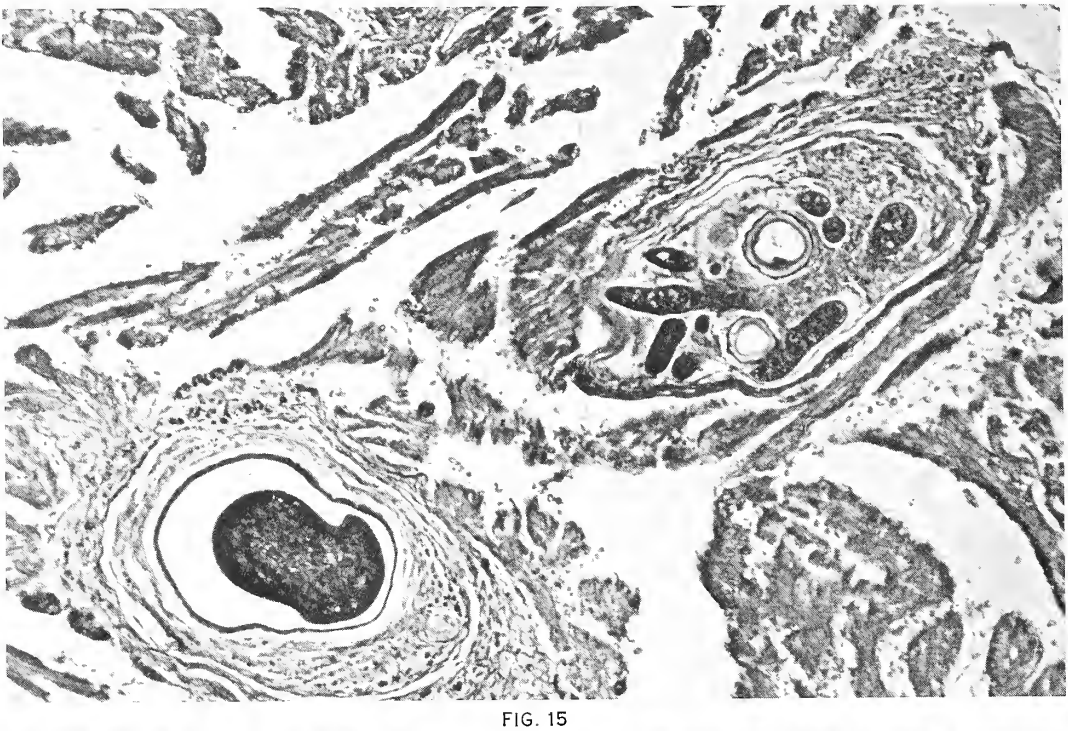


FIG. 14

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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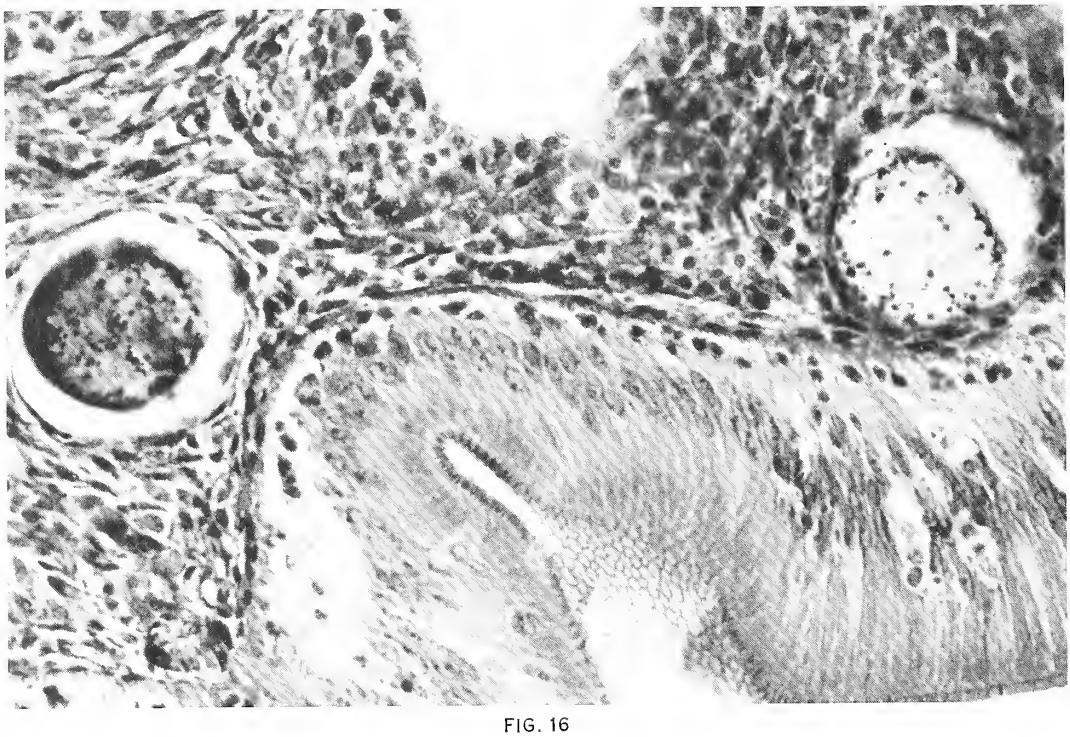


FIG. 16

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

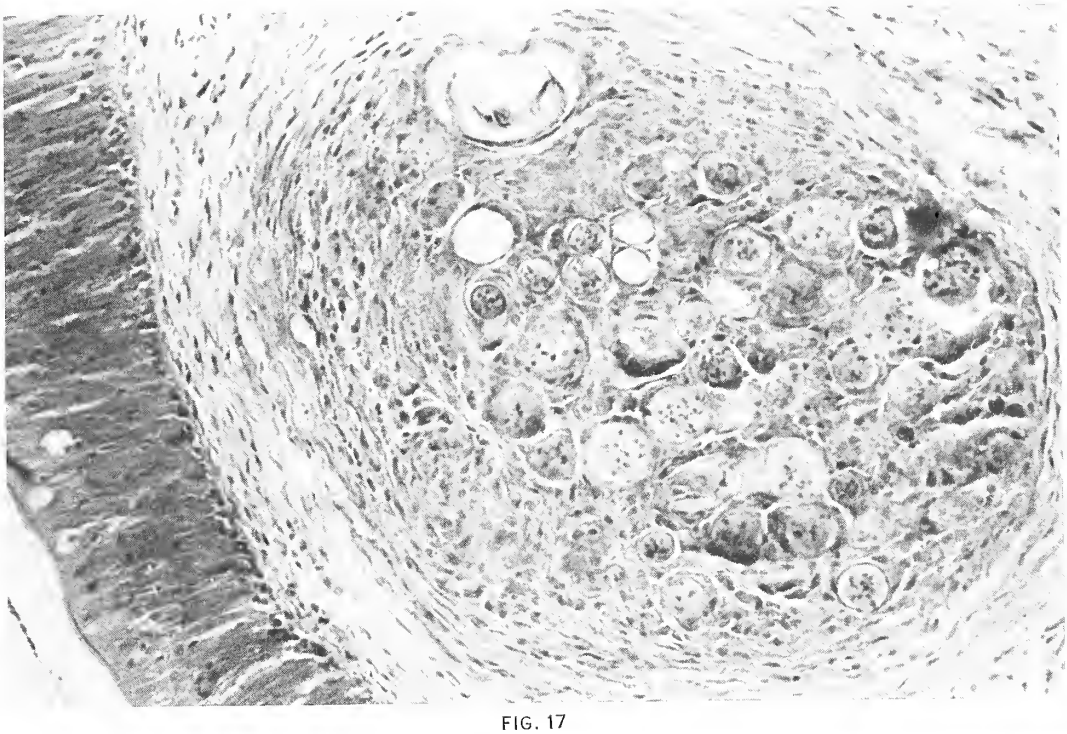


FIG. 17

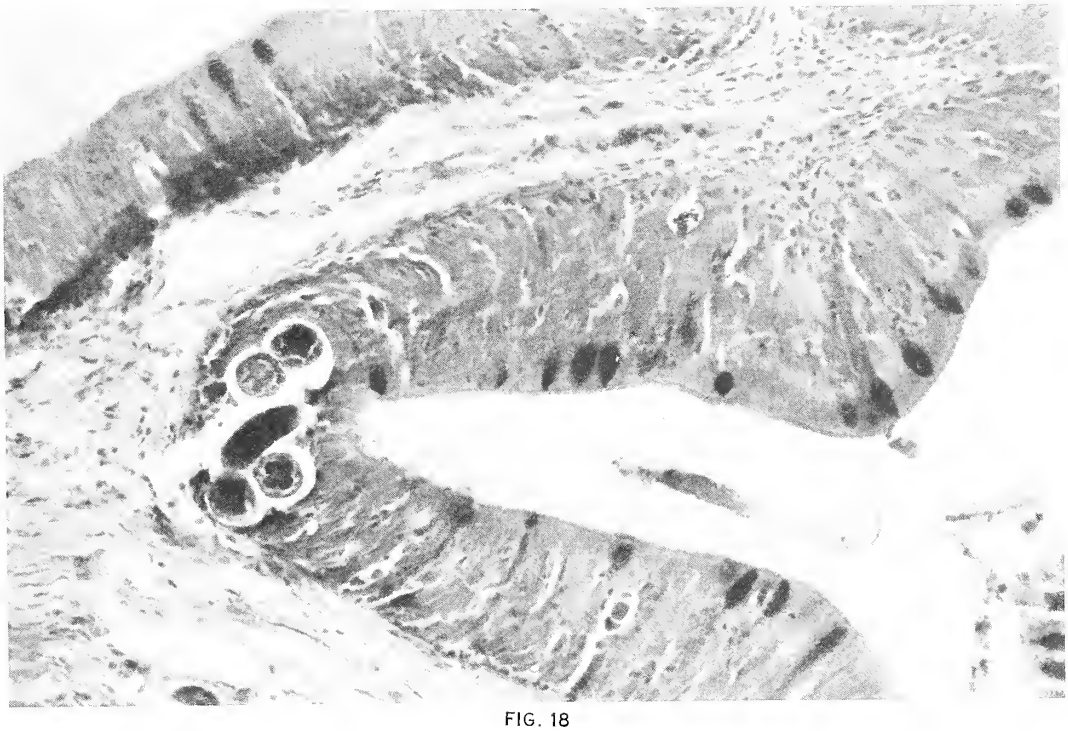
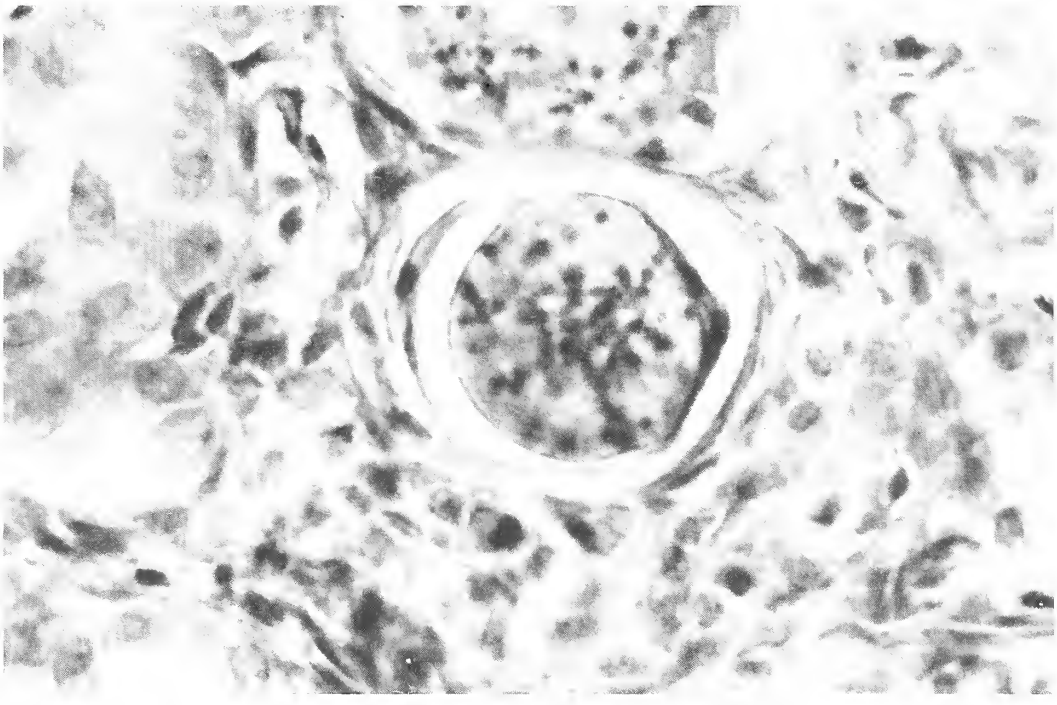


FIG. 18

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS



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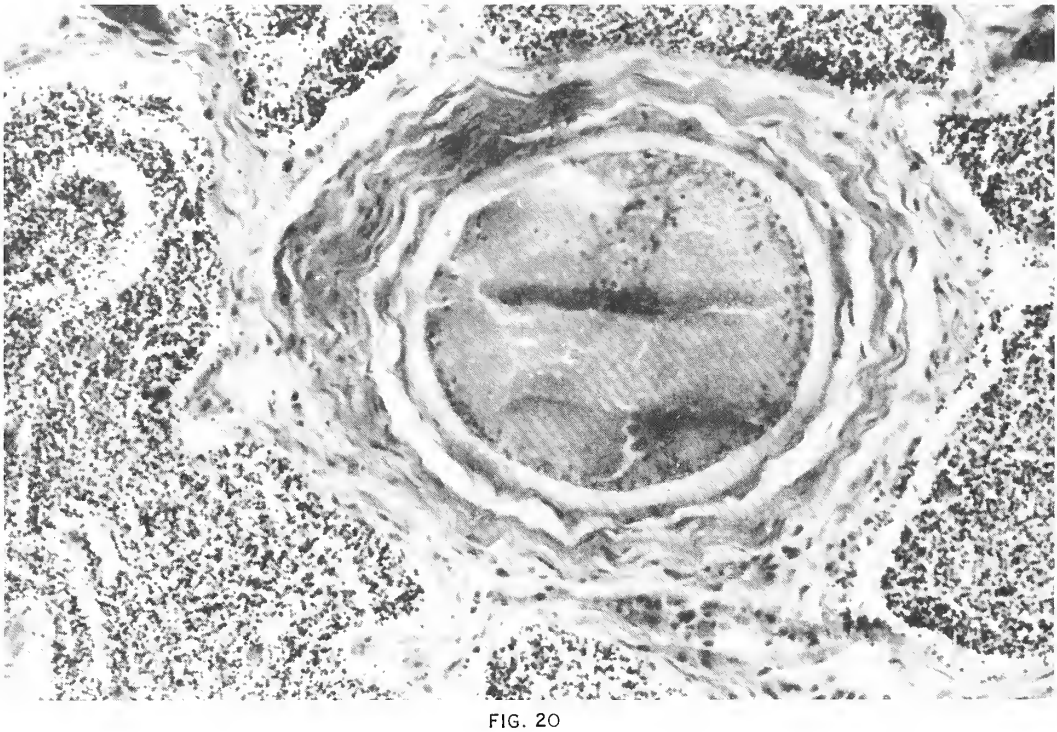

FIG. 20

EPIZOOTICS IN YELLOWTAIL FLOUNDER, LIMANDA FERRUGINEA STORER, IN THE WESTERN NORTH ATLANTIC CAUSED BY ICHTHYOPHONUS, AN UBIQUITOUS PARASITIC FUNGUS

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5

Gonadotrophin in the Urine of a Pregnant Indian Elephant—A Case Report

Eho Fujimoto,[1] Natsuki Koto,[1] Tatsuo Imori,[2] and Sanenori Nakama[2]

(Plates I-II)

In 1963, at Takarazuka Zoo, Japan, a young female Indian elephant became pregnant, and in May, 1965, she gave birth to a very large stillborn calf (weighing 133.3 kg, male). The time of conception was problematical, but it was assumed as April or May of 1963, hence the gestation period may have been 24 or 25 months, a little longer than average.

Pregnancy diagnosis was attempted during the early and middle gestation period. For exploration, an urinary gonadotrophin was checked by the Friedman and Aschheim-Zondek tests on the whole urine samples collected twice in August 1963. Results showed apparantly positive responses in both tests.

However, the samples collected in May and September, 1964, showed negative in the three tests, including a male frog (*Rana*) reaction which was subjected to the concentrated urine samples.

So, probably a gonadotrophic substance may have been excreted in urine of this elephant at some time of the early pregnancy, and this may be more like FSH than LH in its activity.

Introduction

The zoo at Takarazuka, Hyogo, Japan, has maintained two Indian elephants for several years. In 1962, the male was 14 years old and the female 15 years old. Mating behavior was first observed in April 1962 and was frequently observed during the day and night. The female became pregnant and gave birth in May 1965 to an abnormally large, male stillborn calf in posterior presentation. It weighed 133.3 kg. The time of conception was problematical, but it was assumed that conception occurred in April or May, 1963. Hence, the gestation period was about 24 to 25 months, a little longer than average (Nalbandov, 1964; Parkes, 1956; Perry, 1953).

Pregnancy diagnosis was attempted during the early and middle stages of gestation period. Urine samples were examined for gonadotrophins by the Friedman, Aschheim-Zondek, and male frog tests. The Friedman and Aschheim-Zondek tests on whole urine collected twice in August 1963 were positive, but whole and concentrated urine samples collected in May and September, 1964, were negative on these three tests.

Thus, it appeared that a gonadotrophic substance was excreted in this pregnant elephant's urine during the third to the fourth month of gestation, but was not present in the urine at the twelfth to thirteenth and the sixteenth to seventeenth month of a 24 to 25 months gestation period.

Materials and Methods

Urine samples were collected at three periods of time during the elephant's pregnancy; in the second and the fourth week of August, 1963; in the first week of May, 1964; and in the second week of September, 1964. As a control, urine from a 17-year-old non-pregnant female elephant, which had been raised with another female for years at the Hanshin Park Zoo, Nishinomiya, was collected the fourth week of August, 1963.

Urine samples were collected directly with a ladle (Pl. I, fig. 2) during urination. Each urine sample collected had a pH of 8.6 to 8.8. The urine was weakly acidified to pH 5.0 to 6.0 with acetic acid and filtered through both clean absorbent cotton, that had been washed in ethanol and ether and then dried, and paper. The urine was then washed with three volumes of ethyl ether in a large separatory funnel for about three

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minutes for the purpose of removing possible toxic substances and steroids. The washed urine was placed in a warm water bath of 37 C, for seven to eight minutes. While stirring with a glass rod, a stream of nitrogen gas was blown on the washed urine to drive off the small amount of ether that remained in it. The control urine was treated similarly.

A modified Friedman test was done. The urine sample for injection was separated into two equal portions. Each portion was injected into each of two Japanese white rabbits, one young and the other adult. Twenty-four hours before the first injection, the ovaries and uterus of each rabbit were examined by laparatomy. After confirming that no large, protruding hemorrhagic follicles or corpora hemorrhagica existed on the ovaries and that the uterus was normal, the first half-dose of urine was injected into the ear vein of each rabbit. Twenty-four hours later the second-half dose was given, and autopsy was done 48 hours after the first injection (Table 1). Thirty I.U. of human chorionic gonadotrophin (HCG) dissolved in saline solution was injected into another rabbit as a positive control.

The Aschheim-Zondek test used in this experiment was also modified. Each of five immature female rats, Wistar strain, weighing 29 gm to 37 gm was given injections subcutaneously with urine, six times in three successive days, and an autopsy was performed 116 to 120 hours after the first injection (Table 2). Beside the findings of follicles or corpora hemorrhagica on the ovaries of each test rat, the weight of the ovaries of test and control rats were compared and recorded.

The urine concentrate was prepared by the addition of 1/20 amounts (v/v) of 20 percent acid-washed kaolin suspension to the urine, as an adsorbing agent, and processed by the method suggested either by Cutler (1949), and Bradbury *et al* (1949).

Each 500 ml of whole urine was finally concentrated to a 25 ml solution according to each method. The concentrated urine samples were used in the frog test, the Friedman test, and Aschheim-Zondek test.

The male *Rana nigromaculata* Hallowel (Japanese rana frog) was chosen as the test animal for the frog test. Two ml of the above concentrated solutions was injected into the dorsal lymph sac at each side of five frogs. Thirty I.U. or HCG dissolved in saline solution was injected into a frog for a positive control. The fluid in the cloaca was pipetted every 30 minutes for a two hour period and was examined under micro-

Table 1. Result of the Friedman Test with the Whole Urine of a Pregnant Indian Elephant

No. of Urine Samples	*No. and Body Weight of Rabbit (gm)*	*Ml. of Urine injected (i.v.)* *1st*	*2nd*	*Autopsy Findings in Ovaries (1) in Uterus (2)*	*Judgment*
1	1 (1340)	3.5	3.5	(1) 9 hemorrhagic follicles (2) enlarged & hyperemic	positive
	2 (2200)	6.0	6.0	(1) 11 hemorrhagic follicles (2) enlarged & hyperemic	positive
2	3 (1330)	3.3	3.3	(1) 8-9 hemorrhagic follicles (2) enlarged & hyperemic	positive
	4 (2740)	6.5	6.5	(1) 9-10 hemorrhagic follicles (2) enlarged & hyperemic	positive
control	5 (2620)	6.5	6.5	(1) many small follicles (2) small	negative

(1) Samples: 1 — whole urine, second week, August, 1963
2 — whole urine, fourth week, August, 1963
control — whole urine, non-pregnant, fourth week, 1963

(2) Time of injections:
1st inj.: at 0-hour, 2nd inj.: at 24-hour

(3) Time of Autopsy: at 48-hour

scope. When examinations at 30, 60, 90, and 120 minutes showed no spermatozoa in the cloacal fluid, a "negative" report was given.

Results

Whole urine samples 1 and 2, of August 1963, were both positive on the Friedman test as indicated by the presence of hemorrhagic follicles on ovaries of the four test rabbits. The whole urine of the control female elephant was negative.

In the Aschheim-Zondek test using immature Wistar rats, weighing 29 gm to 37 gm, the positive reaction was weak with whole urine sample 1, but stronger with whole urine sample 2 (Table 2). The average weight increase of ovaries of test rats was 2.4 and 3.5 times, respectively, that of the control rat. The concentrated and whole urine samples collected in May and September, 1964, (sample 3 and 4) were negative in the frog test or the other three tests (Table 3).

Though urine samples 1 and 2, collected at the third or fourth month of gestation were positive, unfortunately, no further examinations were done in 1963 to follow the excretion pattern of gonadotrophic substance in urine of this elephant.

Thus it could be concluded that in the third to fourth month of gestation, some gonadotrophic substance was excreted in this elephant's urine, and it had disappeared by the twelfth to thirteenth month of gestation.

Discussion

The details of the behavioral observations throughout estrus, mating, and parturition of this elephant were made in 1966 (Koto and Fujimoto). Nalbandov (1964) noted that it has been reported that the elephant (as the mare) forms accessory corpora lutea, but only from about the end of the sixth to the ninth month of the 24 months gestation. He inferred that elephants secrete a gonadotrophic substance similar to the one produced by pregnant mares. So, there seemed to be some difference in time in the secretion or excretion of gonadotrophin, between the findings obtained by them, six to nine months, and by us, three to four months. However, the time of conception may probably be hard to judge in most pregnant elephants. Actually in the experiment recorded here, "mating behavior" was often observed over a long period of time, apparently even in the pregnant period. True or successful copulation, however, was only observed once at midnight by an attendant (June 16, 1962) and the mating was infertile.

Table 2. The Aschheim-Zondek (Rat) Test on Urine from a 3-4 Month Pregnant Indian Elephant

1. Technic of Test

Day	*Injection of Urine*		
	Morning	*Noon*	*Evening*
1	1st	—	2nd
2	3rd	4th	5th
3	6th	—	—
6	Autopsy (at 116 to 120 hours after the 1st injection)		

2. Results of Test

No. of rat	*Volume of Urine per Injection (1st to 6th)*	*No. of Samples*					
		1		*2*		*control*	
		Reaction	*Judgment*	*Reaction*	*Judgment*	*Reaction*	*Judgment*
1	0.3 ml each	I		I		0	
2	0.5 " "	II		II		0	
3	1.0 " "	I	pos.	II	pos.	0	neg.
4	1.5 " "	II		II		0	
5	2.0 " "	I		II		0	

(1) Reaction: 0 — very small follicles only
I — large follicles
II — hemorrhagic follicles
III — hemorrhagic corpora lutea

(2) Urine samples:
No. 1 — whole urine, second week of August, 1963
No. 2 — whole urine, fourth week of August, 1963
control — whole urine, non-pregnant elephant, fourth week of August, 1963

TABLE 3. SUMMARY OF RESULTS OF TESTS ON THE URINE OF A PREGNANT INDIAN ELEPHANT

Sample No.	*Time in Gestation, urine collected*	*Urine*	*Test*	*Result*	*Controls*
1	3-4 mons. (Aug., '63)	whole	Friedman Asch.-Zond.	pos. pos.	pos. (HCG) neg. (saline)
2	3-4 mons. (Aug., '63)	whole	Friedman Asch.-Zond.	pos. pos.	neg. (urine) neg. (urine)
3	12-13 mons. (May, '64)	concentrated by method (A)*	Frog (male)	neg.	pos. (HCG) neg. (saline)
4	16-17 mons. (Sept., '64)	1. whole 2. concen'ted by method (A) 3. concen'ted by method (B)**	Friedman Asch.-Zond. Frog (male) Friedman Asch.-Zond.	neg. neg. neg. neg. neg.	pos. (HCG) neg. (saline)

* method(A), Cutler

** method (B), Bradbury *et al*

In this experiment, the time of conception was judged by the following observations. The female began to refuse the male, when he was going to mount her starting in May 1963. She became more gentle and quiet. She never bathed in a water pool in the zoo since the later part of May 1963, although she had previously been very fond of it, even in the cold winter season. The body weight of the fetus, 133.3 kg, was above the average, 70 to 122 kg, of newborn Indian elephants in zoos or circuses. So conception might have occurred a little earlier than April 1963.

From the information reported here we cannot determine the nature and time of excretion of a possible gondotrophin in the urine of elephants in pregnancy, and we have been unable to find another pregnant elephant for further study. Although it may be presumptuous to speculate from one case on the nature of this gonadotrophin in the urine of a pregnant elephant, from the results obtained in the Friedman test and Aschheim-Zondek test, it seems more like FSH than LH in its activity.

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EXPLANATION OF PLATES

PLATE I

FIG. 1: "Mating behavior," incomplete copulation – June, 1962.

FIG. 2: Collection of urine by a ladle.

PLATE II

FIG. 3: Parturition and a stillborn calf.

FIG. 4: "Positive" Friedman reaction on an ovary of a test rabbit. (ca. x 5)

FIG. 1

FIG. 2

GONADOTROPHIN IN THE URINE OF A PREGNANT ELEPHANT

FIG. 3

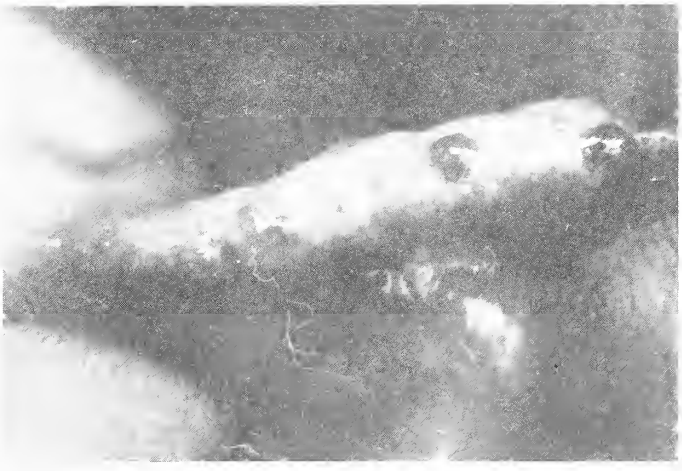

FIG. 4

GONADOTROPHIN IN THE URINE OF A PREGNANT ELEPHANT

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