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A Study of the Biology and Behavior of the Caterpillars, Pupae and Emerging Butterflies of the Subfamily Heliconiinae in Trinidad, West Indies. Part I. Some Aspects of Larval Behavior[1,2]

Anne J. Alexander

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(Plate I; Text-figures 1-8)

[This paper is one of a series emanating from the Tropical Field Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the Station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, and the annual rainfall is more than 100 inches.

[For further ecological details of meteorology and biotic zones, see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe, Zoologica, 1952, 37 (13): 157-184.

[The success of the present study is a large measure of the cooperation of the staff at Simla, especially of Jocelyn Crane and Constance Carter, the former contributing much of her knowledge of the animals, the latter helping with recording of observations].

Contents

I. Introduction

THE phylogeny of the neotropical subfamily of butterflies, the Heliconiinae, is interesting, partly because most species are distasteful and aposematically colored, partly because of the possibility of Müllerian mimicry. The determination of relationships within the group must clearly utilize information of behavior, physiology and ecology of the butterflies as well as their anatomy. Indeed, work has already been going on along former lines (see the study of comparative ethology of the adults, Crane, 1955 and 1957) and that of breeding experiments and wing patterns (Beebe, 1955). It is obvious, however, that all such studies must relate not only to the adult stages but also to egg, larva and pupa. The present paper represents an attempt to study comparatively the larval behavior of as many species of heliconiines as were available over a period of four and one-half months spent in the laboratory at Simla. Because of limitations of material and time,

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[1] Contribution No. 1,007, Department of Tropical Research, New York Zoological Society.

[2] This study has been aided by a grant from the National Science Foundation, G6376. Thanks are also due to the Royal Commission for the Exhibition of 1851 and to the South African Council for Scientific and Industrial Research for financial support.

the project was necessarily of a preliminary nature and there are many unavoidable gaps in information. The significance of some late larval behavior was clearly to be sought in the pupa and possibly the emergent adult; limited observations were thus made on these stages as well. These, together with information on molting of the larvae, will be presented in Part II. Implications of the phylogeny of the species observed are discussed tentatively but in relation to this study only, and it is hoped that a later paper will correlate evidence from the several fields and workers on the subjects of relationships among the Heliconiinae.

For descriptions and illustrations of the external characteristics of the larvae to be discussed, see Beebe, Crane & Fleming (1960) and Fleming (1960).

II. Materials and Methods

Of the 14 species of heliconiines known to occur in Trinidad, three were not available at all during the time of this study and any reference to their behavior is from the notebooks of the Simla staff. These species are *Heliconius wallacei wallacei* Reakirt, *Heliconius numata ethilla* Godart and *Philaethria dido dido* Clerck. Of the others, observations on *Heliconius doris doris* (Linnaeus) were limited to two days, while only a single specimen of *Dryadula phaetusa phaetusa* (Linnaeus) was obtained. *Dione juno juno* (Cramer) is gregarious and a single group of 37 healthy individuals was observed; these were, however, already in their second instar when found. Lastly, information on *Heliconius sara thamar* Hübner and, to a lesser extent *Heliconius erato hydara* Hewitson, was limited, as healthy stocks were unobtainable for much of the period. Observations on the emergence of *H. sara* are due entirely to Constance Carter to whom I owe many thanks. The remaining six species consist of *Heliconius melpomene euryades* Riffarth, *Heliconius ricini insulana* Stichel, *Heliconius aliphera aliphera* (Godart), *Heliconius isabella isabella* (Cramer), *Dryas iulia iulia* (Fabricius) and *Agraulis vanillae vanillae* (Linnaeus).

After initial observations of caterpillars on single leaves in glass jars, it was found more satisfactory to keep the larvae on lengths of vine, one to three feet long, the ends of which were thrust into narrow-necked bottles of water. Fresh vines were added to the bottles every few days and the caterpillars were free to move onto them, or were very occasionally transferred by hand. In such conditions the caterpillars remained on the vines, apparently content. With the exception of the two species mentioned earlier, all larvae were very healthy. Well-formed butterflies emerged from the pupae and individuals of those species whose normal courtship is known behaved as would be expected of healthy adults.

Most of the observations of larval behavior were made on vines in an isolated room on the fringe of the forest around Simla. Conditions of light, humidity and wind were therefore close to those of the natural habitat of at least some of the species outside (see Beebe, 1952, for details of ecological conditions in the Arima Valley).

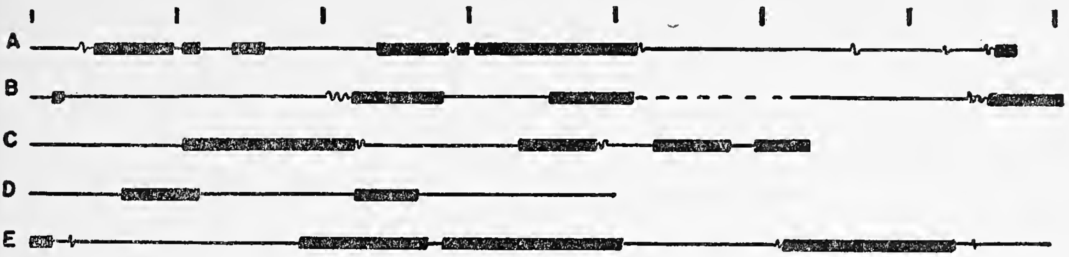
Late larval or prepupal behavior was watched outside the laboratory among rows of *Passiflora* vines planted by Simla staff. Where the host vine was not available in the vine rows, prepupational larvae were kept on extra large vines stuck into bottles in the laboratory. Observations of feeding patterns and choice of pupational site were also made in the field where possible. In no such case was any discrepancy found between these observations and those made under laboratory conditions.

At night caterpillars were watched beneath either a red or very dim white light. Individual animals were identified by peculiarities of anatomy or color or, in cases where they were very similar, by keeping them on separate vines. Numerous mirrors had to be used to render the activities of the caterpillars visible without disturbing them.

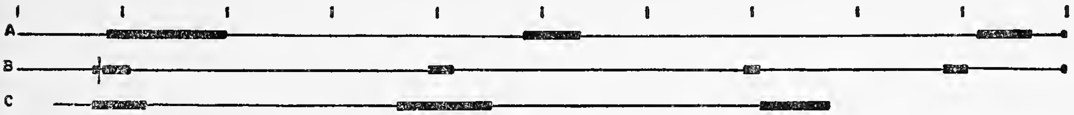
III. Feeding Rhythms

It has generally been recognized that most, if not at all, lepidopterous larvae show rhythm in their behavior patterns. Thus Crowell (1943) reported that a large number of species have feeding periods of about 20-30 minutes alternating with rest periods of similar duration. The caterpillars of at least ten species of heliconiines from Trinidad are no exception (see Text-fig. 1 for examples).

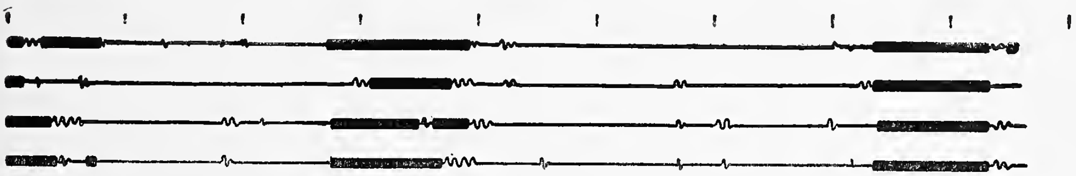
As Ford (1945) has recorded for caterpillars such as the Silver-studded Blue (*Plebejus argus*) and the Black Hairstreak (*Strymonidia pruni*), most of these species show rhythmic bursts of feeding evenly throughout both day and night. An examination of the activity patterns suggests that there are specific differences in the details of this rhythm, but the picture may be blurred by changes in the pattern during the course of an instar. Thus the duration of feeding periods immediately after a larval molt is shorter than of those immediately before (Text-fig. 2). Again, the rhythm differs between instars, while just before pupation there are long, almost uninterrupted periods of feeding.



TEXT-FIG. 1. Feeding rhythm in caterpillars the day after their fourth molt. Thick straight lines = feeding; thin straight lines = resting phases; thin wavy lines = walking or other movement. The time intervals on the upper line = 30 minutes. **a**, *D. iulia;* **b**, *H. isabella;* **c**, *H. aliphera;* **d**, *H. melpomene;* **e**, *H. erato.*



TEXT-FIG. 2. The feeding and resting periods in a caterpillar of *H. melpomene*: **a**, the day before the fourth molt; **b**, immediately after eating the cast fourth exuvium; **c**, the following morning. The convention used is as in Text-fig. 1. The line drawn down through the first feeding period of 2b indicates an interruption.



TEXT-FIG. 3. Synchronization of feeding and resting in a group of four *H. doris* caterpillars.

While the majority of the species feed throughout the 24 hours, *H. doris* does not normally feed at night, a habit which Ford (1945) records for the Dark Green Fritillary (*Argynnis aglaia*) and the Swallow-tail (*Papilio machaon*). Furthermore, two species, *D. iulia* and *H. melpomene*[3], show a tendency, during their fifth instar, to rest throughout the day[4].

Ford has recorded a similar change of feeding habit in the last larval stage of the Scotch Argus (*Erebia aethiops*) which feeds only during the night at this period, although earlier it was not so restricted.

In naturally gregarious species, such as *D. juno*, *H. doris*, *H. sara* and to a lesser extent *H. ricini*, the caterpillars synchronize their feeding and resting periods (Text-fig. 3). The beginning of the feeding period is somewhat less strictly coordinated than its end; this reflects the possibility that synchronization is controlled by at least two factors.

An internally controlled rhythm could initiate feeding periods. In caterpillars which have been deprived of food or for some other reason are not eating, *e. g.*, the nocturnal phase of *H. doris*, there are indications of a persistence of rhythmicity expressed as alternating periods of rest and locomotor activity. If indeed the locomotor activity corresponds to the feeding activity, it will follow that the onset of the activity period is determined not simply by a reflex due to lack of food, as suggested by Crowell (1943), but by some endogenous pattern. The rest periods are usually induced by factors which affect all the animals simultaneously, possibly such extraneous stimuli as a sudden wind, the passing of an ant or the touch of an observer. The last-mentioned stimulus has in fact been noticed to end the feeding periods in various

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[3] From the laboratory notebook of Barbara Young (1957), it seems probable that *P. dido*, a species which I have not observed, is similar in this respect to *D. iulia* and *H. melpomene*. The characteristic may, however, appear earlier in the life of the caterpillar, possibly by the third instar.

[4] Using the amount of dung deposited during a certain time as a measure of the intensity of feeding, it seems that, in these latter cases, light may be a direct inhibitory stimulus. Thus a caterpillar of *D. iulia*, if kept in darkness during the day, eats more than twice as much as it does in daylight, producing 2.4 pellets per hour, as opposed to 0.9.

TABLE I. RELATIONSHIP BETWEEN LARVAE OF THE SUBFAMILY HELICONIINAE AND PLANTS OF THE FAMILY PASSIFLORACEAE IN TRINIDAD

Key:
*—Host plant.
+—Will accept if put on plant.
–—Refuses if put on.
(*)—Very occasionally found on.
(*–)—Eggs found on it but larvae refused it.
(S.T)—South Trinidad.

Species	Species of *Passiflora*								
	auriculata HBK	*vespertilio* Linnaeus	*tuberosa* Jacquin	*rubra* Linnaeus	*quadriglandulosa* Rodschied	*serrato-digitata* Linnaeus	*laurifolia* Linnaeus	*lonchophora* Linnaeus	*foetida* Linnaeus
1. *Dione juno*	+–			+		*	(*–)		+
2. *Agraulis vanillae*	+			+			+	(*)	*
3. *Dryadula phaetusa*			+	(*)	+–				(*–)
4. *Dryas iulia*	+		*	+			–	–	–
5. *Philaethria dido*			–				*		
6. *Heliconius isabella*				+		*(S.T)	*		
7. *Heliconius aliphera*			+–	*	? *(S.T)		+	*	+
8. *Heliconius melpomene*	+		(*)+				*	+	
9. *Heliconius numata*								*	
10. *Heliconius erato*	+	*(S.T)	*				(*)+		
11. *Heliconius ricini*			–				*		
12. *Heliconius sara*	*						+	+	
13. *Heliconius wallacei*					*				
14. *Heliconius doris*						*	(*)		

species. If this stimulus is given early in the feeding period, it will have no effect or merely occasion a momentary halt. Towards the natural end of the period, however, it usually causes premature resting. These considerations may also apply to the habits of the non-gregarious caterpillars, for these tend to fall into a synchronized rhythm of eating and resting when kept on the same or neighboring leaves—a phenomenon which has been observed especially in *H. aliphera* and *H. melpomene*.

Regardless of how such synchronous eating is controlled, any selective advantage of such behavior would surely come from the limit which it sets to the time during which any caterpillars are moving. Movement of the prey is

TABLE II. VEGETATIVE CHARACTERISTICS OF THE VINES ON WHICH THE CATERPILLARS FEED

Species	Vine Size	Texture of the Stem	Leaf Texture	Covering of Leaf	Abundance of Leaves	Leaf Shape
Passiflora auriculata	Slight, maximum 10′, climbing	Slender, smooth	Tender-medium	Smooth	Fairly scarce	Simple, entire
Passiflora rubra	Medium, bushy, climbing	Medium, branches more than other vines	Medium	Medium	Abundant	Widely bi-lobed
Passiflora tuberosa	Slight, effervescent	Slender	Tender-medium	Mat	Scarce	Bi-lobed, narrow-wide
Passiflora quadrigrandulosa	Climbing, reaches height of 30′	Tough-medium	Tough-medium	Rough-mat	Fairly scarce	Bi-lobed, unequal
Passiflora serrato-digitata	Medium, sprawling	Smooth, sturdy	Tender-medium	Smooth	Fairly abundant	5-7-palmate
Passiflora laurifolia	Climbing, reaches up to 30′	Tough, frequent branching	Tough-medium	Smooth	Abundant	Simple, entire
Passiflora foetida	Sprawling, medium	Tough-medium, hairy	Medium	Hairy & glandular	Fairly abundant	Tri-foliate
Passiflora lonchophora	Slender, climbing up to 40′	Tough	Tender-medium	Smooth	Fairly abundant	Tri-foliate

important to insect and reptilian predators and, furthermore, would assist in calling the attention of any bird to a caterpillar. Thus with the exception of "agonistic" movements when disturbed, the caterpillars would do well to remain as motionless as possible during non-feeding periods. Without synchronization of feeding movements, some animals could always attract the attention of predators to the resting caterpillars, as well as to themselves.

IV. FEEDING

Although heliconiine imagines feed on nectar from a variety of flowers, their larvae feed only on various species of passion vines (Family Passifloraceae). Usually feeding is confined to the leaves, but in some cases tendrils, stalks, flowers and hairs are also eaten. Ten species of vine growing in Trinidad have been found to support one or more of 14 species of heliconiine caterpillars (see Table I). These vines vary fairly widely in their vegetative characteristics (see Table II) and are, moreover, somewhat variable within each species.

One factor which determines which species of vine a caterpillar eats is, of course, the fairly high specificity in respect to oviposition site shown by the heliconiines; the eggs are almost invariably laid on the "natural" food-plant of the species. Occasional "mistakes" are, however, found in the field (see Table I).

Tolerance of an "unnatural" host vine may alter according to what a caterpillar has eaten previously, the stage and number of its instar and possibly its water load immediately before a test. Thus *H. aliphera* raised to the fifth instar on *P. lonchophora* accepts *P. rubra* almost immediately and with no change of its previous patterns of eating and resting. The converse is not true. *H. aliphera*, raised on *P. rubra* to the equivalent stage and then transferred to *P. lonchophora,* wanders and rests without eating for several hours before beginning to feed. In this species there is a tendency for later instars to show less specificity in their food preferences than the earlier ones. The matter has not been investigated in detail among other species, but tests made on the day before pupation indicate that host preference is then less strict.

Sometimes when a butterfly oviposits on an abnormal species of vine, the caterpillars refuse to eat altogether, *e.g., D. juno* laying on *P. laurifolia* (Table I). In other cases they may accept the abnormal food plant but their growth may be reduced and/or retarded. Text-fig. 4 shows the latter effect on a group of *D. juno* fed on leaves of *P. auriculata* and *P. rubra* as opposed to one fed on the natural food-plant (*P. serrato-digitata*). The number of instars is greater, molting is more protracted and most growth periods are longer. It has not been established whether they actually eat less in

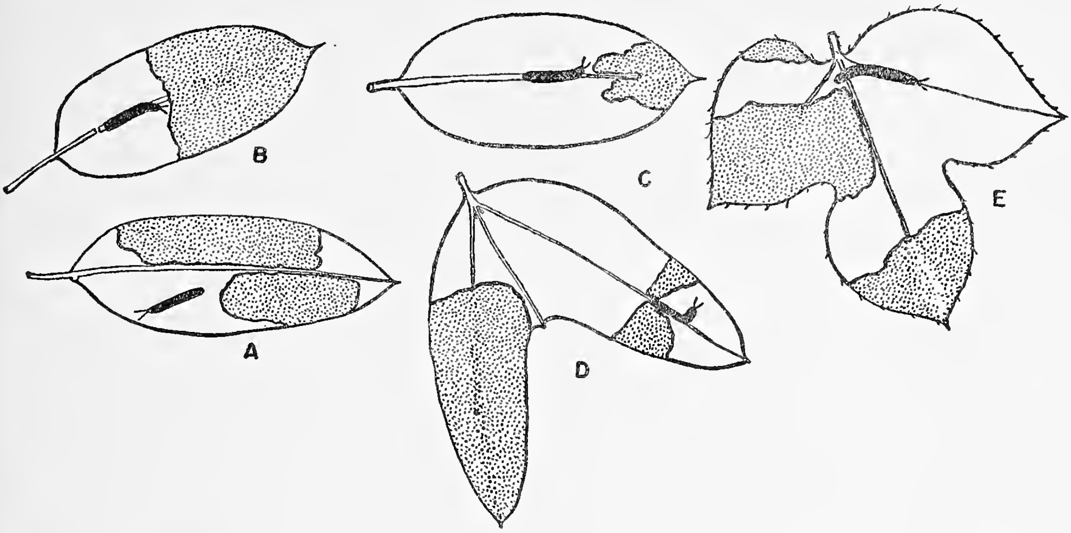
TEXT-FIG. 4. The effect of food plant on instar number and length in *D. juno*. Growth and feeding are indicated by the black lines, molting by the white lines. Crosses indicate the time of hanging up and triangles the shedding of the last larval skin. Time interval = 24 hours, and the arrow indicates when the second group were collected as they molted into the second instar. **a**, Four caterpillars on *P. auriculata* and *P. rubra*: **b**, 13 caterpillars on *P. serrato-digitata*, their normal food plant. Information for the upper picture was kindly supplied by Jocelyn Crane.

unit time under the unfavorable conditions. There are, moreover, behavioral changes in such cases; for instance the synchronization of feeding is lost. This may reflect a more basic physiological disturbance, for the synchronization of molting and pupation also disappears.

It is not clear how the caterpillars distinguish between a vine which they accept and one they reject. Some rejections occur after the material has actually been chewed. Thus *H. ricini* refused to eat *P. tuberosa* after tasting it and rejected an aqueous extract of *P. tuberosa* leaves, though it would drink similar extracts made from its food-plant *P. laurifolia*. In other cases caterpillars can certainly make a distinction without having to taste the material. Thus neither *H. isabella* nor *D. iulia* will attempt to eat *P. foetida;* in fact the former cannot be persuaded to remain on this vine, commonly dropping off as soon as it is put on a *P. foetida* leaf. It seems possible therefore that in this species of caterpillar, behavior is controlled by more than one factor, in keeping with Dethier's (1937) findings on larvae of the gypsy moth. Such an effect might explain conclusions such as those of Merz (1959) who declares that among the larvae of monophagous species of Lepidoptera many are less specialized than had been previously supposed. The technique she used in testing the larvae was to present them

TABLE III. INFORMATION RELATING TO CROWDING OF EGGS AND THE SITE WHERE THEY ARE NORMALLY LAID BY THE HELICONIINE BUTTERFLY

Species	Gregariousness	Leaf Size	Leaf Surface	Tendril
Dione juno	60/90 in raft almost touching	Medium (but tender)	Under	
Agraulis vanillae	Single	Leaves indiscriminately	Upper	
Dryadula phaetusa	Single	Medium		
Dryas iulia	Single	Medium	Upper	Fresh, dry
Philaethria dido	Single	?	?	Thick
Heliconius isabella	Single or few scattered	Medium-large	Under	
Heliconius aliphera	Scattered 5/6 or single	Medium-large	Under	
Heliconius melpomene	Single	Subterminal leaflets, medium	Upper	Young
Heliconius erato	Single	Subterminal leaflets, medium leaves	Upper	
Heliconius ricini	4/12 loose cluster	Among leaf buds		
Heliconius sara	About 25 in tight cluster	Among leaf buds		
Heliconius wallacei	25/30 fairly loose cluster	Among leaf buds		
Heliconius doris	36/52 in raft almost touching	Medium leaf	Upper	



TEXT-FIG. 5. Patterns left on leaves by the feeding of caterpillars, the stippled part being that eaten. The caterpillars concerned are shown in their typical resting positions and postures on the appropriate leaves. **a**, *H. isabella* on *P. laurifolia;* **b**, *H. melpomene* on *P. laurifolia;* **c**, *H. ricini* on *P, laurifolia,* chewed to give the ragged effect rather than the straight across, shown for *H. melpomene* and often produced by *H. ricini;* **d**, *D. iulia* on *P. tuberosa;* **e**, *A. vanillae* on *P. foetida.*

with dried leaves moistened with sugar water. Regardless of additional complications of the possible attraction of sugar water itself, the physical properties of the leaf are vastly changed by desiccation.

a. *Leaf Patterns*

Since the butterflies further show consistent preferences for oviposition in particular sites on the vine (Table III), the food first accepted by the caterpillars will be affected by this choice, for the newly-hatched animals usually eat the food nearest to them. Thus *H. isabella* and *H. aliphera* begin eating the undersurface of a leaf, although they will feed when placed on its upper surface, a position in which they are never found in the field. Again, a high percentage of *H. melpomene* start by eating a tendril. However, of a batch of 20 caterpillars from eggs laid on tendrils and offered a choice of tendrils and young leaves, 55% ate leaves, disregarding the tendrils after they had investigated them. Thus, in certain cases at least, the feeding sites of first instar larvae are determined simply by the normal oviposition site and where, as with *H. melpomene,* some selection might be exercised, this cannot find expression.

Besides showing specific preferences for a single or various species of vine, the larvae leave characteristic patterns (Text-fig. 5) on material which they have been eating, a phenomenon which is well known in many phytophagous insects (see Hering, 1926).

The following features are among those distinguishing different patterns in the heliconiine species of this study.

1. The green cells alone may be scraped away from the surface of a leaf, leaving only a layer of transparent epidermal cells on the far side. This is in contrast to an actual hole being chewed in the leaf or its being eaten away from a margin so that bays or channels are left.

2. The midrib of the leaf or leaflet may remain when the caterpillar abandons the leaf, or it may be eaten together with the blade of the leaf or, in complex patterns, it may be ignored while the caterpillar eats most of the blade but is finally eaten before the leaf is abandoned.

3. Whether it is finally eaten or not, the midrib may also be the focus for other specific attentions. *H. melpomene, H. ricini* and, in special cases to be mentioned later, *H. erato,* will chew a small chunk out of the ventral midrib of the leaf on which they are feeding. Occasionally there may be two such "furrows" chewed across the same midrib but usually a single one is cut between the base of the leaf and the level at which the caterpillar rests between feeding periods. On the other hand the midrib may be involved in complicated channeling and

TABLE IV. FEEDING BEHAVIOR OF TEN SPECIES OF HELICONIINE CATERPILLARS
(Information relating to *Philaethria dido* supplied by Constance Carter)
Key: + — Eaten.

Species	Scraping, holes, margin chewing	Midrib eaten, eaten later, or left	Midrib treatment	Leaf margins eaten	Leaf tips eaten	Amount of leaf abandoned
Dione juno	Scraping in first instar at least	Left	?	+	Dropped	Frill left on leaf, no petiole eaten
Agraulis vanillae	Holes chewed	Eaten later or ignored		+	+	Frill left on leaf, no petiole eaten
Dryadula phaetusa	1st instar, 2nd, chews channels in from margin	Eaten later	Channeling and bridging	+	+	Frill around base of leaf or petiole chewed
Dryas iulia	Chews channels in from margin even in first instar	Eaten later	Channeling and bridging	+	+	Often frill around base of leaf, but more usually petiole also eaten, sometimes stem as well
Philaethria dido	Chews channels in from margin even in first instar	?	?	?	?	?
Heliconius isabella	Scraping 1st, 2nd instar	Left, occasionally eaten later		+	Dropped	Base of leaf, midrib and tip are left
Heliconius aliphera	Scraping 1st, 2nd instar	Sometimes eaten later, usually not		+	Sometimes definitely discarded	Always base of leaf is left
Heliconius melpomene	Very occasional scraping, usually channels or bays chewed from margin	Eaten together with blade	Chews furrows across under surface	+	+	½ leaf or frill around base is left
Heliconius erato	Never holes or scraping but channels or bays from margin	Eaten together with blade	May chew furrow in petiole	+	+	All leaf, and frequently petiole and often part of stem, is eaten
Heliconius ricini	Never scraping, but holes or channels and bays from margin	Eaten, often left by groups of larvae	May chew furrows across under surface	Often left, especially when eating as a group	+	About half leaf if larvae is solitary, if in group may eat even stem
Heliconius sara	As in *Heliconius ricini*	Eaten	May chew furrows across under surface			If larvae in a group, no leaf left; even stem chewed
Heliconius doris	Chewing from margin	?	?	?	?	?

bridging behavior shown by *D. iulia* and *D. phaetusa.*

4. The margins of a leaf may be left by *H. ricini* as tattered edges or they may be eaten with the rest of the leaf.

5. Leaf tips may be regularly eaten, as by *H. ricini* and *H. melpomene;* if the tips are not eaten they may be allowed either to fall to the ground or to remain with the midrib.

6. When a caterpillar moves over to a new leaf, as much as half the old leaf may be left, only a small stub may remain or the caterpillar may have eaten it entirely. Some species (*e. g., D. iulia* and *H. erato*) normally eat the petiole, while *H. erato* eats the stem as well.

Table IV shows the distribution of these six characteristics among the species studied.

Many of these specific features appear to have no very obvious significance in the lives of the caterpillars. Some are extremely consistent and appear to be endogenously differentiated while others occur only under certain conditions and it seems that environmental factors are at least partly responsible for their appearance. The case of *H. aliphera* and *H. isabella* will be considered first. These scrape the cells from the surface of leaves instead of chewing through as do other larvae which eat the same host vines, *P. lonchophora* and *P. laurifolia* respectively. The scraping is seen only in the first, second and to some extent third instars of both species, which gives the impression that it occurs when the animals are too small to do otherwise. The same idea is also gained from the fact that *H. isabella* occurs on the tougher leaves of *P. laurifolia* while young *H. melpomene* and *H. ricini*, which feed on the same vine and do not use the scraping method, are limited to thin and tender leaves. Moreover, on several occasions a first instar *H. melpomene* did scrape for a short while when it was on a tougher leaf than is normal for this species. Finally, it is possible to induce a third instar *H. isabella*, which has been scraping at a tough leaf, to eat holes by putting it on a tender one, though it reverts to scraping when replaced on the tough leaf.

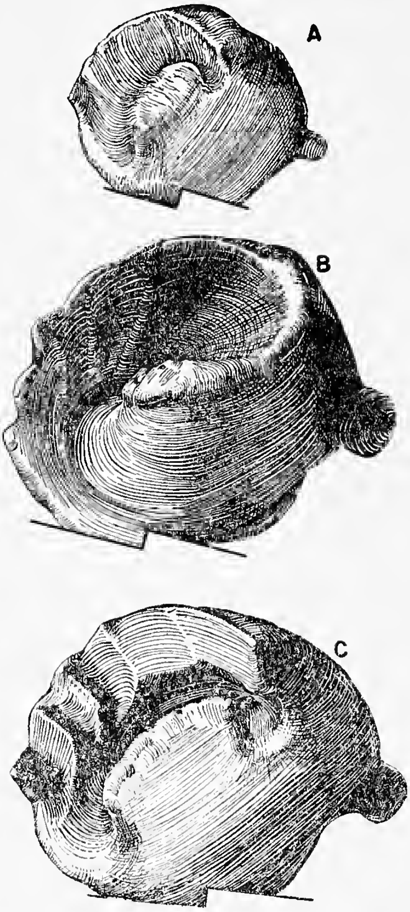
These observations suggest that the scraping habit of *H. isabella* is determined by purely mechanical considerations. This cannot be the full explanation, however, for the first and second instar *H. isabella* still scrape when put on tender leaves through which *H. melpomene* will chew. Further *H. aliphera* scrapes on the softest of *P. lonchophora* leaves. Nor can the effect be attributed in any simple way to the differences which occur in the sculpturing of the mandibles for *H. aliphera* and *H. isabella* are capable of chewing through both the upper and lower surface of a leaf at which they nevertheless only scape if left on one side. Thus it does not seem that these caterpillars eat only the one epidermal layer because they are incapable of dealing with the other. Scraping would rather seem to be, at least in part, an inherited pattern in *H. aliphera* and *H. isabella*, although it is lost later and can be modified by external circumstances. It is probably present in the early instars of some species such as *H. melpomene* but is not expressed in the conditions in which these normally live.

Eating or leaving the midrib is another characteristic for which there seems to be a simple, mechanical explanation. Although both *H. isabella* and *H. melpomene* feed on *P. laurifolia* leaves of similar texture, the appearances of the leaves left by the two species is quite different. *H. isabella* leaves the tip and the midrib entire and only the blade on either side is chewed away. (Text-fig. 5a). *H. melpomene* eats the tip of the leaf and straight across the blade, midrib included. (Text-fig. 5b). If *H. aliphera* is made to eat the leaves of *P. laurifolia*, its feeding pattern is the same as that of the *H. isabella*. It could be suggested that *H. melpomene* has more effective mandibles than *H. isabella* or *H. aliphera*. Indeed, an individual of *H. isabella* or *H. aliphera* does leave small veins projecting along a margin where it has been eating, as if those parts which are slightly fibrous are less readily taken. Further, when *H. isabella* feeds on *P. serrato-digitata*, with a more succulent and tender leaf, it does not invariably reject the midribs.

Another species which rejects the midrib, at least in the third, fourth and early fifth instars, is *D. juno*. This caterpillar feeds on *P. serrato-digitata*. If the same mechanical explanation applies here, we would expect its mandibular apparatus to be still less efficient.

The only measure of efficiency of mandibular apparatus comes, at the moment, from considering the sculpturing on the biting surface of the mandibles (Text-fig. 6). On this factor the argument about the ineffectiveness of chewing in *H. aliphera* and *H. isabella* seems to be borne out, for these two possess none of the ridges and cusps which are clear on the maxillary edge of *H. melpomene* and *H. ricini* mandibles and the molar process is distinctly lower, flatter and smoother (*cf. H. aliphera* and *H. melpomene*, Text-fig. 6a and b). On the other hand, such studies fail to support the suggestion that chewing apparatus of *D. juno* is inefficient, for this caterpillar (Text-fig. 6c) has distinct cusps and ridges on the maxillary edges of its mandible although they are admittedly broader and less well formed for cutting than those of *H. melpomene*. The molar process, like that of *H. aliphera*, is well separated from the maxillary edge but is nevertheless somewhat cusped and there are small auxillary cusps lying part way between molar and maxillary edges. Thus, arguments from simple considerations such as mandibular sculpturing do not throw light upon feeding differences, if indeed it is legitimate to expect them alone to serve as an index of chewing efficiency.

It is important, moreover, to recognize that the actual feeding patterns of these caterpillars are distinct. *H. melpomene* (and also *H. ricini*) eat across a leaf, taking both blade and midrib in a single action, whereas, if they accept the midrib, *H. aliphera* and *H. isabella* first eat the blade of a leaf and then, subsequently, the midrib which remains. Thus, whether or not mechanical factors determine whether the midrib can or cannot be eaten, these do not exert



Text-fig. 6. Biting surface of the right mandible of a fifth instar caterpillar, showing the maxillary edge along the upper margin of the drawing and the molar process in the mid foreground. The projection on the lower right is the point of articulation. **a**, *H. aliphera;* **b**, *H. melpomene;* **c**, *D. juno.* Drawing by F. Waite Gibson.

an immediate influence over the pattern of feeding behavior.

b. *Furrowing Behavior*

H. melpomene and *H. ricini* share another very distinct behavior pattern in regard to the midrib. Caterpillars of either species will be found on the undersurface of *H. laurifolia* leaves, eating from the tip of a leaf back toward the base. Some time after a caterpillar has begun eating, it will turn around, walk a short way up the midrib and chew out the furrow mentioned earlier (Text-fig. 5b). At its deepest point this furrow is one-third of the depth of the midrib. It may be chewed out immediately after a bout of eating, during a rest period or just before the animal returns to the tip of the leaf to eat. It seems improbable, therefore, that the material is eaten merely for its nutritive value.

Initially it seemed possible that such furrows might serve to control the flow of water into a leaf; perhaps when the water content of the leaf blade rises too high for a caterpillar, it chews furrows across the midrib, so reducing the water flow. Experiments do not support this hypothesis; desiccated caterpillars and those in normal water balance still chew furrows both in wilting and in normal leaves. Thus it seems improbable that the primary function of the activity is that of upsetting water transport in the leaf. Further, the furrow often does not go deep enough to injure the vascular bundles in the midrib.

A second possibility is that furrows represent a simple way of preventing other caterpillars' coming down the midrib and disturbing or even attacking the larva beyond the furrow; it may serve as a form of territory marker. No unequivocal evidence has been found for or against this theory. When an intruder has turned back and away after reaching a furrow, the response might have been mediated by movements made by the furrow-owner. Conversely, on one occasion when the owner had been removed, an intruder walked on down the midrib, crossing the furrow with scarcely any hesitation. This could be attributed to the intruder's being aware that there was no caterpillar beyond the furrow and that it could therefore ignore the "warning." Slight indications that this may be at least part of the explanation come from the fact that both *H. ricini* (semi-gregarious) and *H. sara* (gregarious) do not produce these furrows when they live together in a group but if individuals of either species are kept isolated they may do so.

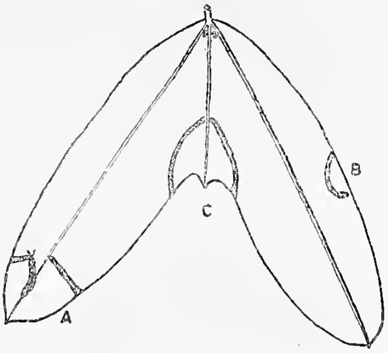
Another point of interest in regard to this furrowing habit relates to *H. erato.* In many respects the behavior of this caterpillar is clearly related to that of *H. melpomene* and *H. ricini* and it might therefore be expected to show some signs of chewing furrows. Its normal food-plant is *P. tuberosa,* whose ventral midrib hardly protrudes at all, and on this vine it makes no attempt to chew furrows. Raised on *P. rubra* or *P. auriculata,* however, *H. erato* produces furrows indistinguishable from those of *H. melpomene.* This latter species will, however, attempt to chew furrows when kept on *P. lonchophora,* another vine with negligible midrib. These furrows are quite recognizable, although they may go rather deep and even be developed into a hole. Thus *H. erato* may, on an unusual host plant, make furrows if the midrib is strongly developed while *H. melpomene* will retain its normal pattern on an unusual host even if the midrib is almost absent.

Compared with *H. melpomene* and *H. ricini*, *H. erato* displays very aggressive behavior towards other caterpillars. This can be interpreted in terms of the suggestions made above that one function of furrowing is as a territory marker. Possibly *H. erato* lived previously on a *P. laurifolia*-like vine with its thick veins. After the change, furrowing was difficult and its role in territorial defence was replaced by the development of more aggressive behavior.

c. *Channeling Behavior*

The channeling behavior of *D. iulia* and *D. phaetusa* (possibly also *P. dido*) is in some respects reminiscent of the furrow-chewing of *H. melpomene, H. ricini, H. sara* and *H. erato*. Both *D. iulia* and *D. phaetusa* have been seen on *P. tuberosa* while the former has shown precisely the same pattern on *P. rubra* and on the simple-leafed *P. auriculata*. A larva walks down the midrib to the tip of the leaf or leaflet, then it turns and walks back a variable distance, often about one-third of the length. If its body is long enough to reach from the midrib to the margin of the leaf, the caterpillar stretches out and begins to chew a channel across from the margin to the midrib of the leaf. The channel usually slopes slightly towards the leaf base as it nears the midrib but sometimes is almost at right angles to the midrib. As soon as this first narrow channel reaches the midrib, the caterpillar stretches across to the other side and begins a second which will extend inwards as did the first. When it, too, has reached the midrib, the caterpillar is on a small island of leaf, bridged merely by the midrib of the leaflet (Text-figs. 5d and 7a). Usually the caterpillar then chews at the midrib and spins some silk across the bridge. Sometimes, though not invariably, the larva crosses back to the main part of the leaf, walks along the proximal margin of its channel and bites at it. This does not appear to remove any material but just dents the edges slightly. The caterpillar then returns to the distal side of the bridge and rests along the midrib or begins to eat the island.

Although it is usual for this channeling behavior to occur in relation to the midrib of a leaflet, it may also relate to the margin of a leaflet (Text-fig. 7b), especially in the early instars, or even to the central vein running down between the two leaflets (Text-fig. 7c). After the first island has been eaten away completely, midrib and all, the caterpillar walks further up the leaflet and repeats the procedure once or twice more before crossing over to the other leaflet.



TEXT-FIG. 7. *D. iulia* feeding on *P. tuberosa*. **a**, A caterpillar chewing the second of two channels which will cut the tip of the leaflet almost free of the base; **b**, channel chewed in relation to the margin; **c**, a channel chewed in relation to the vein between the two leaf lobes.

Channeling is shown by caterpillars of *D. iulia* and *D. phaetusa* from the first to the fifth instar, although both the first and fifth tend to show it less distinctly. Immediately before pupation, larvae lose the channeling habit and eat leaves either from the tip or lateral margin, the pattern being very like that shown throughout life by *H. erato* on the same vine.

Channeling could serve the same function of territory-marking as has been postulated for the midrib furrows shown by *H. melpomene, H. ricini, H. erato* and *H. sara*. If a number of *D. iulia* caterpillars are put onto a stem with an abundance of leaves, they establish themselves, each one on a separate leaf, or at most one to each leaflet of a leaf. When a caterpillar is moved onto a leaf occupied by another animal, it walks down until it reaches the bridge and then turns back. This may be due to some movement on the part of the owner, who often crosses the bridge and swings at the intruder with its head. Two observations of a caterpillar turning back although the owner had been removed are offset by several in which it did not.

The question may finally be raised as to whether there is any direct relationship between furrowing and channeling. It seems unlikely, because *D. iulia*, though normally found on *P. tuberosa* which has only a slight midrib, will continue to channel and bridge when cultured on *P. rubra* and *P. auriculata*, both of which have thick midribs. It shows no tendency to furrow. It seems therefore more probable that the furrowing and channeling patterns have been independently evolved.

d. *State of Abandoned Leaves*

H. ricini is the only one among the species studied here which ever abandons a leaf in the state illustrated in Text-fig. 5c. Parts of the blade remain adhering to the margins, which are left almost entire. Even the midrib is not chewed level. The caterpillar lies with most of its body along the midrib as it eats, just stretching its head out towards the leaf margin. Where the leaf is too wide to allow the caterpillar to reach the margins without moving its body off the midrib, the animal may just leave the margins untouched. There is some indication that this particular pattern may be correlated with the gregariousness of *H. ricini.* When eggs are laid singly or larvae isolated by the experimenter, the feeding pattern of the caterpillars recalls the "straight across" action of *H. melpomene.* Several animals together produce a more ragged effect. Similarly *H. sara,* when part of its normal group, shows no clear-cut feeding pattern, though when a caterpillar is raised alone it eats in the typically *H. melpomene* fashion, straight across blade, midrib and margins and even chews furrows in the midrib.

The amount of leaf material left when a caterpillar abandons a leaf (Table IV) and moves to a new one is to some extent characteristic of the species, although external factors, such as whether or not there is more food available, do have some influence. There is also a tendency for earlier instars to abandon a leaf with a higher percentage of it remaining than in the case of later ones. Four species, *D. iulia, D. phaetusa, H. erato* and *H. sara,* habitually eat the petiole of their leaf and sometimes continue and eat the stem as well. The first three species eat *P. tuberosa* and it could be that the stem of this vine is more palatable than that of others. This seems somewhat improbable. Moreover, other caterpillars, *e.g., H. melpomene* and *H. aliphera,* eat neither petioles nor stems when they are fed on *P. tuberosa.*

It is possible to relate this habit to the vegetative characteristic of the vine on which the caterpillars normally live. Thus the natural food of *H. sara* is the small *P. auriculata* vine, while *P. tuberosa* is also slight and slender and its leaves are frequently very scanty (see Table II). Presumably there would be strong selective pressure for any animals living on such vines to practice economy as far as possible and this might explain this aspect of the behavior of *D. iulia, D. phaetusa, H. erato* and *H. sara.* It is relevant that, when *H. erato* is fed on *P. laurifolia* or *D. iulia* on *P. rubra,* they both still eat the petiole as well as the leaf. This facet of their feeding behavior is thus, at least to some extent, independent of environmental control.

e. *Feeding Positions*

It has already been said that positions adopted by feeding caterpillars differ specifically. The most obvious difference in this respect is whether or not the midrib is used as an orienting feature. A caterpillar of *H. melpomene, H. erato, D. iulia* or *D. phaetusa* usually has at least part of its body along the midrib while it eats a leaf (Table V). If the leaf is so wide that the animal cannot stretch to the margin, it lies parallel to the midrib (see p. 16 for the exception in the case of *H. ricini*). This adherence to the midrib may possibly have some selective advantage in relation to camouflage from predators but whether this is so or not, it certainly gives the caterpillars a more secure hold on the leaf. It is much easier to dislodge an individual of *H. melpomene* placed on the blade of a leaf than one that has been allowed onto the midrib.

While *H. aliphera* and *H. isabella* occasionally orient themselves parallel to the midrib as they eat, they do not lie along it. Normally their orientation bears no relation to it. *D. juno, A. vanillae, H. doris* and *H. sara* appear sometimes to orient to it or along it but at other times to pay it no attention. *D. juno, H. doris* and *H. sara* are gregarious and the lack of a definite and consistent orientation in feeding may be correlated with this habit; certainly *H. sara* when isolated from its fellow caterpillars will lie along the midrib, while a group of *D. juno* walk only along the midribs of the leaflets when they search for a new feeding place. *A. vanillae,* however, shows no signs of any consistent orientation of its body with respect to the form of a leaf in feeding. Neither *D. juno* nor *H. doris* has been tested singly on a leaf.

In the field the caterpillars of most species feed on the under or abaxial surface of a leaf (Table V), which is almost invariably the ventral one. This is not necessarily so in the laboratory and it has been found that the different species tend to differ in their responses to a leaf whose position has been reversed (Table V). *H. aliphera* and *H. isabella* both return to the ventral position, even though this means that they will not be eating from the true upper surface. *D. iulia, H. melpomene, H. erato* and to a lesser extent *H. ricini* still choose the under surface though this is now dorsal. Thus *H. aliphera* and *H. isabella* seem to be using different criteria for their choice than the other four species.

Feeding in first instar *H. ricini* and *H. sara* is noteworthy in that the caterpillars are found on the upper surface of a tender leaf which is still young enough to be at least partly folded.

TABLE V. FURTHER INFORMATION RELATING TO FEEDING BEHAVIOR

Key: +— Behavior in column above does occur.
—— Behavior in column above does not occur.

Species	Oriented along midrib	Eats standing on petiole or stalk	Holds loose piece in forefeet and eats	Eats from upper or under leaf surface	
				Normal leaf	Leaf inverted
Dione juno	Sometimes	Only just before pupation	—	Both	Both
Agraulis vanillae	Sometimes	—	—	Both	Both
Dryadula phaetusa	Usually	+	?	Under	?
Dryas iulia	Usually	+	+	Under	Upper
Heliconius isabella	No	—	—	Under	Under
Heliconius aliphera	No	—	—	Under	Under
Heliconius melpomene	+	—	—	Under	Upper
Heliconius erato	+	+	+	Under	Upper
Heliconius ricini	+ Except when grouped together & when parallel to it	—	—	Upper in 1st instar, lower or both later	Upper
Heliconius doris	?	?	?	Both	Both
Heliconius sara	Sometimes	—	—	Both	Both

They congregate between the two blades of the leaf, chewing away from inside their cover. This habit, which in *H. ricini* may continue into the early part of the third instar, differs from the behavior of the older larvae, which remain as far as possible on the under surface of the leaf (Table V). In *H. ricini,* however, the attraction does not lie in the upper surface of the leaves as such but in the fact that the blades provide a cover. If a young leaf is bent in the opposite way from normal (*i. e.*, with two under surfaces together), young *H. ricini* still collect between the two folded blades, although they are now on the under surface.

The surface of a leaf on which a caterpillar feeds may in some cases determine the feeding pattern adopted. When *H. melpomene* is placed on the under surface of a *P. lonchophora* leaf, it orients along the midrib of one of the leaflets, starts from the tip and eats straight across, midrib and all. If, however, it is placed on the upper surface it orients as *H. aliphera* and *H. isabella,* and eats from the margin of the leaflet, tending to leave the midrib. Thus the straight-across type of feeding is probably dependent to a large extent on the larva's having the midrib for orientation and this feeding pattern may be a specialization of one not dependent on the presence of a marked midrib.

f. *Eating Actions*

The third column of Table V shows that only two species, *D. iulia* and *H. erato,* are known to use their legs for holding a loose fragment of leaf while they eat it, though it is possible that *D. phaetusa* may also behave in this way. *D. iulia* and *H. erato* are the two species which normally eat even the petioles of their leaves, so it is not surprising that they have evolved a behavior pattern allowing them to make use of small fragments which are lost to other species. On the other hand, when it is the exuvia cast after a molt that are being eaten, all species which have been watched use their first legs and usually their second in manipulating the empty skin and its scoli. This is perhaps explicable if the exuvia-eating pattern is an old and stable one within the subfamily, uninfluenced by evolutionary adaptations relating to new food plants.

H. erato, D. iulia and *D. phaetusa* also share what seems to be a related behavior pattern, in which they sit near the base of a leaf, on the petiole or even the stem, and chew at the distal end of the leaf, holding it with their legs and bending it back towards themselves. This habit of "pulling up" the leaf would also seem to be associated with the particular vine on which these species normally feed. *P. tuberosa* is one of the few species with medium-sized leaves still flexible enough to allow such bending. The behavior occurs when the larvae are in the fourth or fifth instar and are already beginning to become heavy for the thin and flexible leaves.

H. erato and *D. iulia* appear to twist their heads more freely in eating than do the other species, while *H. melpomene* and *H. ricini* seem more flexible in this respect than *H. aliphera* and *H. isabella.* These differences are also reflected in the fact that, when they rest between a series of bites, *H. erato* and *D. iulia,* and to a lesser extent *H. melpomene* and *H. ricini,* have their heads overlapping the margin where they have been chewing.

There is no indication of "right-handed" or "left-handed" caterpillars, in that the head is never twisted to one side more than another. It goes to whichever side is the more convenient, considering the surface to be eaten and the disposition of the caterpillar's body as a whole.

Eating movements of all these species are alike in that the caterpillar extends and twists its neck. The mandibles then bite into the leaf. The head is drawn a little closer to the body and the jaws bite a second time. This is repeated until the head is against the body or the edge of the food is reached. The number of bites made during this movement of the head varies from 3 to 15, depending upon the length of material available for eating and its nature–more than one bite is given at a point where the material is tougher, such as a leaf vein.

While the head is being brought in from the extended position, the legs in all species are usually involved to some extent in shifting the thorax backwards. Sometimes all three pairs move but usually only the first two. There is a suggestion that the hind legs of *A. vanillae* are more active in this backward movement than those of other species. This may flatten the numerous glandular hairs with which *P. foetida* is covered. As will be seen later, this species shows a locomotory specialization which may also have evolved in relation to the problem of the thick, sticky hairs on this plant.

In eating, *H. isabella* and *H. aliphera* move their legs somewhat differently from the other species studied. A front leg, the one on the side to which the head is turned, beats rhythmically as the caterpillar makes its series of bites. The beats are generally made in the air but occasionally the foot will touch the leaf surface. No suggestion can be made concerning the significance of this movement and it is mentioned merely as a pattern shared by *H. aliphera* and *H. isabella* and appearing in no other species observed.

g. *Eating of Egg-shells*

Recently emerged larvae of the solitary *H. melpomene* and *H. erato* eat their own egg-shells within 10 to 20 minutes of leaving them. If they come across remnants of the egg-shells of other caterpillars, they chew these too. When they find an egg which still has an embryo within, they show no inhibitions about eating into the shell and devouring the embryo. Were such behavior present in gregarious species such as *H. doris, H. sara, D. juno* or *H. ricini,* a whole batch of eggs might be destroyed by the first few larvae to emerge.

If, however, those caterpillars which are gregarious are less attracted to egg-shells they would be less likely to eat their fellow-larvae. This should be reflected in the absence of a tendency to eat their own empty egg-shells. Twelve *H. sara eggs* have been investigated in this respect and it was found that they had not been chewed other than at the emergence holes. *H. wallacei* has apparently a similar inhibition about eating its own or other egg-shells. This does not, however, hold for *D. juno* nor *H. ricini,* so that either a different method of preventing cannibalism has evolved here or inhibition of egg-shell eating is in no way associated with protecting developing embryos from predation by other caterpillars.

h. *Eating of Cast Skins*

The only question relating to the eating of cast skins that has been investigated, is whether or not foreign skins were acceptable and for how long. The answer is that any one species will accept the skins of any other if they are fresh and from first to fourth instar animals. Skins more than 24 hours old may still be eaten but they are often rejected. Similarly the skins cast by larvae molting out of the fifth instar and into a pupa are occasionally eaten (with the exception of the head capsule) but are usually rejected.

In testing the edibility of these various skins, the practice was to tie a piece of cotton thread tightly around the skin to be tested and then tie or tape the thread to the stem or leaf at the point from which the test animal's own skin had been removed. It was often the thread which the caterpillar ate when it moved to investigate the skin. Part of the attraction to the skin might lie in the spikiness of the spinules of the scoli, for the cotton threads had numerous minute threads attached to them, projecting like the spinules on the skin.

i. *Drinking*

Caterpillars of 10 species have been seen to drink water from small droplets on their leaves and there is little doubt that the other species also do this. The mouth is applied to the water, the mouthparts move and then remain still for the rest of the time. There is no indication that the drinking pattern is derived from that of eating or vice versa.

In the field even during the dry season there is abundant dew at night so that caterpillars have opportunity to drink adventitious water even when there has been no recent rain. They appear to thrive better in captivity when given free water to drink than when they are kept at a very high humidity but given no free water.

A pattern which is most noticeable in *H. aliphera* and *H. sara,* although it occurs in caterpillars of other species as well, is that of regurgitating a drop of green fluid when severely disturbed. After the effect of the disturbance has passed, the caterpillar takes up the fluid again, using the same pattern as when drinking water.

V. DEFECATION

Caterpillars all defecate at intervals throughout their periods of eating and resting and during locomotion. The rate of defecation varies, as measured by the time between the production of two fecal pellets, rising during any one instar with the increase in the duration of feeding periods. On the other hand the size of the pellets increases from one instar to the next, so that the range over which the defecation rate changes is roughly the same for the different instars of any species. As Nagasawa (1957) reported for the larvae of the gypsy moth, *Lymantria dispar* Linnaeus, there is no stepwise increase in the size of the fecal pellets as there is in head capsule size.

A fecal pellet is extruded as a small green cylinder, the form being very similar in all species. It dries and darkens to a brownish-black mass over a period of 2 to 3 hours. The defecatory behavior patterns of the species of heliconiine caterpillars studied here are all alike; the anal prolegs release their hold of leaf or stem, the hind end is raised into the air and the pellet expelled with more or less force from the anus. The rectal opening then closes and opens several times, moving rhythmically with the pads of the anal prolegs. Finally the hind end is once more lowered. *H. isabella* is the only species in which there is a noticeable waggle of the hind end immediately after defecation and even in this species it does not always occur. A caterpillar of any of the ten species watched (*H. doris, H. numata, H. wallacei* and *P. dido* have not been observed), will turn its head to its anus and with its jaws remove a pellet which has become stuck. Sometimes even after the pellet has been thrown free, the caterpillar turns and chews in the region of its anus, presumably at particles which remain.

When caterpillars are on a vine in a natural position, the fecal pellets are either shot off or roll free of the leaf. Raised in dishes in the laboratory, however, the caterpillars frequently come across their own cast pellets. In these conditions a larva picks up the pellet with its mandibles, lifts its head and releases the pellet so that it may fall off the leaf or roll away. There is a slight tendency to jerk the head as the pellet is released so that it is actually thrown. In a dish in the laboratory a caterpillar invariably comes across the same or another pellet within a few minutes. It continues to reject pellets for a variable length of time and then apparently accommodates to the situation, ignoring pellets which are right beside its head. Constance Carter, who has raised large broods of *H. melpomene* for genetical work, reports that her caterpillars finally ceased to show the pellet-throwing behavior.

H. aliphera and *H. isabella,* when presented with fecal pellets, may attempt to throw them as do the other species. They may, however, show a variation which is peculiar to them. Instead of picking the material up in its mouth, a caterpillar bobs its head several times in the direction of the pellet, knocking it with the long head scoli. If the pellet is merely caught on some irregularity on the leaf, this treatment sometimes frees it and it falls away. In laboratory rearing dishes it is of course not usually effective. What is of interest is that the head bobbing or beating with the head scoli is also a response which both *H. isabella* and *H. aliphera* give to other more general disturbances.

D. juno is the only caterpillar in which an activity takes place that might be called "social defecation." Instead of a caterpillar turning and pulling a pellet free of its own anus, *D. juno* may have the pellet removed and rejected by another larva which is passing at the time. Usually the second caterpillar assists when the pellet is almost free, but sometimes it is almost dragged out of the defecating animal. It is not known how early in larval life this habit appears, but it persists into the late fourth instar before disappearing in the fifth. During the period over which the behavior occurs, a caterpillar is perfectly capable of ejecting pellets alone or of turning round and freeing a pellet from its own anus.

VI. RESTING

After an interval of feeding, caterpillars stop eating, turn away or walk backwards a short distance, and go into a resting phase. Both the position of a caterpillar relative to the leaf and its posture are, to a large extent, specific characters.

TABLE VI. RESTING BEHAVIOR OF TEN SPECIES OF HELICONIINE CATERPILLARS

Species	Main /general larval pattern of resting position in relation to leaf	Any change in resting position with prepupal stage	Posture of body
Dione juno	No definite orientation except to each other		Either straightened out or with thorax elevated
Agraulis vanillae	No definite orientation	Tends strongly to rest on stem or petiole	In "J" during early instars, later straight and extended on stem
Dryadula phaetusa	Along midrib		Usually "J" but straighter in later instars
Dryas iulia	Along midrib	Some tendency to rest on stem	Occasionally straight especially late 5th instar. Usually in "J"
Heliconius isabella	As in eating or facing opposite direction		Invariably straight, or with very slight curve
Heliconius aliphera	As in eating or facing opposite direction		Thorax usually curved to side. "J" less acute
Heliconius melpomene	Along midrib	None except coming off stem onto bottle in morning	Straight but usually thorax just out of line in front
Heliconius erato	Along midrib or stem	Strongly tends to rest on stem	Straight
Heliconius ricini	Along midrib, parallel to midrib or an oblique position		Straight but often thorax just out of line in front
Heliconius sara	No definite orientation except to each other		Straight, sometimes thorax elevated
Heliconius doris	?	?	?

a. *Resting Position*

Resting positions on a leaf are indicated in Text-fig. 5 and the last column of Table VI. In the same way that many behavior patterns change during the larval period, so does the choice of resting position. These positions can be divided into three main categories:

1. Those in which the caterpillars (*H. aliphera* and *H. isabella*) are oriented neither to the midrib or margin of the leaf nor to gravity. The only describable regularity is that in the resting position a caterpillar's body is oriented along the same line (or one parallel to it) as that taken during the previous period of feeding. The hind end may be pointing directly to or away from the site where the caterpillar finished eating.
2. Those (*e. g., D. iulia, D. phaetusa, H. melpomene, H. ricini* and to a lesser extent *H. erato*) which rest with the main part of their body along the midrib, irrespective of their last feeding position. After two individuals of *H. melpomene* have been eating side by side on a *P. laurifolia* leaf, they both crawl up and rest on the midrib, one behind the other.
3. The final group contains those species whose resting position shows no consistent relationship to the form of leaf. The details vary from species to species. Thus *A. vanillae* sometimes rests along the midrib, sometimes faces its feeding place, sometimes faces the opposite direction and often lies with its body having no particular orientation to anything at all. In later instars, however, this species most frequently rests on the petiole or stem of its plant.

In social resting among those species which are to some extent gregarious, the caterpillars usually lie side by side, frequently but not invariably facing in the same direction. In natural conditions the orientation of *D. juno* is in relation to the other caterpillars of the group rather than to environmental markers. The same would appear to be true of *H. doris* and also *H. sara*. If there has ever been a tendency to orient their bodies to the leaf in a particular way during the resting phase, it seems to have been lost in these gregarious species. The same explanation might apply to the slight tendency which the semi-gregarious species *H. ricini* shows away from the *H. melpomene*-type orientation, for it does not invariably rest along the midrib, but may lie obliquely or parallel to it.

Part of the pattern of resting during the day, or at least in the morning, shown by fifth instar *H. melpomene* and *D. iulia*, has been the selection of a particular resting position, away from the leaf on which they were eating. In many cases it has been a large leaf near the base of the vine but in the laboratory caterpillars have frequently chosen to rest on the bottle in which their vine was stuck. The same position was selected day after day.

b. *Resting Posture* (see Table VI).

The posture adopted by these various species of heliconiines during their resting phase must also be considered. A resting pose which may well be basic to the subfamily Heliconiinae is one in which the greater part of the caterpillar's body shows a particular orientation on the leaf but the head and thorax are turned to one side, giving what may be called the "J" position. This is shown in its most extreme form in *D. iulia* and *D. phaetusa* (Text-fig. 5d). *H. aliphera* usually has a far less acute bend on the "J" and *H. isabella* such a gentle curve (when it is present at all) that it hardly resembles a "J" (Text-fig. 5a). *H. melpomene* and *H. ricini* rest in a "J" position in which there is no suggestion of a hook at the base of the "J", only a slight deviation from the straight line of the body, (Text-fig. 5b, c). When a number of *H. ricini* are kept together as a group, the "J" tends to straighten further.

Those species which show distinctly gregarious tendencies, *H. sara, H. doris* and *D. juno,* all rest with their bodies straight out or with the head and thorax arched up, the latter habit being especially marked in *D. juno*. This would seem to be merely a modification of the straightened position, allowing more crowding while preventing the animals' heads being buried. *H. erato* is somewhat anomalous for, although a solitary species, it rests with its body not only in a straight line but also extended; all the other species when they rest have their bodies contracted to some extent. *A. vanillae* in its fourth and fifth instars does, however, extend its body in resting on the stem.

The contraction of its body is most marked in a second anomalous case, that of *D. iulia* (Plate I). Here the whole thorax is drawn in towards the abdomen and this results in a marked humping of the first and second abdominal segments. Consequently the scoli borne on this region do not keep their normal orientation but all point forward along with those of the meso- and meta-thoracic segments, giving the animal a hunched appearance. The dorsal scoli of the second and third thoracic segments mingle with those of the first and second abdominal. The first lateral scolus, that belonging to the second thoracic segment, is always quite distinct in the resting pose, but the lateral scoli of the metathoracic segment lie with the supralateral ones of the first two abdominal segments. This rest position is highly characteristic of *D. iulia* and has not been seen in other species. It is interesting in that it is extremely like the position taken up when the caterpillar is disturbed by a blast of air (not a touch), the only difference being that a disturbed caterpillar contracts so that the first lateral scolus also lies in the bunch of scoli, instead of being separate as it is in the rest position.

VII. WEAVING

When caterpillars are kept on fresh vines which are acceptable to them as food, they do not walk about very much. Between eating and resting a caterpillar may turn and walk away but often *H. melpomene, H. erato* and *H. isabella* just shuffle slightly backwards. Characteristic of even this brief walking, however, is behavior which may be called "weaving." The head is swung regularly from side to side and the spinneret behind the mouth spins a silk thread, touching it down to the surface so that figure-of-8 tracks are left behind the caterpillar.

Even when a caterpillar walks as fast as it may, it still trails a silk thread behind it, though in such conditions it is not attached as frequently.

This spinning of silk throughout the entire larval stage is undoubtedly of value to the caterpillar in that it always has a safety line attaching it to the leaf or stem and there is thus less danger of its being swept away from its vine. If a caterpillar falls, it hangs suspended on its thread of silk and subsequently climbs up this and back onto the point where it was attached.

A mat of silk threads is also spun over the surface of a leaf and this provides a secure footing for the claws and for the crochets of the prolegs. By watching the individual movements of a caterpillar's feet in walking, it is clear that the claws rely to a great extent on the silk trail to provide a foothold. A limb may make several movements and only when its claws make contact with the silk does it grasp. The effect can also be demonstrated by comparing the footholds of caterpillars on surfaces upon which they have been allowed to weave. Parts of a glass plate and of a smooth leaf (*P. lonchophora*) were covered by the weaving of *H. aliphera*. A caterpillar was then dropped gently onto one surface or the other and as soon as it was on its feet, the glass or leaf was turned upside down. Occasionally the animal on the glass hung down for a short time, its prolegs not grasping the surface immediately, but there was never any risk of its actually falling off. On the silk-covered leaf even the prolegs were attached quickly enough to prevent the hind end from hanging loosely. Conversely the caterpillar fell off clean glass, although occasionally it managed to remain for some time on the clean leaf. During this time it would rapidly weave on the under-surface of the leaf and as soon as its claws and crochets came in contact with the silk attachments, risk of their slipping decreased. An ability to remain on glass plates when these are reversed may have little value in the natural life of a caterpillar of *H. aliphera,* yet it is a fact that *H. aliphera* and *H. isabella* are the two species which weave most markedly and are also the animals which rest under the smooth blades of their leaves. It has already been suggested that part of the advantage of resting on the midrib is that it allows a more secure foothold, so a species living on smooth leaves and not utilizing the purchase of the midrib might well increase the extent of its weaving so that silk could be used in place of the midrib.

A caterpillar of *H. aliphera* or *H. isabella* will touch its spinneret to the substratum, then stretch its head back and then finally forward and down again. This behavior may be called "yawning" and though all species do yawn during the construction of the silk pad just before pupation, only *H. aliphera* and *H. isabella* have been seen to do it during earlier larval life. In spinning the pupational pad, yawning draws the silk so that a loop is formed. *H. aliphera* and *H. isabella* yawn predominantly when they return to their resting positions after feeding and it seems that these two species rest on a mat of somewhat looped silk instead of one with plain attachments like the other species. This would presumably provide them with a more secure foothold.

Weaving may be expected to occur whenever the foothold of a caterpillar is precarious. In fact, it seems to be elicited by any surface which is strange to the caterpillar—even if it has already been covered with silk by another animal. There are exceptions to this rule, the ambulatory phase immediately before a larva hangs up to pupate being a clear example. During this period the caterpillar walks onto many strange surfaces but will not begin to weave until it reaches a potential site.

Another example of the weaving behavior was first noticed in the laboratory dishes. Fresh leaves were put in for the caterpillars each day and frequently when those of the previous day were being removed they were found to be tied firmly onto the dish and/or each other with silk threads. If there were a remant of the petiole left it was almost invariably this part which was attached to the dish, though sometimes another projection might be used. This phenomen is explicable in terms of what has already been said about weaving. The caterpillars remain on their leaves for most of the time, refusing to abandon a leaf in order to walk onto the glass. When they encounter the glass, however, there is a bout of weaving as they touch the strange surface; thus at this point the stem and glass become attached by a series of silk threads. The species on which these observations were initially made comes onto or deserts a leaf, walking along the midrib, so that caterpillars in the dishes would usually have come into contact with the glass from one or the other end of the midrib. Thus it was here, the petiole or sometimes the tip of the leaf, where the silk attachment was formed.

There are observations which suggest that such "tieing-up" behavior is not unnatural and that it occurs in the field. In a number of instances caterpillars, living freely on large pieces of vine, have been seen to weave steadily between the petiole and the stem to which it is attached, thus reinforcing the natural junction. The behavior has been particularly noticed in *H. melpomene* but also occurs in other species. The same or a very closely related pattern is sometimes observed in *H. sara,* where a group of caterpillars will tie two or several leaves together with silk. Bell (1920) records how the larvae of the oriental lycaenids, *Vivachola isocrates* and *V. perse,* bind the stalk of the fruit on which they are feeding onto the branch with silk, and such behavior doubtless occurs among many other lepidopterous larvae.

Tieing the whole leaf onto the stem would seem a possible safeguard against the leaf's becoming detached while the caterpillar eats from it—an event which has in fact been seen on two occasions in Trinidad. The same explanation could be true of the behavior of gregarious *H. sara,* for *P. auriculata* leaves will sometimes drop off while still green and while being chewed by the caterpillar. It is also an advantage when the leaf concerned is chewed loose by animals proximal to it. Any caterpillars which are feeding distally are then still able to cross by the silk bridge onto another leaf and so back to the vine. This also has been observed in the laboratory.

Even if its leaf comes loose or another animal chews away the link between leaf and vine, there is still a possibility that a caterpillar may be able to climb up and regain its place. This cannot be said for a pupa in the same circumstances, because its power of movement is so limited. It is therefore not surprising that a caterpillar, preparing to pupate on a leaf petiole, old flower stalk, tendril or even a leaf, should frequently weave between the object on which it will pupate and the main body of the vine. Such behavior would certainly be selectively advantageous and has been recorded in many lepidopterous larvae (see Ford, 1945, and Hinton, 1955). Indeed, in the present study there have been four instances in *D. iulia,* one in *A. vanillae* and one in *H. melpomene* in which the slight silk attachment alone was holding the pupal support on the vine. The consequences of one of these pupae falling to the ground will be considered later.

The final point about weaving is that it may occur in situations in which the caterpillar seems merely to be generally disturbed. *H. aliphera* is especially prone to weave very actively on the surface of its leaf if it is poked, blown on, shaken or if another caterpillar comes near it. This could perhaps be regarded as an example of a displacement activity, though it may also be argued that a behavior pattern which ensures a more secure attachment to the substratum would be appropriate in a situation in which the animal is being attacked in any way. It seems very similar to behavior described by Dethier (1943) in lepidopterous larvae removed from their plant food and which he interprets as a "visual searching movement."

VIII. LOCOMOTION

Locomotion which occurs between feeding periods is normally of short duration. Nevertheless some specific differences are apparent. *H. aliphera, H. isabella, H. ricini, H. melpomene, H. sara, D. juno* and *D. phaetusa* make practically no movement at all, sometimes just walking slightly backwards, sometimes turning and walking a few centimeters. *H. erato, D. iulia* and *A. vanillae* often walk an appreciable distance, up the leaf, onto and along the stem. *D. iulia* walks very much more quickly than the others, directly and without stops. Conversely *H. erato* barely seems to be moving at all and frequently stops altogether. *A. vanillae* walks slowly, eating the hairs from the leaf, petiole and stem as it goes.

Both *A. vanillae* and *D. iulia* have peculiarities in their mode of walking but these do not always appear. *A. vanillae* has a strange, jerky stride; the anal prolegs are raised, carried forward and then oscillated back and forth just above the stem several times before they are finally put down in the new position. The head and legs also show this jerky motion but not as strongly as the hind end. It is possible that the action normally results in flattening down the glandular hairs with which *P. foetida* is liberally covered, thus producing a clear area for attachment of the anal prolegs. These caterpillars seem rarely to leave their vines, even pupating on them; this might explain why they sometimes walk in this jerky way even in situations where there are no hairs to flatten, as when walking down a piece of wire. The peculiarity in walking of *D. iulia* also consists of a movement of the hind part. In this case the last segment with the anal prolegs is lifted and lowered sharply several times during each short burst of forward locomotion. No explanation is offered for this pattern.

The locomotor stage which frequently occurs just before pupation will be considered more fully later. Suffice it to say here that in most species it starts very sharply and that it may last as long as three hours, during which time the animal may cover as much as 50 meters of ground. With the exception of *A. vanillae* and occasionally *D. iulia,* the walking motion is smooth. *H. aliphera, H. isabella, H. erato* and *D. phaetusa* will seldom walk as far as a meter without stopping for one or more short rests. During this walking stage the animals show no obvious photopositive or photonegative orientation. There is, however, slight evidence of a negative geotaxis, at least in *H. aliphera, H. isabella* and *D. iulia.*

IX. SOCIAL BEHAVIOR

Information which allows an estimate of the degree of social behavior among larvae is available for only 10 of the 14 species of Heliconiinae in Trinidad. Of *D. phaetusa, P. dido, H. numata* and *H. wallacei* it can be said only that the last-mentioned alone among them is gregarious.

The other 10 species can be arranged in a series from the typically aggressive, asocial caterpillars of *H. erato* to those of *D. juno* which are not only gregarious but show signs of actual social behavior. As has already been said (Table III), the eggs of some of the species are laid together in a group so that the larvae of these species, *D. juno, H. ricini, H. sara* and *H. doris,* start living communally. The eggs of *H. isabella* and *H. aliphera* are never laid in a group but the female is not averse to laying more than one egg —up to six in the case of *H. aliphera*—on a single leaf. This contrasts strongly with the behavior of the female *H. erato* which flies away to another vine rather than lay an egg on a set of leaflets where one is already glued.

The degree of intolerance of one caterpillar for others, either of its own or another species, is greatest in *H. erato,* which is never found sharing a leaf without fighting. *D. iulia, H. aliphera* and *H. isabella* also remain solitary on their leaves but when more than one caterpillar are together, they do not bite at each other as *H. erato* would do. A specimen of either of these three species will swing its head and thorax at an intruder or will shake the whole of its anterior half but there is very seldom any contact at all between such caterpillars. Two individuals of *H. aliphera,* for instance, may try for several hours to share the same leaf and it seems to be only their mutual disturbance which eventually results in their separation. Further, newly-emerged larvae of *H. erato* will attack and eat other larvae or eggs, while such behavior is comparatively rare in *D. iulia, H. aliphera* and *H. isabella.*

H. melpomene, while it eats eggs and newly-emerged larvae during its first instar, appears later to become more tolerant and will share a leaf with another caterpillar of approximately its own size even when there is an ample amount of leaves available. *A. vanillae* shows no violent reaction to sharing its leaf with others of its own or other species and has, furthermore, never yet been found to eat eggs or newly-emerged larvae. *H. ricini* shows even less response to other caterpillars than does *A. vanillae,* not even swinging its head at them as a rule. There is nevertheless no great attraction evident between *H. ricini* caterpillars. Although they may eat, rest and molt together as a group, one or two will frequently remain separate from the others, an occurrence which is almost never seen in *H. sara, H. doris* or *D. juno.*

Except in cases of food shortage, there is never any aggression between caterpillars of *H. sara, H. doris* and *D. juno.* In fact, individuals of the last-mentioned species seem distinctly unsettled when separated from others of their group. Even small groups of three or four caterpillars do not remain discrete in the presence of a larger collection, but join it. One specimen of *D. juno* appeared less unsettled when allowed to share a leaf with *H. ricini* than when it was alone.

Molting has not been watched in conditions approaching normal for *H. doris.* In glass dishes, however, they clearly orient to each other. Groups of *H. sara* and *D. juno* molt on the stem of their vine and are oriented to each other. *D. juno* form a double ring around the stem, bodies parallel to each other and to the stem, most with their heads pointing to the center. *H. sara* shows a far less strict orientation although the bodies of the larvae are parallel to each other.

As has been said in the section on feeding, gregarious species and in fact those which are not normally gregarious but are kept in groups by the experimenter, establish synchronization of feeding and resting. The degree of coordination varies from the strict effect in *H. doris* and *D. juno* to the more ragged one typical of *H. ricini* and the naturally solitary species. Similarly the synchronization of molting and pupation is more marked in the truly gregarious species.

Finally, *D. juno* alone has been seen to practice what has been termed social defecation, one larva discarding a fecal pellet from the anus of another. It has not been established whehter *D. juno* will remove pellets from the anus of a species other than its own, nor is it clear whether or not there is any selective advantage to be had from social defecation. Certainly it would appear an easy habit to acquire in that all species of heliconiines have been seen to throw free pellets which they come across.

X. Defensive Behavior

Study of these caterpillars was started with a view to using their defensive reactions as clues in physiological work on their sense organs. It soon appeared that such responses are very variable and probably depend on the basic activity of the larva at the time. It was therefore necessary to expand the project so as to get some idea of these "basic activities." The caterpillars were disturbed as little as possible by the observer, the result being the information presented in sections I and II of this study. A further result was that defensive behavior was seldom elicited except in encounters between the caterpillars themselves or in fights with insects such as ants or mantids. It should therefore be held in mind that caterpillars may be capable of far more drastic and clear-cut defense responses than are described for them here.

The stock response is that of turning from side to side or banging the head and thorax continually towards the side that was stimulated.

In *H. isabella* alone the caterpillar may loosen the hold of its anal and posterior prolegs and thump its tail end up and down when disturbed. It is noticeable that this species is alone in having its posterior segments a bright contrasting color (see Beebe, Crane & Fleming, 1960). Two apparently otherwise unrelated species, *H. sara* and *H. aliphera,* are both prone to regurgitate contents of the gut, though such behavior can be elicited in other species as well. *H. aliphera* and *H. isabella* both beat at an object or intruder with their long head scoli, a habit already mentioned in the section on defecation.

Although *H. melpomene* and *D. iulia* also have long scoli on their head capsules, they have never been seen to use them as do *H. isabella* and *H. aliphera.*

XI. Phylogenetic Discussion

The observations recorded here were part of what was essentially a preliminary study, an attempt to expose problems which allow experimental analysis, and consequently any phylogenetic conclusions must necessarily be extremely tentative. It would appear that they are worth discussing, nevertheless, if only because they define more clearly what further information is needed.

The activity patterns have not revealed any striking similarities or differences between the species. While it would obviously be of great interest to investigate the control of these, it seems improbable that information on this score will contribute to knowledge of relationships. The nocturnal feeding in *D. iulia* and *H. melpomene* has probably been acquired independently—it is clearly a later specialization, taking effect only in the fifth instar. It certainly seems worth discovering whether the method of control is the same in both.

On the basis of feeding behavior, the 10 species studied fall into three major groups. This is true of patterns in the first instar larvae as well as later on and may be further correlated with the resting position and posture taken up between feeding. Within these three there are other, closer associations of species.

The first group is that comprising *A. vanillae, D. juno, H. aliphera* and *H. isabella.* None of these habitually orient their bodies along the midrib of a leaf or leaflet, either during feeding or rest. It is not known for certain how the first instar *A. vanillae* eat but certainly some individuals have been seen to chew holes in their leaves. The other three species all scrape the green cells from the surface of leaves, and it seems probable that this pattern is fairly generally present among heliconiines even though it is not in all cases the one naturally shown. If a leaf is thin enough, the caterpillar is likely to produce small holes where it has scraped and it seems possible that this is what occurs in the case of *A. vanillae.*

While *A. vanillae* and *D. juno* do not orient in relation to the midrib, *H. aliphera* and *H. isabella* never lie along or on it and appear to orient away from it. Their feeding patterns, especially that of *H. isabella,* result in the midrib's being left on an abandoned leaf. This tendency is perhaps reflected in two other characteristics shared by *H. aliphera* and *H. isabella.* First, these two show a greater development of weaving behavior than any other species. This would be of importance when the foothold provided by a midrib is unavailable (see p. 18). Second, there is the trend seen in *H. aliphera* and characteristic of *H. isabella* towards attaching the pupa to the smooth blade of a leaf rather than the midrib of some other vein or protrusion (see section II).

H. aliphera and *H. isabella* both wave a single foreleg rhythmically during their feeding, a further indication of the sharing of behavior patterns.

The second group consists of *H. melpomene, H. ricini, H. erato* and *H. sara* (possibly *H. doris* as well). When these caterpillars are solitary their feeding position is sharply oriented towards the midrib, in that they lie along it during the rests between chewing as well as during feeding. When alone, all of these species eat straight across the leaf, cutting through the midrib as well as the blade. When raised in a group *H. ricini* and *H. sara* both become inconsistent in their patterns but this is to be expected, for the animals now tend to orient in relation to each other rather than to the leaf.

Furrowing across the midrib occurs in some circumstances in all of these four species. Until its function has been established, too much emphasis should not perhaps be laid on this. Yet it is suggestive of a fairly close relationship among the species, especially as it is in *H. erato,* at least, what might be called "vestigial behavior"—a pattern elicited only when the caterpillar is in somewhat abnormal conditions.

It seems possible that *H. erato* comes from a stock which lived on *P. laurifolia,* as do *H. melpomene* and *H. ricini.* When it migrated to the slender *P. tuberosa* vine, *H. erato* lost the sharp orientation to the midrib from its natural repertoire of behavior patterns and this only reappears now if it is fed on *P. laurifolia.* On *P. tuberosa* feeding and resting are still relative to the midrib but the habit of midrib furrowing is not displayed. Chewing straight across the blade and midrib is replaced to some extent by behavior such as the "pulling-up" technique. This enables *H. erato* to utilize even the tips of slender leaves onto which it is too heavy to climb. The flimsiness of many *P. tuberosa* leaves may also have led up to the evolution of the practice of resting on the stem instead of the leaf.

H. sara and *H. ricini* share the characteristic of eating holes or channels in subterminal leaflets while these are still folded together. This might be taken to indicate a close relationship.

Another explanation is, however, possible. Their eggs are laid in batches and are smaller than those of *H. melpomene* or *H. erato;* the maximal diameters of the eggs of *H. sara* and *H. ricini* are 0.65 and 0.70 mm. respectively; corresponding minimal diameters are 0.92 mm. for *H. melpomene* and 0.81 mm. for *H. erato*. Correlated with the smaller eggs are smaller first instar larvae and it is possible that their size allows the caterpillars of these species to pass between the folded blades of the young leaves. The significance of egg size is not clear. It might be related to the habit of laying eggs in batches, *i. e.*, laying a large number at once instead of a few each day. If this is indeed the case, the similar early larval habits may be more a reflection of adult behavior and physiology than a simple case of larval similarity.

The third group contains *D. iulia, D. phaetusa* and almost certainly *P. dido*. Feeding and resting orientation, as in the second group, are in relation to the midrib. Bridging and channeling behavior, however, is quite distinct from furrowing. In *D. iulia* such behavior is very consistently present and occurs on *P. auriculata* and *P. rubra* exactly as it does on *P. tuberosa*. The leaves of these two vines are furrowed by *H. erato* and *H. sara*, however, so that there is no question of channeling behavior being distinct from the furrowing simply because they occur on different plants.

Here again the feeding of first instar larvae is fairly distinct from that of the other two groups. *D. iulia, P. dido* and almost certainly *D. phaetusa* chew long channels, starting at the margin. This pattern is in fact clearly related to their channeling and bridging behavior which occurs later.

Despite the fact that *D. iulia* and *H. erato* have been relegated to separate groups here, there are nevertheless a number of similarities in their feeding patterns; both will bend a leaf back and chew it, both eat the last remnant of leaf, then the petiole and often even the stem. The sculpturing of the mandibles is superficially very alike in *H. erato, D. iulia* and *D. phaetusa*, the large cusps on the mesial end of the maxillary edge being especially well developed (see Text-fig. 8a, b, c). All these seem to be adaptations that might well be produced in species living on vines with few, flimsy leaves and where behavior and chewing apparatus were evolved in relation to minimal wastage of plant material.

The feeding pattern of the larvae of the first group, and especially *A. vanillae*, is considered to be more primitive than those of the others. This view is based on two facts. First, an individual from the second or third group, when forced to feed without the orienting signal of a midrib, shows a pattern which could easily have been produced by a caterpillar of the first group. Thus *H. melpomene* placed on the upper surface of a *P. lonchophora* leaf eats the margin instead of the tip of the leaf, frequently leaves the midrib and between periods of feeding orients relative to the place where it has been chewing, not the central part of the leaf. Second, *P. laurifolia* has a very well-developed midrib. This feature or the smoothness of the blade might influence a caterpillar towards orienting on the midrib; both together should be more effective. *A. vanillae* will indeed orient its body along the midrib of a *P. laurifolia* leaf. However, it neither eats in the specialized straight-across manner of *H. melpomene*, isolated *H. ricini* and *H. sara*, nor does it normally orient to the midribs of other vines.

Resting positions on the leaf have already been discussed. The basic pose of the body during rest seems to be a curve or bend and, with the exception of *H. erato* and larvae living in groups, the caterpillars of all species show this at some stage. *H. isabella* is less inclined to do so than *H. aliphera*, another fact indicating that it is somewhat further along a line of specialization than *H. aliphera*.

There are clear differences between the postures of *D. iulia* or *D. phaetusa*, that of *H. aliphera* and that of *H. melpomene* or the solitary *H. ricini*. The first two take up a distinct "J" position, with only the head and thorax twisted to one side. The bend in a resting caterpillar of *H. aliphera* is approximately at its second abdominal segment, while *H. melpomene* lies in a straight line with only its head and thorax slightly to one side (see Text-fig. 5b).

All species which rest along the midrib, *H. erato* and in their later stages *D. iulia* and *A. vanillae*, hold their bodies straightened out but this seems to have no more significance than that most stems or midribs are straight. *H. erato* and the later stages of *A. vanillae* rest in an extended position, but the fact that young *A. vanillae* rest in a contracted and bent posture suggests that this is more probably a case of convergence than relationship. *D. iulia* alone produces its peculiar humped-up rest pose, with its characteristically bunched spines, and no sign of this has been seen elsewhere, not even in *D. phaetusa*.

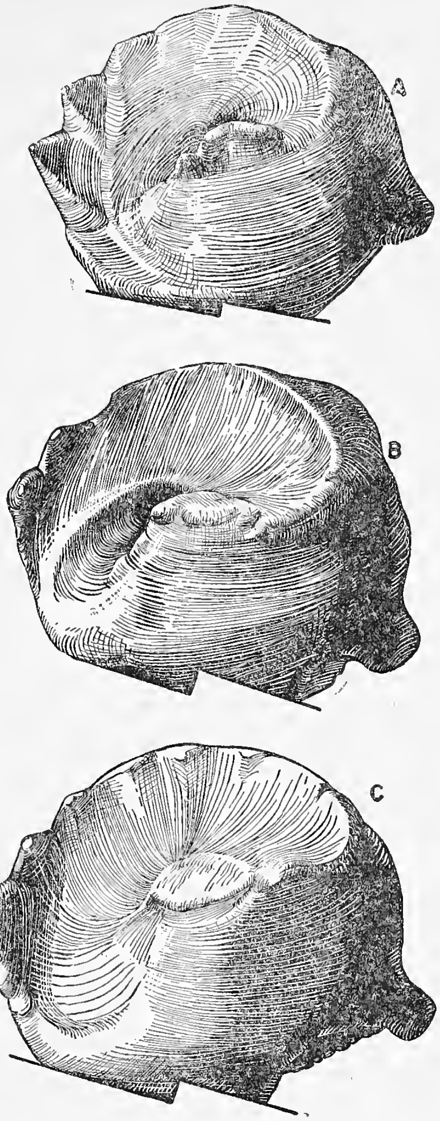
The gregarious species, *D. juno, H. doris, H. sara* and to some extent *H. ricini*, usually rest with their bodies laid straight along the substratum though this is less marked the fewer animals there are in the group. In some instances the caterpillars rest with their thoraxes elevated. This usually occurs when animals would otherwise overlap each other. It may appear even when there is no suggestion of crowding and occasionally occurs when a caterpillar rests alone.

It seems clear that the resting heliconiine caterpillar has its body bent laterally and that those species which rest in a straight line are the exceptions. However, there is no suggestion concerning the control of the behavior in each individual, for *D. iulia* will occasionally be found in a "J" pose even when sitting on a straight stem. The anterior part is in this case free in the air. Nor is there any indication of a possible selective advantage of such a bend to the larvae.

The range of social behavior does not at all reflect the groupings suggested on the basis of feeding and resting. *H. erato* is fairly distinct in its sharp intolerance for other caterpillars of any species. On the other hand the remaining species of the group in which *H. erato* has primarily been classified vary between vague tolerance on the part of *H. melpomene* to the distinct gregariousness of *H. ricini, H. sara* and *H. doris*. The question of social behavior in *D. phaetusa* and *P. dido* is largely an open one; if they are at all like *D. iulia,* they show neither violent aggressiveness nor any signs of gregariousness. The third group varies from slight intolerance shown by *H. aliphera* and *H. isabella* through the tolerance of *A. vanillae* to very distinct gregariousness in *D. juno*.

On the information available here there is nothing to distinguish the trends towards social behavior seen in *H. ricini, H. sara, H. doris* and *D. juno*. There is no indication that these have been independently achieved although this would be inevitable on evidence from feeding and resting. The matter of eating egg-shells (p. 14) has not been taken far enough to serve as more than a pointer to the need for further information.

In conclusion, larval behavior as estimated from feeding, defecation, locomotion, resting and weaving is specifically distinct. It seems likely that the differences reflect phylogenetic relationships within the group. If this be so, the present study suggests that *H. isabella* and *H. aliphera* are closely related to each other, *H. aliphera* being nearer the other species of *Heliconius*. Of these other *Heliconius, H. erato* is specialized away from *H. melpomene, H. ricini* and *H. sara* but shows indications of relationship nevertheless.



Text-fig. 8. The biting surfaces of the right mandible of **a**, *H. erato;* **b**, *D. iulia;* and **c**, *D. phaetusa,* showing the common development of the first maxillary cusp and the separation of the maxillary and molar parts. Drawings by F. Waite Gibson.

D. iulia, D. phaetusa and almost certainly *P. dido* form a group which in many ways parallels that of *H. melpomene*. Of these *D. phaetusa* is less specialized in behavior patterns and shares to a lesser extent similarities which seem to have been independently evolved in *H. erato* and *D. iulia*.

D. juno and *A. vanillae* are less alike but on the whole they resemble each other more than they do any other species. In most respects these two, and more particularly *A. vanillae,* would seem fairly closely related to primitive heliconiine stock.

XII. SUMMARY

1. Larval behavior of 11 of the 14 species of heliconiine butterflies of Trinidad was observed in the laboratory and to a limited extent in the field.
2. Periods of feeding alternate with quiescent phases, the extent of each depending somewhat on the species but varying in different instars and during any particular instar.
3. Feeding behavior is described in terms of preference for particular species of the food plant, vines of the Family Passifloraceae, the pattern left on the leaves and the movements made during eating. The first two differ from species to species but the last is very similar in all species examined.
4. Observations are recorded on how the larvae eat egg-shells, drink and defecate
5. Resting is described in relation to position on the leaf and posture of the body.
6. The activity of spinning silk threads is described, its significance discussed and the different emphasis on such behavior in different species pointed out. Slight peculiarities of locomotion in some species are mentioned.
7. The extent to which a caterpillar will tolerate others of the same or other species is discussed, together with evidence for gregarious behavior in regard to feeding, resting, molting and defecation.
8. Defensive behavior is briefly mentioned.
9. On the evidence of the behavior described above, the grouping of species is discussed, It does not agree fully with that of the present taxonomy although it is itself consistent within its limits.

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EXPLANATION OF THE PLATE

PLATE I

FIG. 1. Resting posture of *D. iulia,* showing the contracted state of the first abdominal and last two thoracic segments and the consequent bunching of the anterior scoli. Photograph by Russ Kinne.

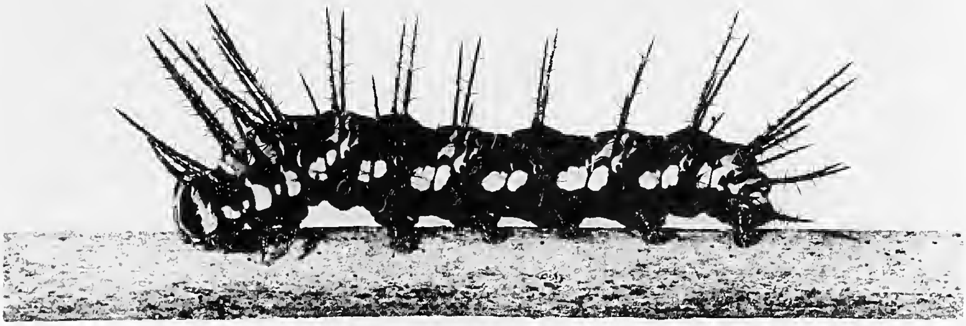

FIG. 1

A STUDY OF THE BIOLOGY AND BEHAVIOR OF THE CATERPILLARS, PUPAE AND EMERGING BUTTERFLIES OF THE SUBFAMILY HELICONIINAE IN TRINIDAD, W. I.

2

Hybridization Experiments in Rhodeine Fishes (Cyprinidae, Teleostei). An Intergeneric Hybrid between Female *Rhodeus ocellatus* and Male *Acanthorhodeus atremius*

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(Plate I)

In an attempt to determine whether any genetic affinities still exist between rhodeine species, a number of interspecific and intergeneric crosses have been performed under laboratory conditions. This paper deals with results obtained by crossing *Rhodeus ocellatus* (Kner) with *Acanthorhodeus atremius* (Jordan & Thompson), both of Japanese origin.

One male *A. atremius* and four female *R. ocellatus* were placed in an aquarium, together with three South African freshwater mussels (*Aspatharia wahlbergi* Krauss). Although spawning behavior of these fishes differs slightly, spawning occurred. At short intervals fry were released by the mussels, and 15 arbitrarily selected larvae have been raised to the adult stage. A representative specimen of the adult hybrid form is illustrated in Plate I, Fig. 1.

All hybrids showed a male phenotype. In the breeding season they displayed full nuptial colors. Tubercles were abundantly present on the top of the snout. Spermatogenesis was normal. Their taxonomic characteristics will be described in a separate publication.

Four of these hybrids were allowed to breed freely with six females of the parental species, *R. ocellatus,* in the presence of six South African najads (*Aspatharia wahlbergi* Krauss and *Unio caffer* Krauss). Offspring were produced in the course of the following six weeks.

Twenty-five larvae, which had escaped from the mussels during one day, were placed in a separate aquarium and raised to the adult stage. Eighteen of them developed into functional females while the remaining seven specimens showed a male phenotype. The females developed a long ovipositor, and normal spawning behavior was observed. They were uniform in body size and shape and intermediate between both ancestral forms. A representative specimen is illustrated in Plate I, Fig. 2 (top). The males, however, were not uniform in size. The largest ones attained the body sizes of adult male *R. ocellatus* and the smallest ones those of adult male *A. atremius.* Representative specimens showing these extremes are illustrated in Plate I, Fig. 2. In all males, spermatogenesis was normal.

The above-mentioned back-cross generation was again allowed to interbreed freely, and another interfertile experimental population was obtained. Not full grown but sexually mature female and male specimens from it are illustrated in Plate I, Fig. 3. It is still too early to decide whether the characteristics of this population are similar to those of the ancestral stock or not.

The taxonomic significance of the present work will be discussed and forthcoming experimental populations will be reported in future publications.

Summary

The successful intergeneric hybridization of female *Rhodeus ocellatus* and male *Acanthorhodeus atremius,* both of Japanese origin, is reported. All the hybrids were males. The offspring produced by crossing the hybrids back to the female parental species consisted of functional females and males. The females were uniform in body size and shape, and intermediate between both ancestral forms, but the males were not. The breeding of further generations from this back-cross stock is now in progress.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor Tokiharu Abe and Dr. Yoshitsugu Hirosaki for supplying the species of Japanese bitterling, and to Mr. F. G. du Jardin for the photographs of the hybrid specimens and their offspring. This investigation was generously supported by the Council for Scientific and Industrial Research of the Union of South Africa.

ADDENDUM

Since the preparation of the manuscript, the following interspecific and intergeneric hybrids have been reared to the adult stage:

FEMALE	MALE
Acheilognathus lanceolata	× Acanthorhodeus atremius
" "	× Acheilognathus limbata (Japan)
" "	× Acheilognathus rhombea
" "	× Acheilognathus tabira
" "	× Rhodeus ocellatus (Japan)
" "	× Rhodeus ocellatus (Korea)
" "	× Rhodeus spinalis
" "	× Tanakia tanago
Acheilognathus rhombea	× Acheilognathus lanceolata
" "	× Acheilognathus limbata (Japan)
" "	× Acheilognathus tabira
" "	× Rhodeus ocellatus (Japan)
" "	× Rhodeus ocellatus (Korea)
" "	× Rhodeus spinalis
" "	× Tanakia tanago
Acheilognathus tabira	× Acheilognathus lanceolata
" "	× Acheilognathus limbata (Japan)
" "	× Rhodeus ocellatus (Japan)
" "	× Rhodeus ocellatus (Korea)
" "	× Tanakia tanago
Rhodeus ocellatus (Japan)	× Acanthorhodeus atremius
" " "	× Acheilognathus limbata (Japan)
" " "	× Rhodeus ocellatus (Korea)
" " "	× Rhodeus spinalis
Rhodeus ocellatus (Korea)	× Rhodeus ocellatus (Japan)
" " "	× Rhodeus spinalis
Rhodeus spinalis	× Acheilognathus lanceolata
" "	× Acheilognathus limbata (Japan)
" "	× Acheilognathus tabira
" "	× Rhodeus ocellatus (Japan)
" "	× Rhodeus ocellatus (Korea)
" "	× Tanakia tanago
Tanakia tanago	× Acheilognathus lanceolata
" "	× Acheilognathus limbata (Japan)
" "	× Rhodeus ocellatus (Japan)
" "	× Rhodeus ocellatus (Korea)

Ichthyologists who are interested in the description and publication of the taxonomic characteristics of these specimens, which are new to science, are invited to communicate with the author.

EXPLANATION OF THE PLATE

PLATE I

FIG. 1. A representative adult intergeneric hybrid obtained by crossing *Rhodeus ocellatus* ♀ with *Acanthorhodeus atremius* ♂. Standard length 48 mm.

FIG. 2. Three representative adult specimens of the back-cross generation (see text), one female (top) and two males of the same age but of different size. Standard length: 38, 51 and 39 mm., respectively.

FIG. 3. Two representative, not full grown but sexually mature, specimens of the "interbreeding population" (see text). Standard length of female (top) 25 mm., that of male 38 mm.

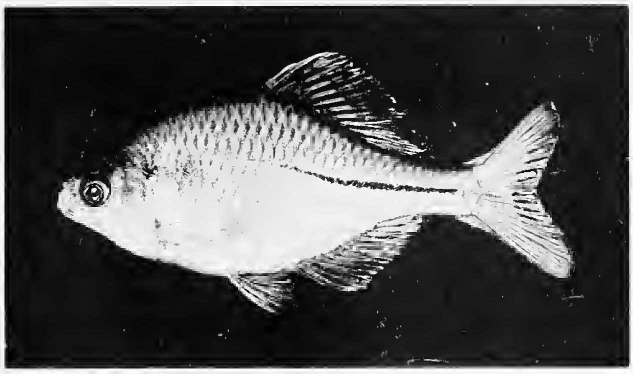

FIG. 1

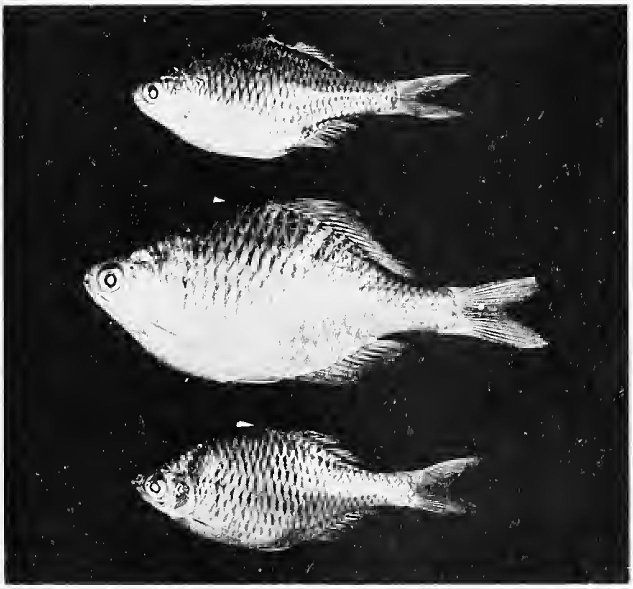

FIG. 2

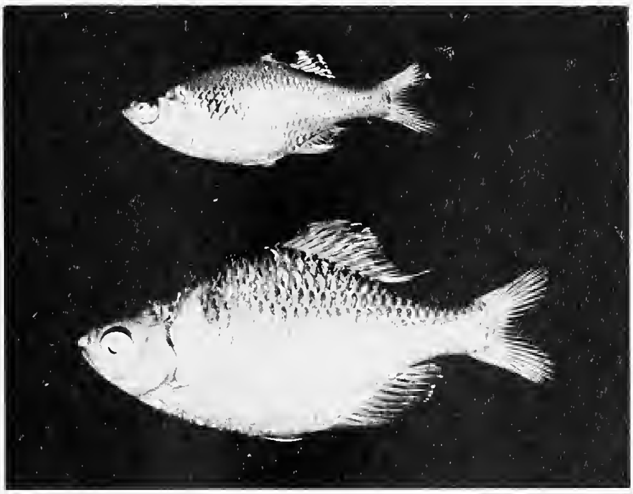

FIG. 3

AN INTERGENERIC HYBRID BETWEEN FEMALE RHODEUS OCELLATUS
AND MALE ACANTHORHODEUS ATREMIUS

3

The Natural History of the Oilbird, *Steatornis caripensis,* in Trinidad, W.I. Part 1. General Behavior and Breeding Habits[1,2]

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(Plates I & II; Text-figures 1-6)

[This paper is one of a series emanating from the tropical Field Station of the New York Zoological Society at Simla, Arima Valley, Trinidad, West Indies. The Station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest reserves. The laboratory of the Station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, with an annual rainfall of more than 100 inches.

[For further ecological details of meteorology and biotic zones see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe. (Zoologica, 1952, Vol. 37, No. 13, pp. 157-184).].

Contents

Introduction

Humboldt's original account established the main features of the Oilbird's unique way of life (Humboldt, 1817; Humboldt & Bonpland, 1817). In 1799 he visited the now famous cave near Caripe in the mountains of northern Venezuela. He described how he found it filled with hundreds of screaming birds, of the size of a fowl but with the aspect of vultures. He reported that the birds left the cave only at night to feed on the fruits of forest trees, spending all day, and nesting, deep within the cave, where their ear-splitting shrieks and snarls made them seem, to the intruder, more like devils than birds. The scientific name which he chose, *Steatornis,* marked another memorable feature: that the young birds become exceedingly fat; he described how they were collected and boiled down by the local inhabitants to give oil for cooking and for lamps.

Humboldt's two original specimens were lost at sea and it was not until 1834 that the first specimens reached Europe (l'Herminier, 1834). As more specimens became available they attracted a great deal of attention from the bird anatomists of the day (especially Müller, 1842; Sclater, 1866; Garrod, 1873; Parker, 1889; see also Wetmore, 1918). These investigations showed that *Steatornis* is almost certainly closer to the caprimulgiform birds than to any other group, but that even to them the relationship is

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[1] Contribution No. 1,008, Department of Tropical Research, New York Zoological Society.

[2] This study has been supported by National Science Foundation Grant G 4385.

very distant, while in certain characters they resemble the owls, perhaps due to convergence. Analysis of egg-white proteins corroborates the relationship to the Caprimulgiformes (Sibley, 1960).

For over 100 years far more was known in detail about the Oilbird's anatomy than about its ecology and behavior. Among a number of accounts of visits to Oilbird caves, most of which added little that was new, mention must be made of the more prolonged visit to the Caripe cave by Funck (1844), the first naturalist to visit it after Humboldt and Bonpland, and of the observations made by Stolzmann (1880) in Peru, which contain a number of points of great interest (quoted less fully in Taczanowski, 1884). Then Griffin investigated the Oilbird's method of orientation inside the caves. He showed that they are able to avoid obstacles when flying in pitch darkness, and that they do so by a method of acoustic orientation akin to that of bats, except that the note given out is easily audible, not supersonic as in bats (Griffin, 1954). More recently, Pietri (1957) has given an account of the Oilbird in Venezuela containing some interesting observations on the birds' behavior when feeding. A short preliminary account of the present study has already been published (Snow, 1958). Apart from these, little has been written about the Oilbird in life that is not anecdotal. The remoteness of most of the caves where they live, and the inaccessibility of the nesting ledges, have prevented sustained field study.

A small colony of Oilbirds inhabits a gorge near the head of the Arima Valley in Trinidad, about three miles from the New York Zoological Society's Tropical Field Station. This colony is the most easily accessible in Trinidad; furthermore, the nests are more easily reached than those in any other Trinidad colony, and probably more easily reached than in any colony throughout the bird's range. The gorge is situated on a private estate and is carefully protected. A further advantage is that a good deal of daylight enters the gorge, which is only partially roofed over, and around midday the nests are well enough illuminated for the birds' behavior to be easily observed. The present paper is based mainly on observations made at this colony over a period of 3½ years.

METHODS

Much of the information gained has come from frequent routine visits to the colony, usually once or twice a week but sometimes daily for short periods. A total of some 250 visits have been made, and they are being continued. At each visit the contents of the nests are checked, a food sample is usually taken, and any necessary weighing, measuring or banding of young birds is carried out. The food samples have been collected in catching trays made of fine wire mesh, slung on the slopes below the nests, and from the nests themselves.

From 1958 onwards all the young reared in the cave have been banded. In addition four adults have been caught and banded. No attempt has been made, however, to band all the adults, since the handling of an adult bird causes great alarm among the whole colony and makes the birds shy for some time afterwards. The handling of young birds has no such effect.

In December, 1957, a platform was erected, spanning the gorge at the same height as the nests and about 15-25 feet away from them. A blind was set on the platform and from it observations were made on the birds' behavior by day and night. By day most of the birds, accustomed to my repeated visits, returned to their nests and behaved normally soon after I entered the hide, though some of them remained aware of my presence. Between 10.00 and 14.00 hrs. all details of their behavior can usually be seen, unless the weather is overcast; before and after this time the light is dim and less can usually be made out. By night much can be learned by listening from the blind, and by occasional quick inspections of the nests with a flashlight. This has been the usual method employed. In addition some observations were made at night by means of a battery-operated infra-red "Snooperscope," but technical difficulties have so far limited the success of this method.

ACKNOWLEDGMENTS

I am grateful to several persons and institutions for help in this work, and especially to the following: my wife, for help with the field work, particularly in catching the adults and banding adults and young; Mrs. Frederick B. Bang, for details of the Oilbird's olfactory apparatus; Mr. J. Barlee, for help in understanding the Oilbird's flight adaptations; Dr. William Beebe, for notes on the colony in the Arima gorge in earlier years, and for continuous interest and encouragement in the course of the work; Dr. W. G. Downs, for copies of photographs taken in the cave; Mr. J. Dunston, for making routine visits to the colony at times when I was unable to do so; and Professor J. K. Loosli, for analysing samples of the Oilbird's food. Above all I am indebted to Mrs. H. Newcome Wright, the owner of Spring Hill estate where the colony is situated, for her help and hospitality during the whole of the work. It is through her efforts that this colony, which is very vulnerable to human disturbance, has been able to survive.

This investigation, part of a wider program of studies on the ecology of neotropical birds, has been substantially aided by a grant from the National Science Foundation.

GENERAL APPEARANCE, STANCE AND LOCOMOTION

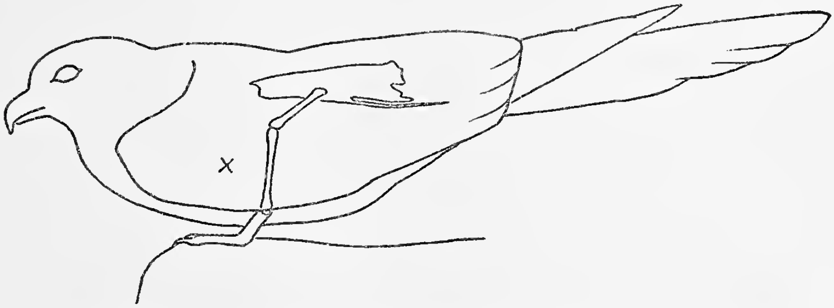
Several points about the Oilbird's general appearance deserve mention. It is a large bird, about 18 inches from beak to tip of tail and with a wing span of 3 to 3½ feet. The plumage is mainly rich brown with a scattering of white spots that are especially conspicuous on the wing coverts and outer secondaries. The body feathering is short and rather soft. The beak is strongly hooked and the upper mandible is notched on the cutting edge. The gape is very wide and the tongue short. Long vibrissae surround the beak and project mainly forward, beyond the tip of the beak. The legs are unfeathered and very short, but not weak; the claws are not strongly hooked. The tail is rather long, ample and markedly graduated. When it is folded the arrangement of the feathers is unusual: they form in transverse section an acute-angled inverted V.

Soon after observations were begun from the hide a slight but consistent color difference between the sexes was noticed. Males are a grayer, slightly darker brown, females paler and more rufous. Funck (1844) also noticed this difference, which has been of value in interpreting the behavior of pairs at the nest. (The sexed museum specimens that have been examined also show this difference, except that a proportion of the males tend towards the female coloring. These may be young birds, or in some cases perhaps "foxed" skins). Wing length is very variable in both sexes, but males average larger than females (nine Trinidad males, 307-333 mm., mean 320; eight females, 292-321, mean 307).

Oilbirds spend most of the daytime perched on the more or less flat surface of their nests. On such a surface they normally rest with the head held low, the body nearly horizontal but tilted somewhat forward, and the tail pointing slightly upwards. The feet are placed far forward, so that the bird appears to be crouching over them (Text-fig. 1; Plate I, Fig. 1). This "down-by-the-head" position is unusual for a bird; it is due to the fact that the Oilbird's legs are very short and come free from the body at a point rather farther forward than is usual in short-legged birds, while the center of gravity also lies well forward, the pectoral musculature being well developed, the sternum deep and the head large. Under such conditions a stable resting position on a flat surface can be achieved only by straightening out the leg joints as much as possible and rotating the whole limb as far forward as possible, and tilting the body head-downward, so that the breast is just above the feet. In this position the articulation of the femur with the pelvic girdle is well above the center of gravity. The bird thus rests with its weight, as it were, slung between the two more or less upright struts formed by its legs. This arrangement, which as a resting position is perhaps unique in birds, makes it mechanically impossible for the Oilbird to stand on one leg.

Ingram (1958), from an examination of specimens, concluded that the short, thick tarsus functions as an integral part of the foot, and that when the bird is perched both lie flat on the substratum. This is not so, however (Text-fig. 1; Plate I, Fig. 1). The tarsus is held within about 30° of the vertical, as in most other birds. Three of the toes point forward while the hallux projects inward approximately at right angles to the line of the body. Movement on the nest, or on any other flat surface, is effected by very short shuffling steps, a method well adapted to prevent the bird from suddenly stepping off the edge.

From the mobility of the hallux it has been



TEXT-FIG. 1. Usual stance of Oilbird on nest, showing position of pelvic girdle and leg-bones, and approximate center of gravity (X).

suggested (Ingram, 1958) that when the Oilbird clings to narrow ledges all four toes point forward, as in the swifts (the pamprodactyl arrangement). In fact, in such a situation the toes are spread out more than when perched on a flat surface. The second and third toes point forward as usual, while the fourth (outer) toe may be held more to the outside of the foot than usual. The hallux still points inward or sometimes even backward (Plate I, Fig. 2). Bock & Miller (1959) have shown how a rather similar arrangement is most effective in enabling woodpeckers to cling to rough vertical surfaces, and for the Oilbird too it probably gives a surer grip on the rough ledges to which it clings than if all four toes pointed forward. For additional support, the tail is fanned a little and pressed hard against the rock face.

An Oilbird cannot, however, cling to a vertical surface, like a woodpecker on a tree trunk or a swift on a wall. Its claws are not very strongly hooked and its tail feathers are not stiffened. As Plate I, Fig. 2 and Plate II, Fig. 3, show, when an Oilbird clings to a small ledge or rough slope the feet are held far back, not forward, as in the woodpeckers, swifts and other birds adapted for clinging. An Oilbird can only cling in such a place if it can bring its center of gravity inside, *i.e.*, to the cliff side, of its feet; otherwise it would simply fall off. To do this, it must not only hold its feet well back but must also keep its head and breast well into the cliff side, an inefficient method which shows that Oilbirds are not primarily adapted for clinging to rock faces.

As mentioned above, Oilbirds are unable to support themselves on one foot. This is clearly seen when they scratch their heads. As in many non-passerine families, the foot is brought to the head directly from below, not from behind the wing. When the bird scratches, it lowers the wing on the same side as the foot which is raised, so that the carpal joint takes the bird's weight. Occasionally both wings are so lowered.

It has been supposed, from the conformation of the tarsus and foot, that Oilbirds are unable to perch in trees (Ingram, 1958). However, Stolzmann (1880) reported that they do so occasionally, and Pietri (1957) gives a detailed account of Oilbirds perching on the bare branches of trees at night. They are also able to alight on quite slender perches. When the birds were suddenly disturbed in a semi-open cave with a top entrance, a few miles east of the Arima gorge, a bird perched for a few moments on a slender woody vine that hung across the cave mouth. Since Oilbirds may be absent from their caves for six hours or more at night (p. 41), it is likely that they make frequent use of their ability to perch on trees. The scene in the well-known illustration in Brehm's *Tierleben* of Oilbirds perched on trees outside a cave apparently by day, though fanciful, is not physically impossible.

Aerodynamically the Oilbird is highly specialized for its way of life. Life in caves demands that it should be able to fly very slowly, hover, and turn and twist with agility, all within narrow confines. Its method of feeding, on bulky fruits often collected far from caves, again demands the ability to hover, and also to carry considerable weight. I am indebted to J. Barlee for showing how well these demands have been met, and for making the calculations given in Table I.

The Oilbird's wing combines, to a striking degree, low wing-loading with an extremely low aspect-ratio. (Text-fig. 2). Low wing-loading (weight/wing-area) enables a bird to fly slowly, manoeuvre easily and carry large loads. The very low aspect-ratio (wing-span/mean width) enables the Oilbird to achieve the necessary low wing-loading without having a large wing-span. This must be of special importance in negotiating the narrow passages of caves. The Oilbird's wing-loading is comparable to that of a harrier (*Circus* sp.) or an owl, both slow-flying birds which carry considerable weights (Table I), while its aspect-ratio is, Mr. Barlee informs me, one of the lowest known for birds of large size.

There are further refinements in addition to this major adaptation of wing shape. The wing has plenty of wing-tip slotting, to reduce stalling speed, and is deeply cambered, to give high lift at low speed. The ample tail further improves manoeuverability and gives extra supporting area when the bird is flying slowly and hovering (Plate II, Fig. 4). Barlee suggests that these adaptations may give the Oilbird a flight speed as low as one or two knots, which is in agreement with observation.

Stolzmann (1880) explained the inverted-V arrangement of the tail feathers as an adaptation to hovering. He described how, when hovering, the Oilbird rhythmically elevates and depresses the tail, as some hummingbirds do when feeding. He suggested that, owing to the inverted-V arrangement, the downward movement of the tail generates lift, while the upward movement meets with little resistance from the air. I have never seen a bird hovering for long enough, in good light, to be able to see the tail movement which Stolzmann describes. He appears to have been an acute observer and his suggestion deserves consideration.

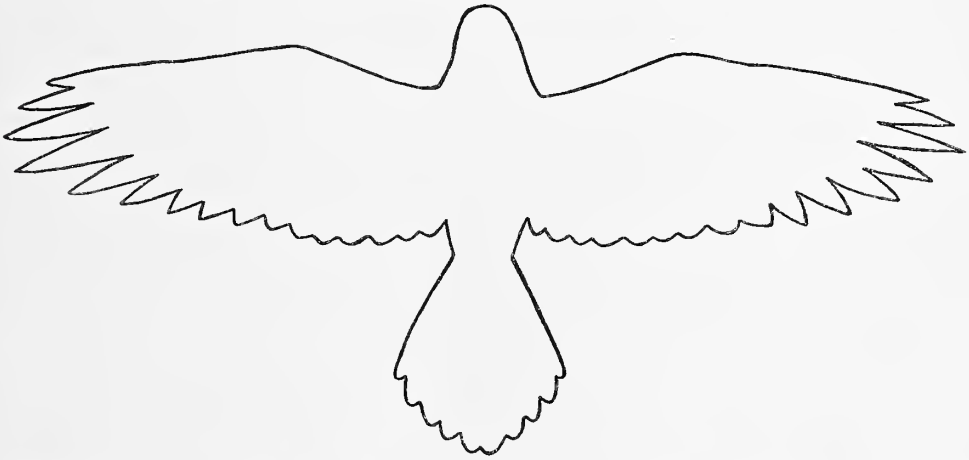
TABLE I. AERODYNAMIC CHARACTERS OF OILBIRD WING COMPARED WITH MARSH HARRIER AND LONG-EARED OWL

	Weight (gm.)	Span (cm.)	Wing-area (cm.^{2})	Aspect-ratio (span/mean width)	Wing-loading (weight/wing-area)	Pectoralis major/supracoracoideus
Oilbird	415	96.5	1450	6.4	0.29	15
Marsh Harrier (*Circus aeruginosus*)	510	124	1820	8.5	0.28	22
Long-eared Owl (*Asio otus*)	291	95	1270	7.2	0.23	12

High-speed flash photographs show that in slow flight the wingbeat is deep and the upstroke of the wing is propulsive (Plate II, Fig 5), as it has been shown to be for pigeons in rising flight (Brown, 1951). In hovering, too, the upstroke (which, with the body in a half-upright position, becomes a backstroke) must generate lift as well as the downstroke. Hence it would be expected that the muscles that raise the wing would be highly developed. The supracoracoideus, which is usually considered to be the chief muscle raising the wing, is however quite small (weight 2 gm., compared with 30.5 gm. for pectoralis major). In this the Oilbird agrees with most other birds of low aspect-ratio. Probably most of the power for the upstroke comes from the deltoid muscles, which are well developed (weight 2.5 gm.) and have a broad attachment along half the length of the humerus, rather than from the supracoracoideus, which in addition to being smaller has a mechanically less efficient attachment to the humerus.

In the open, at night, the Oilbird's flight is rather different from its flight in caves. The wingbeat is rapid and shallow. Doubtless the wings are held in a more sweptback position, and flying speed is thus increased by the reduction in wing area. As in the caves, the flight is quite silent. Stolzmann, who clearly had excellent opportunities for observing them by night, saw them occasionally dive down like falcons, with wings half closed. Speed of normal flight in the open has not been determined by observation, but Barlee suggests that it may be about 16 miles per hour. Flight speed may be important ecologically, as it must have a bearing on the time taken to fly to the food trees, and hence on the number of times that the adults can feed the young in the course of the night.

The method of feeding is not easy to observe in detail. In the Arima Valley birds have been watched feeding on trees of two kinds, *Ocotea wackenheimii* and *Trattinickia rhoifolia*. Invariably they have been seen to fly up to the tree, hover, and swoop away a moment later.



TEXT-FIG. 2. Outline of Oilbird with wings and tail fully spread. (Traced from a freshly killed specimen).

Occasionally a quick forward thrust of the head could be seen, as the bird seized a fruit. Pietri's account of Oilbirds feeding on *Persea caerulea* is similar. However, Stolzmann saw Oilbirds cling momentarily, with beating wings, when taking fruit from a lauraceous tree (probably *Nectandra* sp.), and Ingram (1960) has reported that when feeding at a palm (called Sabal, but probably *Livingstona*) they cling to the bunches of fruit for several seconds. In *Ocotea* and *Trattinickia* the fruits do not grow in large compact bunches, as in the palms. It is likely that Oilbirds adapt their methods of feeding to the type of tree, clinging when this enables them to pluck a number of fruit from a single bunch.

An analysis of the Oilbird's food will be given in Part 2 of this paper. Here it need only be said that the fruit taken varies greatly in size, from the small round fruits, about 4 mm. in diameter, of the palm *Geonoma vaga* to the relatively huge fruits, up to 60 mm. long and 30 mm. wide, of the lauraceous tree *Beilschmiedia tovarensis*. All the main fruits eaten are alike, however, in having a single relatively large seed surrounded by a firm pericarp. The pericarp is digested and the seed regurgitated. Regurgitation of the night's feed is completed by about 09.30 hrs. on the following morning.

SENSES

Although orientation by sonar (Griffin, 1954) takes the place of visual orientation within the caves when the amount of light is reduced below a certain point, it is not known to what extent sonar would be effective outside the caves at night; in particular, it is not known how small an object can be detected by this method. This is a question that must be settled by experiment. Observation shows, however, that the Oilbird's eyes are very sensitive to light and whenever possible sight is used instead of sonar, and suggests that sonar is normally never used outside the caves.

Thus in the Arima gorge, when a bird is flying toward a dark recess the echo-locating clicks are uttered, but they slow down or stop as the bird wheels around toward a better-lighted part of the gorge. When the birds were watched in the evening leaving the Oropouche cave, several miles east of the Arima gorge, they clicked continuously as they flew down the narrow passage toward the cave mouth and stopped clicking as soon as they emerged into the open. I have never heard clicks from birds feeding at night. It may be noted that the Oilbird's eyes, though not very large, have a very wide pupil and a tapetum which shines bright red when illuminated with a light held beside the observer's eye.

In locating food it seems likely that the sense of smell is important, though here again experimental work is needed. For the following notes on the Oilbird's olfactory apparatus I am indebted to Mrs. Frederick B. Bang, who recently examined freshly preserved specimens from Trinidad as well as comparable material from other species of birds. The Oilbird has a relatively very large and heavily innervated olfactory organ, with one of the thickest mucous membranes of any bird examined. The nasal chamber is beautifully adapted to carry the incurrent airstream, after being initially filtered by the respiratory concha and anterior concha, straight onto this mucous membrane. It is of interest that the respiratory (middle) concha is relatively enormous, but the functional significance of this is uncertain.

Such a highly developed olfactory apparatus must surely have an important function. That it is used for locating food trees is suggested by the fact that many of the trees on which Oilbirds feed are spicy or aromatic, in particular members of the families Lauraceae and Burseraceae. It seems unlikely, however, that the sense of smell can be used for locating individual fruits; for this, sight is almost certainly used. Very many of the fruits taken are green when unripe and turn dark purple or black when ripe. That the birds nevertheless sometimes make mistakes is shown by the fact that the food samples collected in the caves often contain a small proportion of unripe fruits which have been regurgitated undigested. Pietri's account is particularly significant on this point. A party of Oilbirds watched feeding on *Persea caerulea* settled on trees when the moon was obscured by clouds, and began feeding again when the clouds had passed.

Within the caves the sense of smell could perhaps be used for locating the nest, which with the decaying fruit on it usually has an odor perceptible to the human nose, but it is unlikely that this is of importance in view of the undoubted accuracy of the birds' orientation by sonar. Once the bird is on the nest, the sense of touch probably plays the chief part in orientation with respect to the mate or young, and for this, as Ingram (1958) points out, the long, forwardly-projecting vibrissae are undoubtedly used. It may be noted that the birds themselves have a characteristic musty odor, which may perhaps play a part in individual recognition.

Whatever senses are used in the various activities related to food and to the nest, an extremely highly developed kinaesthetic sense may be postulated. It is therefore of interest that the Oilbird's cerebellum is unusually large (Bang, *in litt.*).

General Behavior and Daily Routine

At all times, whether breeding or not, adult Oilbirds spend most of the daytime in pairs, perched on their nests. Usually they perch side by side, facing outwards. For most of the time they are quiet; sometimes they sleep. Occasionally the silence is interrupted by an outburst of harsh calls, as perhaps when two birds on adjacent ledges engage in a tussle, with beaks interlocked, or an unestablished bird tries to land on a ledge near an occupied nest. Toward evening there is an increase in calling and general activity and birds begin to leave their nests and fly around. This period of restlessness lasts an hour or more before the departure from the cave begins.

In the Arima gorge the evening departure is difficult to study in detail as there are four ways of exit, up the gorge, down the gorge, and by two top holes. On September 25, 1957, watch was kept in the cave from 17.30 to 19.30 hours. From 18.00 to 18.45 there was great activity which gradually decreased as more and more birds left. At 19.00 the departure seemed to be complete. Inspection by flashlight, however, disturbed two birds which flew around for a few seconds and then left. The cave was then empty of adults except for one which was brooding a small nestling. In another watch, on December 24, 1957, most birds had left by 18.15. At 18.30 the flashlight revealed five adults still present, of which four were probably attending nestlings or eggs. On April 16, 1960, almost all had gone by 18.45, and by 19.00 the cave was empty of adults except for two which were attending nestlings.

By contrast with these observations of the departure of a small colony from a cave with several exits, the departure of a large colony from a cave with a single exit takes much longer. A watch was kept at the mouth of the Oropouche cave on the evening of October 25, 1958, a night of full moon. This is a large cave extending back about 400 yards into the hillside, with one rather small entrance hole. The first birds came out at 18.10. By 18.45, 102 had come out, and by 19.40, 62 more. In the next five minutes 11 more came out. Between 19.45 and 20.00 only one more bird came out and the departure seemed to be over. When the cave was entered at 20.05 the flashlight disturbed about 25 birds; almost certainly these were attending eggs or young. The birds had shown considerable hesitation in leaving the cave, repeatedly flying up to the cave mouth and turning back before finally coming out into the open. In addition, the narrowness of the exit passage had apparently forced the birds to "queue up" to leave and it was this, combined with their hesitation in leaving, that made the departure of the colony so slow. Most of the birds were in pairs as they emerged, with a small proportion single or in threes.

The birds seek food as soon as they leave the cave. Almost certainly they fly directly to fruiting trees that they already know. Thus the first birds arrived at a favorite food tree about half a mile from the Arima gorge at 18.20, 18.22 and 18.40 on three successive nights in December, 1958, suggesting that they had flown straight to it on leaving the cave.

I have not spent an all-night watch in the cave at a time when the birds were not breeding, in order to see whether they return periodically to the cave during the night. When they have eggs or young they return at intervals, as would be expected. In any case, the main return to the cave takes place shortly before dawn. On February 10, 1959, when no birds were nesting, one bird was already present when I entered the cave at 04.30. No other birds arrived until 05.35, when two or three returned. The number of birds present then gradually increased until 06.00, by which time all were probably present. On April 16, 1960, when most nests had young, the final return of the adults took place between 05.10 and 05.40.

Oilbirds are occasionally found in the daytime out in the open, sometimes far from a cave. Stolzmann (1880) mentions two such instances; he supposed that the birds had not left themselves enough time to return to their cave, and having been surprised by the oncome of daylight were waiting for nightfall before resuming their flight. But it seems very unlikely that an experienced bird would make such a mistake, and a more probable explanation is that these are mainly recently independent young birds, which have either failed to find enough food and have become weak or have become separated from their kind and lost.

Social Behavior

Since nesting activities occupy a large part of the year, and when they are not nesting Oilbirds still spend most of the daytime in pairs on their nests, there is little doubt that the pair bond must normally be permanent. The formation of pairs has not been observed. It is probable that the behavior connected with pair formation takes place at night, since it is at night that the birds are active and have the opportunity to meet birds from other colonies.

When the birds were watched leaving the Oropouche cave in the evening, twice a trio of birds, on emerging into the open, circled around each other, evenly spaced and with a swift, swooping flight. Similar behavior was seen on two occasions when birds were watched feeding in the Arima valley. After taking food, two or three birds circled around and around each other with low, clucking calls and a long, harsh "karrrr." It may well be that behavior of this kind is involved in pair formation. Stolzmann (1880) observed similar behavior in Peru and interpreted it as courtship.

In the daytime, one member of a pair sometimes preens its mate's head as they sit side by side on the nest. This behavior has only been seen just before the eggs are laid or during the laying period, except in one pair in 1959 which laid no eggs in that year (or lost them as soon as they were laid). The preening bird, with its eyes closed, works carefully over the other bird's head. The latter keeps its eyes open or only half-closes them. In nearly every case when this behavior has been seen, the preening bird has been known or presumed to be the male. The exception was a single instance when the presumed female, having been preened by its mate several times, was seen tentatively to preen its mate's head. This behavior is certainly a form of courtship and, from the times when it has been observed, must be closely associated with the period of copulation, but copulation itself has not been seen.

Relations between neighboring pairs are generally harmonious. When a bird alights on its nest, it sometimes provokes an outburst of excitement and calling from its neighbors, but such outbursts are short-lived. Occasionally, for no apparent reason, neighboring birds spar with their beaks and engage in tussles, gripping each other by the beak and twisting and pulling, with harsh calls, sometimes for several minutes on end. More prolonged fighting occurs when an apparently unestablished bird tries to secure a foothold on a ledge near an occupied nest. On May 10, 1959, two birds were watched trying repeatedly to secure a foothold in the same place, on a steep slope just below an occupied nest. Both were repelled. One of these, which was banded, was a young bird fledged in August of the previous year. These conflicts have been seen only shortly before, or during the early part of, the breeding season.

That there is some sort of cohesion between the adults of the colony when they are out at night, apart from the cohesion of the pairs, is apparent from observations on the times of feeding of the young which will be described later. Except for three nests in which the young were small and were fed more often, returns of the adults with food were concentrated into three main periods of about 20 minutes each, spaced about two hours apart. During these periods each nest, as far as could be ascertained by listening, was visited by one or two adults and the young were fed. This could only have been possible if the adults were keeping company with each other while collecting food.

The few observations that have been made on their feeding behavior also give evidence of a strong social tendency. When Oilbirds have been watched feeding at night in the Arima valley, up to five birds have arrived at the food tree within a minute, and after feeding for several minutes have departed within a short time of each other. Pietri (1957) also refers to their social behavior when feeding and describes how, when one bird of a feeding party was shot, the others called and swooped down low over the dying bird.

Except for the echo-locating clicks, the significance of the Oilbird's various calls has not been elucidated. When the birds are disturbed in a cave, the noise can be almost deafening; the calls range from clucks and rather low-pitched "hawking" sounds, reminiscent of the last of the bathwater going down the drain, to long-drawn-out, harsh screams. The calls made by aggressive birds on their nests, when another bird approaches, are similar. Birds flying in the open at night sometimes utter a shorter, less harsh "karr, karr" or "kuk, kuk," which is probably used in maintaining contact with other individuals. As Griffin (1958) points out, these is no sharp distinction between some of these shorter calls and the longer bursts of echo-locating clicks.

Whatever their signal function may be, the acoustic qualities of these calls are well suited to the conditions under which they are uttered. Many Oilbird caves are full of the noise of running water or breaking waves, so that, as for cliff-nesting sea-birds, very loud calls are essential. The harsh guttural quality of the calls, depending on a rapid succession of staccato sounds of many different frequencies, probably makes it easy for the other birds to detect the position of the calling bird (Marler, 1955). By uttering long-sustained calls, birds flying in the confined space of a cave can make their course known to the other birds. Thus social contact between the individuals can the more easily be maintained. The loud harsh calls are, however, not mere traffic signals. Purely for the avoidance of midair collisions, the echo-locating clicks are sufficient. Thus large numbers of Oilbirds can fly together in pitch darkness, uttering only clicks, but any disturbance will at once elicit a chorus of screams and snarls.

THE NEST

As already mentioned, adults occupy their nests all the time, whether they are breeding or not. The nests in the Arima gorge are placed on narrow ledges, 8 to 15 feet above the stream bed. There are no suitable higher ledges. In other caves nests are normally much higher, partly because the caves themselves are much larger and partly because frequent raids on the caves have caused most of the lower and more accessible ledges to be abandoned.

The nest has a diameter of approximately 15 inches, with a shallow central depression and a slightly raised rim. It seems at first sight to be made of mud, and has been so described. It is, however, made primarily of regurgitated matter. When nest-building, the bird moves its head around the rim of the nest and with quick jerky movements plasters on semi-liquid matter which it allows to exude from the side of its beak. From the firmness of the resulting structure, it seems probable that saliva is important in binding together the regurgitated pulp, but this point needs investigation. The central part of the nest is formed from the accumulation of regurgitated seeds which the birds let fall; nest-building behavior does not, as far as I have seen, include work on any part except the rim. The faeces of the adults contribute little or not at all to the structure, for when defecating they turn and face inwards and shoot the faeces well clear, like other cliff-nesting birds. The young, too, turn when defecating but until they are well grown the faeces are usually deposited on the nest edge, where they contribute a little to the structure.

As nests are used year after year, they grow into low cylindrical mounds. Three of the nests in the Arima gorge have fallen away during the period of observation, as they became too large for the small ledges on which they were based. Two have been partly rebuilt. Parts of other nests have fallen away and been rebuilt. It is presumably by such a process of falling away and rebuilding that year after year the nests remain more or less the same size. The birds build up and repair the nest rim most actively in the few weeks before egg-laying begins, but the behavior also occurs when there are eggs and young.

THE EGGS

The eggs are white ovals, slightly pointed at one end. The surface of the shell is slightly rough. The average weight of ten eggs, weighed soon after laying or early in incubation, was 20.2 gm. (range 17-22.5 gm.). Most eggs become spotted and blotched with brown soon after laying; this has led to erroneous statements that the Oilbird lays spotted eggs. The normal clutch is 2–4 eggs. (Clutch size will be dealt with more fully in Part 2 of this paper).

Although several daytime watches have been made at times when the birds were laying, there has been no record of an egg being laid during a period of observation. It seems probable that when about to lay, the female remains behind and lays her eggs before going off to feed.

The interval between the laying of successive eggs is unusually long and very variable. Daily visits to the colony over a period of up to two weeks may be necessary to ascertain the intervals between the laying of eggs in only one nest. As nests are not always well synchronized, daily visits over several weeks would be necessary in order to obtain exact information for the whole colony. This has not been possible, with the consequence that data on this point are fragmentary. The most accurately ascertained intervals between the laying of successive eggs were 2-4, 5, 6-7 and 6-7 days. In addition, minimum intervals of 6, 7 and 9 days were recorded. Between the laying of the first and third eggs in a clutch, the following intervals were recorded: 6-8, 7-9, 9, 9, 8-10 and 9-11 days. Other less exactly recorded intervals were consonant with these.

INCUBATION

The eggs are covered from the time they are laid. Complete clutches were never seen to be left uncovered (except after the birds had been frightened off the nest), but there were two observations of the eggs in incomplete clutches being left uncovered for 4 and 10 minutes while the parents perched on the edge of the nest. Both sexes incubate the eggs. The eggs lie very far forward under the incubating bird, between the chin and the legs, the whole rear half of the bird being slightly elevated. When the bird is relaxed the head is withdrawn between the shoulders and the eyes may be closed. The other bird, standing beside its mate, is usually alert, with its head held forward.

The lengths of the turns taken on the eggs are usually long, but very variable. Thus in a watch lasting three or four hours in the middle of the day, when the light is good enough to see details, only one or two completed spells may be observed. At nests where the sexes were known, females were recorded incubating for just over twice as long as males (1,129 as against 504 minutes), but this was due almost entirely to one nest, at which the female alone was seen to incubate. At the other nests the total time spent by the male and female on the eggs was nearly equal, and the few completed spells recorded for the two sexes were of similar length: males, 4, 32, 96 and 109 minutes; females, 7, 29 and 95 minutes. These spells are shorter than average, as the majority of the longer spells overlapped the beginning or end of the watch and so their complete length was not known.

The change-over is effected silently and without ceremony. Usually the incubating birds gets up off the eggs and shuffles to one side, while the other bird takes its place. Shuffling around by one or both birds may continue for several minutes before they settle down and become still again. Sometimes the non-incubating bird takes the initiative by becoming restless, shuffling about, and perhaps inserting its head beneath the incubating bird as though to ease it off the eggs. At the nest where only the female was seen to incubate, the male periodically became restless. Once after apparently trying to rouse the female from the eggs he performed nest-building movements, but without regurgitating any material, probably a displacement activity. Once he spent most of the watch on another ledge a few feet away from the nest.

Two experiments suggest that the Oilbird's egg-retrieving behavior is very poorly developed. One egg of a clutch of three was moved four inches from the other two, towards the edge of the nest. The male soon returned and incubated. Sitting on the two eggs, he looked at and occasionally touched the third egg with his beak but made no attempt to roll it back. After about a minute he sat quietly, ignoring the third egg. A few days later at the same nest one egg was moved three inches from the other two. The male again returned to incubate. When he had settled down the third egg was lying about one inch in front of him. He gradually shuffled it underneath him by moving forward himself a little and touching the egg with his beak so that it rolled a little. When the egg was nearly touching him he finally poked it underneath him. The whole process took three minutes.

Nests are usually surrounded by regurgitated seeds but these do not usually remain in the central depression of the nest with the eggs. This suggests that the incubating bird moves them, though this has not been seen. Sometimes, however, as many as three of the seeds of the palm *Jessenia oligocarpa* lie with the eggs. They are easily the largest of the seeds regularly taken by the birds in the Arima gorge and are apparently near enough in size to be accepted as eggs when they are regurgitated into the nest.

The eggs hatch at approximately the same intervals as the intervals between laying. For a day before the egg hatches the young bird can be heard cheeping inside. It emerges from the shell by cutting a circular cap from the broad end of the egg. The hatching process is quick; a young bird that was just starting to chip the shell at 15.30 hours was fully hatched at 17.00 hours. There were several instances of chipped eggs being hatched by the time of the next visit 24 hours later, and no cases of prolonged hatching.

The broken eggshells are not cleared away promptly by the parents; they often remain on the nest for a day or two before, like the regurgitated seeds, they are pushed, or perhaps picked up and dropped, over the edge. During a watch of nearly four hours an adult brooded the newly-hatched young with half an eggshell lying beside her; several times she fumbled with the broken shell but made no attempt to remove it.

The incubation period (measured from the time of laying to the time of hatching) is 32-35 days. Table II gives the most accurately determined periods for a number of marked eggs. In most cases there is a possible error of one day or a day and a half either way, as it was not usually possible to visit the colony frequently enough to give greater exactitude. It will be seen that there are no consistent differences in incubation period between eggs of different position in the clutch, which indicates that the eggs are not merely covered but effectively incubated from the time they are laid.

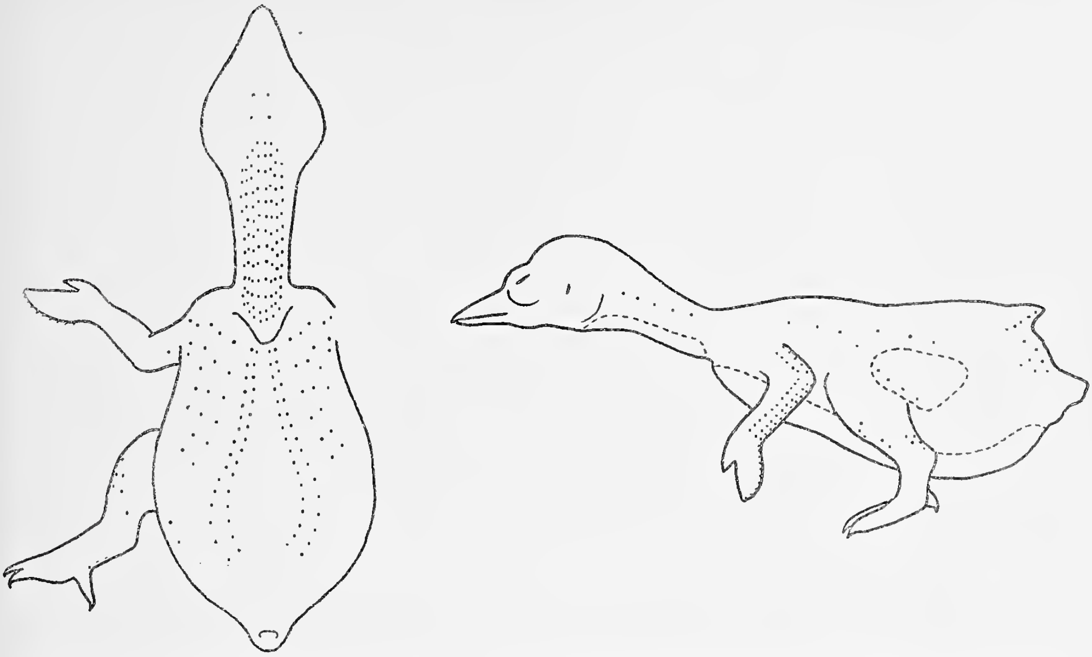
TABLE II. INCUBATION PERIODS

Egg 1	Egg 2	Egg 3	Egg 4
32 (±1)	32½ (±1½)	32 (±1)	34 (±1)
33½ (±½)	34½ (±1½)	33 (±1)	
33½ (±1½)	34½ (±1½)	33½ (±½)	
		34	
		34½ (±½)	
		35½ (±½)	

THE YOUNG

The most striking feature of the development of the young Oilbird is its extreme slowness. Young Oilbirds do not usually leave the nest until they are between 95 and 120 days old. During this time they lay down the great deposits of fat which have led to their being exploited for oil, attaining around the age of 70 days a weight much greater than that of the adult, and then losing weight for the last 30-50 days as their feathers develop.

Growth and Development.—The young bird at hatching weighs from 12 to 15.5 gm. It is naked except for some sparse down, chiefly on the under side. (Text-fig. 3). The amount of down at hatching varies; it is always thickest on the under surface, while on the back and flanks some



TEXT-FIG. 3. Distribution of down feathers on newly hatched Oilbird. Each dot represents one feather. Areas shown in detail in left-hand figure (ventral view) are outlined by broken line in right-hand figure. Area enclosed by broken line behind thigh in right-hand figure contains many feather rudiments visible below skin, but down sprouting only where shown. (Specimens vary individually).

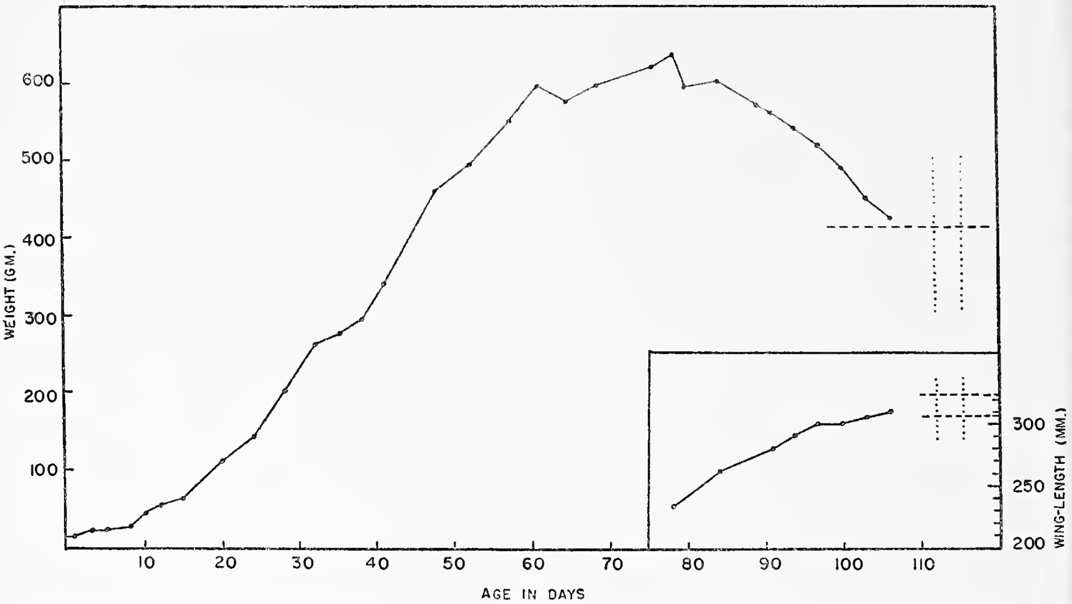
birds have a little down at hatching, or very soon after, while in others it does not burst through the skin until a few days after hatching. These first down feathers are short, pale gray and highly branched. In the second week after hatching a second generation of down feathers appears as black streaks beneath the skin. These second down feathers, which are darker gray and much longer, come from the same rudiments as the first and bear the first on their tips as they begin to break through the skin in the third week. Beneath some of the first down feathers on the ventral surface, especially towards the posterior end, no black streaks appear, and these down feathers are not succeeded by any others.

The first feathers of adult type, those of the tail, secondary coverts and scapulars, burst from their sheaths at the age of about 35 days. Thereafter the feathering of the wings and tail, head, back and underparts, in that order, grows steadily. By the age of about 70 days the nestling is quite like an adult, except that the wings and tail are very short and the body is still mainly downy below. It is noteworthy that there is not only no juvenile plumage but also no juvenile appearance of the head and beak. The nestling, when ready to leave the nest, is indistinguishable from the adult. Being adapted to complete darkness, the nestlings are without the visual signs which elicit parental behavior in other birds.

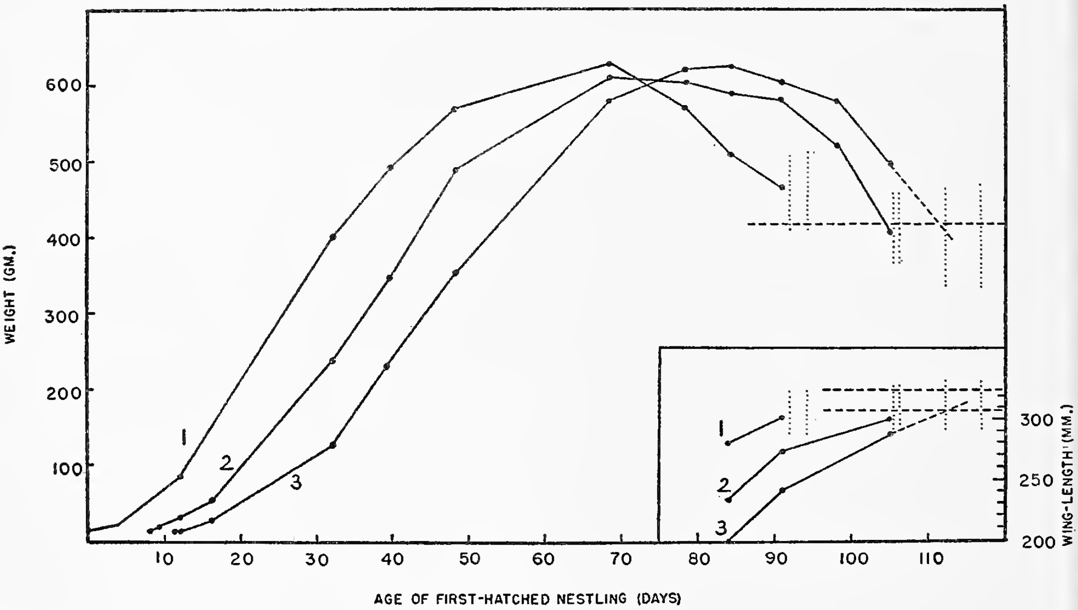
In Table III the main changes in the appearance of the nestling are tabulated. Because of the very long development, an accurate knowledge of these changes is necessary if the breeding season is to be dated from a single visit to a colony with eggs and young. It will be noted that there is great variability in the state of development of different birds of the same age.

Text-figs. 4 and 5 show the growth in weight of a single nestling and of a family of three, with the wing lengths in the last two or three weeks. It will be seen that the young birds leave the nest at the time when the decreasing weight and the increasing wing length have simultaneously reached the adult values.

The Nestling Period.—Whereas small young are sometimes restless during the day, and even beg occasionally (see next section), large young are inactive. Until very shortly before they leave the nest they usually show no tendency to fly when disturbed or even handled, at least during the daytime. (The one exception, a bird 109 days old, flew quite strongly when I rather awkwardly tried to turn it in the nest in order to read its band number). This is probably due in part to their being less active during the day, but also,



Text-fig. 4. Growth in weight and wing length of a single nestling Oilbird. The broken horizontal line in the weight diagram shows the mean adult weight (415 gm.). The two broken horizontal lines in the wing length diagram show the limits of adult wing length. The vertical dotted lines indicate the period within which the nestling left the nest.



Text-fig. 5. Growth in weight and wing length of a family of three nestling Oilbirds. Conventions as in Text-fig. 4.

and more importantly, to their being accustomed to frequent harmless disturbances. Thus when a boatman climbed up to some nests in a sea cave where the birds are still exploited by the local people, two nearly fledged birds left their nests and fluttered down into the sea. For the first few days after the young birds have left the nest they sometimes return to them by day, but

TABLE III. DEVELOPMENT OF NESTLING OILBIRD

Day	Description:[1] feather development	Weight (gm.)	Weight Limits	Wing (mm.)	Wing Limits
1	Nearly naked above, short sparse down below; eyes closed; squeaks when handled	12	12-15.5	—	—
5	As before, but larger	22	20-30	—	—
10	Wing feather rudiments visible as dark points (apparent on day 8)	40	30-75	—	—
15	Vibrissae sprouting; rudiments of second generation of down feathers as short black streaks on body; eye slits beginning to open; bird about 4½ inches long	65	50-120	—	—
24	Down feathers bursting out all over body; eyes open but not widely	145	100-230	—	—
30	Down prominent all over body; bird about 6½ inches long	230	120-300	—	—
35	Secondary coverts and tail feathers just beginning to sprout from sheaths	275	160-440	—	—
40	Tail feathers about 5 mm., secondary coverts 3-4 mm. beyond sheaths; primary coverts sprouting	330	240-500	—	—
50	Head feathers well out of sheaths (burst about day 47); scapulars, wing coverts and tail feathers forming almost complete covering of upper side; whole of body still downy	450	290-570	—	—
60	Body still downy; feathers growing on throat	580	390-610	—	—
70	Back well feathered; feathers growing on under side of body	595	560-650	190	—
80	Appearance like adult, but wing and tail short	595	550-650	240	160-260
90	Appearance like adult	565	520-600	280	200-300
100	Appearance like adult	490	420-550	300	240-305

[1] Description is based on a nestling whose rate of development was average. Weight limits and wing limits are limits for all nestlings which fledged successfully.

as soon as they are disturbed they fly off at once. Thus the fledging period can be measured quite exactly as the interval between hatching and the time when the young bird first voluntarily leaves the nest.

Only a few periods could be ascertained exactly. Usually they could be determined only within a few days, owing to the fact that it was rarely possible to determine both the hatching date and fledging date exactly. All fledging periods ascertained within limits of eight days or less are given in Table IV. There is a great difference between the shortest, 88 days, and the longest, 125 days, but most (71%) fall within 100 and 115 days. There is a tendency for the youngest member of a family to have a longer nestling period than its nestmates. This was certainly in some cases, and probably in all, because their early development was slowed down through competition for food with the older nestlings. Text-fig. 5 shows an example of a family in which this happened.

The full length of a single nesting, from the laying of the first egg to the fledging of the last young, is commonly round 150 days. The longest recorded, for a family of four all successfully reared, was 168 days.

Behavior of the Young.—For about the first 25 days after hatching, the young are brooded by the parents. They lie with their heads under the wing or breast of the parent bird; as they become larger the head often protrudes from between the parent's wing and body. They frequently thrust their heads upward toward the parent's neck, body or wing, apparently seeking contact; having gained it, they will stay motionless with the neck awkwardly kinked and the beak pointing upward. Occasionally they make food-begging movements, thrusting their head up at the parent's beak and nibbling at it. But, except perhaps when they are very small, the young are not usually fed by day (see next section).

The call of the young at hatching, and for a day or two before hatching, is a high-pitched cheeping. Later, by the age of 20 days, it develops into a loud, rather hoarse squeak, which becomes louder as the chick grows older. Large nestlings, when begging, utter a chorus of shrill but rather hoarse squeaks. By the time they are

TABLE IV. FLEDGING PERIODS

	Position in Family[1]			
	(1)	(2)	(3)	(4)
Family of 4	111 (±4)	112 (±2½)	110 (±3½)	125 (±2)
Families of 3	114 (±2)	111 (±3)	125	
	104 (±3½)	100 (±3½)	104 (±4)	
	98 (±3½)	98 (±2)	106 (±2)	
	109 (± 3)	109[2]	114 (±2)	
	93 (±2)	99 (±1)	103 (±2)	
	99 (±2)	104 (±3)	110 (±1½)	
	(Died at 62 days)	112 (±2½)	>116	
Families of 2	119 (±3½)	121 (±3½)		
	108 (±2)	115 (±3½)		
	101 (±2½)	102 (±2)		
	(Died at 42 days)	102 (±2)		
Families of 1	102 (±4)			
	112 (±2)			
	100 (±3½)			
	88 (±3)			

[1] In families in which one of the nestlings died in the first few days after hatching, this nestling has been left out of consideration in placing the other nestlings in their positions in the family.

[2] This bird flew on being disturbed (see text).

well feathered they begin to utter, if alarmed, the harsh screams of the adult.

They begin to preen themselves at the age of about 20 days. From the age of a few days, when defecating they turn, back towards the nest rim, and deposit the faeces on the edge of the nest. When they are larger the faeces are shot clear of the nest edge, as in the adult.

For about the first 50 days after hatching, the young rest with the lower surface of the body, the tarsus and the foot in contact with the substratum. Toward the end of this period they are able to raise the body clear of the substratum when shuffling about the nest. Later they begin to stand with the body clear of the nest, their weight supported only by the tarsus and the foot, and finally, by the age of about 75 days, they can stand on the foot only, with the tarsus held at an angle of 45-60°. Advanced young can clamber efficiently up quite steep slopes. To do this they not only grip and push with their feet but pull themselves up with the beak and dig in the leading edges of the wings. This behavior is of obvious value in enabling them to regain the nest if they are accidentally pushed out. They are very conservative in the position which they occupy in the nest. If taken out and replaced in different positions they shuffle and clamber over each other until they have regained the old positions.

Parental Behavior.—As already mentioned, for about the first 25 days the young are brooded by the parents. Usually only one bird at a time covers the young, but at one nest with four young both parents were seen to cover them for part of a watch. The number of young probably affects the length of time for which they can be brooded; at two nests single nestlings were brooded by day at the ages of 29 and 30 days, a longer time than was recorded for broods of two or more. At night one adult stays with the young while they are small; larger young are left by both parents. Thus during evening watches one adult remained on each of four nests with young 3-6, 12-18, 20 and 30-40 days old, while both parents departed from six nests with young 49 days old or more.

Many hours of watching have shown no evidence that the young are ever fed by day, except perhaps when they are very small. Very small young sometimes become restless, thrust their heads up jerkily toward the parent's head, and utter the food-begging call. Occasionally the parent has then been seen to lower its beak towards the nestling and itself make slight jerky movements. On such occasions some semi-liquid food may be passed to the young, but the adult's position, crouching over the young chick with lowered head, makes it almost impossible to see the details. From the 12th day, and perhaps earlier, the young are fed, at least partly, on whole undigested fruits.

Observations on the feeding of the young were made during an all-night watch on April 16-17, 1960. On this night four nests, all adjacent to one another, contained three young each, aged from 49 to 58 days, while three other nests contained, respectively, three young 12-18 days old, one young 20 days old and four young 30-40 days old (Nest K). When the evening departure of the adults was over, at about 19.30 hours, one parent remained at each of the three nests with smaller young, while both parents had gone from each of the four nests with large young. The first feeding was at Nest K, with four young 30-40 days old. It began at 20.51 and continued, with pauses, until 21.18. The first feed at the other two nests with small young was at about 21.00, but as these nests were more distant from the hide, and very close to each other, further detailed observations were not made on them. Although the darkness was total, it was easy to tell when a family was being fed. The adult on arriving would fly around for a little time, its position being shown by the echolocating clicks. As it approached the nest to land the clicks would become more rapid and then suddenly cease as the bird landed. At once there would be a shrill chorus of squeaks from the chicks, which would continue at greater or lesser intensity while the feeding lasted. The other nests where no feeding was taking place would by contrast be almost or completely silent.

At 21.20, two minutes after the feeding was over at Nest K, inspection by flashlight showed that both parents were still present. At 22.00 only one was present. In the course of the night there were five more bouts of feeding at this nest, and perhaps a sixth: at 22.13-22.27, 23.33-23.44, (00.05-00.06, not certain, and in any case very brief), 01.29-01.37, 03.44-03.50 and 05.22-06.15.

At the four nests with large young, feeding began much later. There was one short feed at one nest only at 23.02-23.06, after which the parent departed again. Nothing further happened till 01.35 when a great burst of feeding activity began and lasted until 02.05. During this period there were at least seven landings by adults on the nests, followed by outbursts of begging calls. At 02.26 there was a single landing followed by a short feed. There was then over an hour without activity. The second main feeding period was from 03.42 to 04.06, when there were at least six, and probably eight, landings by adults followed by bursts of begging calls. There was a minor feed at 04.42-04.43, when only two adults landed, and a final main feeding period beginning at 05.35 and continuing until dawn. During this feeding period at least five adults landed. Thus there were three main feeding periods in the night, during which all or nearly all of the parents brought food, 01.35-02.05, 03.42-04.06 and 05.35-06.15, and three minor feeds by single birds or two birds, at 23.02, 02.26 and 04.42. The total number of recorded landings followed by feedings was 21 or 23. Probably one or two others were missed. Thus each of the eight adults attending the four nests brought food on average about three times during the night. Probably, with some exceptions to account for the three minor feeding periods, each bird brought food once during each of the main feeding periods.

The fact that the main feeding periods were synchronized at the four nests with large young (and also, though less well, at the three nests with smaller young) strongly suggests that the adults were foraging in company. Presumably they were feeding themselves during the six hours after they had left the cave and before the first main feeding period.

As it grew light, at 06.00, the last feeding was still in progress and it was possible to see the birds at the better-illuminated nests. At Nest K all four young were seen craning their heads up toward one of the parents, squealing shrilly. When being fed, the chicks half-turn their heads so that their beaks interlock with the adult's beak. The shrill begging calls cease abruptly at the moment the beaks interlock. Neither beak is opened very wide. As the adult regurgitates the food, its head and that of the nestling with it moves in short quick jerks. At the nests with large young the feeding was most vigorous; in the half-light these nests appeared to be filled with a heaving mass of birds. When the light improved it was possible to see that as adult and young both pushed strenuously during the feeding, with beaks interlocked, they reared up together with the head of the chick pointing obliquely upward and that of the adult downward. The adults were clearly under great physical strain. They could be seen pushing with their feet and would sometimes flap their wings to avoid falling backward. In this attitude both parent and chick would rear and heave together for a minute or more.

It could be seen that there was keen competition between the chicks of each family for the attention of the parent with food. At the only nest with four young, feeding continued longer than at the other nests, and the smallest chick of the four continued to beg for several minutes after the others, when the parents apparently had no more food left. Competition for food probably accounts for the slow early growth of the last-hatched nestling, mentioned earlier, and for the occasional death of small chicks, but there has been no evidence that nestlings have suffered from shortage of food in the later stages, when they need far more.

The Food of the Young.—During the first few days after hatching, semi-digested food is probably given to the young, as nestlings up to the age of ten days have occasionally regurgitated fruit pulp but not seeds. Also, as already mentioned, adults sometimes appear to pass semi-liquid matter to very small young during the day. Later, whole fruits are fed to the young. The earliest age at which a nestling, when handled, has regurgitated a seed is 12 days. A nestling 15 days old, when taken from the nest after its last feed at dawn, regurgitated four seeds in the course of the day, almost certainly too small a number to represent the whole of its last feed. Thus the change from a pulp to a whole-fruit diet is probably gradual.

A full analysis of the Oilbird's food is reserved for Part 2 of this paper. Here only a few special points will be mentioned. Except that they are not given very large fruits, such as those of the palm *Jessenia oligocarpa*, the nestlings are fed on the same fruits as the adults themselves eat. The chief of these, during the periods when the food of the young has been studied, have been the palms *Euterpe oleracea* and *Bactris cuesa;* the Lauraceae *Ocotea oblonga, Phoebe elongata* and one unidentified; the burseraceous trees *Trattinickia rhoifolia* and *Dacryodes* sp.; and an unidentified, probably myrtaceous tree. Like the adults, the nestlings digest the pericarp and usually regurgitate the seeds. Very small seeds, however, may be either regurgitated or passed through the intestine. Most of the seeds of the night's feed are regurgitated by midmorning. Several times nestlings have been removed from the nest soon after their last feed, kept for the day and returned to the nest in late afternoon. Of the total of 428 seeds regurgitated by these nestlings, 306 (71%) were regurgitated before 09.00 hours, and all except 12 (97%) by midday.

On May 14/15, 1960, an attempt was made to estimate the amount of food given to a nestling in the course of the night. Two nests were cleared of all regurgitated seeds in the evening. Next morning three nestlings were taken from these nests at dawn, immediately after the last feed, and all the freshly regurgitated seeds lying by their beaks (hence almost certainly not regurgitated by the parents) were collected. The results, given in Table V, show that each nestling received approximately one-third or one-quarter of its body weight.

Stolzmann (1880) kept a nestling Oilbird for about three weeks. After feeding it at first on various kinds of unsuitable food he was able to obtain fruit of a *Nectandra* sp. (Lauraceae), one of the Oilbird's chief food trees in Peru. From his description the bird was then about 70 days old. Two experiments, made on different days, both showed that the bird was able to eat 14 fruits at a time, and that it regurgitated the first seed half an hour after it had eaten and the last seed one hour after it had eaten. This bird was almost certainly undernourished, which may explain the very short time taken for regurgitation compared with the nestlings studied here. At the same time his experiment shows that regurgitation can begin very soon after the food has been eaten, and suggests that under natural conditions at least a proportion of the seeds from the first feeds of the night will be regurgitated before dawn. This is confirmed by the present study. About one-third of the seeds regurgitated by the three nestlings in Table V had been regurgitated before they were taken from the nest. On the seven further occasions when nestlings have been taken from the nest at dawn (without earlier clearing of the nests and collecting of the fresh seeds regurgitated before dawn), the seeds regurgitated in the course of the day have never represented a feed of more than one-sixth of the nestlings' weight.

These young birds that have been removed from their nests have regurgitated a considerable number of whole, undigested fruits, the proportion varying between individuals and according to the kind of fruit. Fruits of the palm *Euterpe*, with a rather hard pericarp, have been regurgitated whole much more often than any other kind. Proportionally more fruits have been regurgitated whole early in the morning than later. Though the disturbance of being removed

TABLE V. AMOUNT OF FOOD EATEN BY NESTLING OILBIRDS IN THE COURSE OF A NIGHT

Nestling	Age in Days	Weight of Nestling (gm.)	Number of Fruits Eaten	Total Weight of Fruit Eaten (gm.)[1]
Oldest of 3, Nest F	56	525	86	126
Oldest of 4, Nest K	40	350	73	103
Youngest of 4, Nest K	30	120	20	35

[1] Total weights of fruit calculated from the following mean weights of individual fruits: *Bactris cuesa,* 1.9 gm.; *Euterpe oleracea,* 1.3 gm.; *Ocotea oblonga,* 0.6 gm.; *Dacryodes* sp., 2.8 gm.

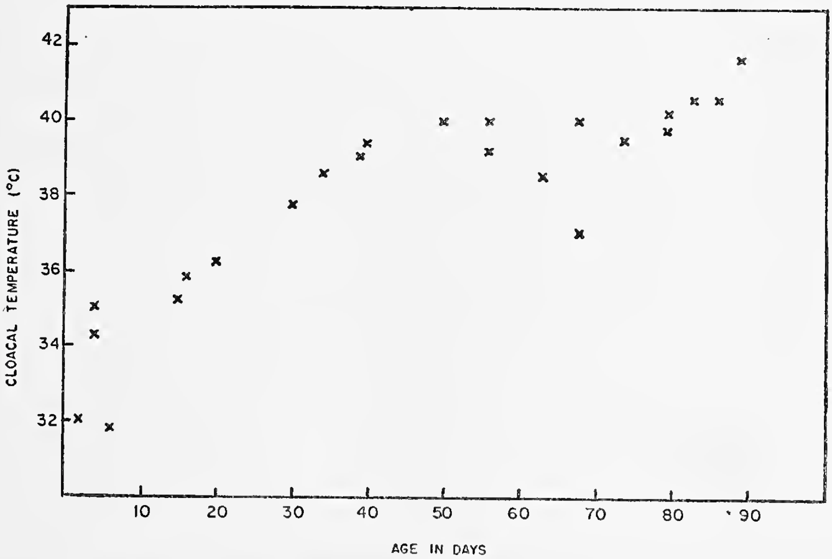
from the nest may cause some premature regurgitation, this is probably not the full reason for the regurgitating of whole fruits, since collections of food in catching trays below the nests regularly contain a proportion of whole fruits, especially during the seasons when the young are being fed.

Temperature Control and the Fat Deposits.—Small young feel cool to the touch after they have been left uncovered for a few minutes, and they rapidly become cooler. In order to study nestling temperatures, cloacal temperatures were taken with a quick-registering mercury thermometer as soon as possible after arrival at the colony (Text-fig. 6). Cloacal temperatures of 31.8° to 35.0° C. were recorded for nestlings up to six days old, while three nestlings 15, 16 and 20 days old had cloacal temperatures of 35.2°, 35.8° and 36.2° respectively. For older nestlings temperatures of 37.0° and over were recorded, most being between 39° and 41° from the age of 40 days onwards. (One very low reading for a nestling 68 days old may have been due to the thermometer lodging in a mass of faecal matter on the point of being expelled).

The ability to maintain body temperature appears to be acquired at the age of about three weeks. Thus the temperature of four young aged 2-6 days fell at the rate of between 1.3° and 2.3° in 10 minutes after being uncovered, while that of three young birds 15, 16 and 20 days old fell at rates of 1.0°, 0.6° and 0.3° respectively, and temperatures of older nestlings have usually not fallen appreciably during exposures of up to half an hour. Air temperatures at the nests are around 22° C. at midday, falling to about 18° at night.

In addition to the acquisition of temperature control, the age of about 25 days marks three other important and related changes in the life of the young Oilbird. The down feathers are bursting out all over the body (Table III). The young bird's weight is increasing rapidly (Text-fig. 4), probably due to a relative increase in fat deposits as well as to an increase in over-all dimensions. At the same time the parents are ceasing to brood the young bird by day, and at night they are beginning to leave it for several hours while they are out foraging.

There is little doubt that the thick down is important in enabling the nestling to maintain body heat, and it seems probable that this is also an important function of the deposits of fat. Deposits of fat in other young birds with slow development, especially Tubinares and swifts, have usually been considered to be reserves against periods of food shortage. These are birds in which the ability to find food is greatly dependent on the weather, and even when conditions are favorable the parents may have to travel long distances in obtaining it. For the Oilbird, however, there is no evidence that the nestlings are liable to undergo periods of fasting. The indications have been that food has been consistently abundant, and the ability of the adults to find the food does not seem to be much affected by weather. Throughout the breeding season in each year, as already mentioned, a proportion of the fruits brought to the nests have been regurgitated intact and dropped over the edge of the nests.



Text-fig. 6. Cloacal temperatures of nestling Oilbirds.

ADAPTATIONS TO CLIFF-NESTING

Cliff nest-sites are safe from most predators; indeed it is because of this that natural selection has favored their use in various groups of birds. If they are also in darkness, they are of course completely safe from visual predators. But suitable cliffs are not numerous, whether in caves or not. Hence cliff-nesting birds tend to defend their nests jealously once they have gained possession of them, but at the same time tolerate the close proximity of birds of their own kind and often of other species. Furthermore, in order to nest safely on a cliff a bird's behavior must be such that it does not knock the eggs or young off, and the young themselves must have behavioral adaptations preventing them from falling off. Cullen (1957) has shown how many of the morphological and behavioral characters of the Kittiwake (*Rissa tridactyla*), which distinguish it from other gulls, are attributable to its cliff-nesting habit. In the Oilbird, too, several adaptations to cliff-nesting are apparent, though in this case there are no close relatives with which it can be compared.

Nest sites suitable for Oilbirds are extremely limited in number, being restricted to a rather small number of caves in Trinidad and parts of northern South America. This has probably been the chief factor in the evolution of their highly social nesting behavior. It also probably accounts for the continuous occupation of the nest throughout the year, since a pair, once dispossessed, would find it very difficult to establish themselves again.

In the Kittiwake, cliff-nesting is associated with the relaxation of various anti-predator features. The same tendency is evident in the Oilbird; in particular, there is complete relaxation of all features that help to protect the nest against visual predators. Thus the adults are rather tame when on the nest, predators are not attacked, the eggs are white, and the chicks are not camouflaged.

If the nest site is safe, slow development of the eggs and young is not a serious disadvantage. Several authors have commented on the general correlation between safety of nest site and length of incubation and fledging period in birds, although no detailed study has yet been made. The Oilbird's development is exceptionally slow; among land birds only the California Condor (*Gymnogyps californianus*) is known to have a longer fledging period (Koford, 1953), while that of the Bateleur Eagle (*Terathopius ecaudatus*) is almost exactly the same (Brown, 1955). Such a slow development could hardly have been evolved if the nests were subject to such heavy predation as are those of most tropical birds (see, *e.g.*, Skutch, 1945); and in fact the evidence is that they are subject to little disturbance except from human beings. However, it is unlikely that the slow development can be attributed simply to the great safety of the nest site. It is probable that the Oilbird's specialized diet, of low protein content and containing a large indigestible fraction (the seeds), necessitates a slow development. If this is so, cliff-nesting and fruit-eating must have been intimately bound up with one another in the Oilbird's evolution.

Egg-rolling behavior is of little use to a cliff-nesting bird, whose eggs, if they are not in the concave nest-cup, are likely to be lost over the edge. It is not surprising therefore that the Oilbird is in marked contrast to the ground-nesting nighthawks, some of which are known to be able to move their eggs many feet, by pushing them or carrying them in the beak, and which do so repeatedly if disturbed. Likewise, when the young Oilbird has hatched, it is safe only if it remains in the nest-cup, and here again its tendency to stay still and maintain contact with its nest mates may be contrasted with the mobile behavior of young nighthawks. Remaining still in the middle of the nest is not due to inability to move, as even when they are quite small young Oilbirds move backward to the nest edge in order to defecate. Their ability to climb with feet, beak and wings gives them a chance to save themselves if they should nevertheless go over the edge of the nest. The parents' contribution to the safety of the young, as of the eggs, is limited to their tiny shuffling steps, which prevent them from kicking anything off the nest, or indeed from effectively moving any obstacle from their path.

ECOLOGICAL FACTORS IN THE EVOLUTION OF THE OILBIRD

The Oilbird's combination, unique for a bird, of fruit-eating and nocturnal habits is undoubtedly due to its evolution from an originally nocturnal or crepuscular ancestor. As already mentioned, anatomical evidence suggests that the Oilbird's closest affinities are with the Caprimulgiformes, though it has certain characters in common with the owls. A consideration of feeding behavior, however, makes it almost certain that they are in fact closest to the Caprimulgiformes. All other caprimulgiform birds seize insects or other small animals in the mouth and swallow them whole, the feet not being used at all. Owls on the other hand seize their food in the talons and tear it up with the beak. Oilbirds, as we have seen, pluck fruits with the beak and swallow them whole. It is an easy transition to this method of feeding from the typical caprimulgiform method, but from the owl's method the transition is almost inconceivable.

We may then regard the Oilbird as the most extreme product of the rather limited adaptive radiation of the caprimulgiform stock. Since many of the larger diurnal birds of tropical forest are mainly or entirely frugivorous, it is perhaps not surprising that this food supply should have been exploited by one nocturnal bird. However, for an originally insectivorous caprimulgiform bird, the change to a fruit diet must have involved a number of ecological problems. The Oilbird's solution of these problems has had effects on every aspect of its life.

For the fruit-eater, forest trees in fruit are, essentially, temporary and discontinuous pockets of abundant food whose location is always changing. For a nocturnal fruit-eater, the short distance at which such pockets of food can be seen, or otherwise perceived, is an added problem. For a strong-flying bird there is no apparent advantage in searching for such food singly or in pairs, or in maintaining feeding territories, and in fact the parrots, toucans and other large fruit-eating forest birds are generally social feeders, as also are the fruit-eating bats. It is probable, therefore, that the change from an insect to a fruit diet in the ancestral Oilbird stock involved the enhancement of social and gregarious behavior at the expense of territorial behavior.

It is probable, too, that the Oilbird's large size, compared with that of most other caprimulgiform birds, was another consequence of the change to a fruit diet. It must have been a great advantage to be able to exploit the larger fruits, up to two inches or so long, of the tall forest trees, which form the main food of the large diurnal fruit-eating birds, since these not only give more nourishment for every fruit taken but are also much more conspicuous at night than the smaller fruits of second-story trees and shrubs which provide much of the food of the smaller frugivorous birds.

Increasingly social habits, increased size and a diet of fruit must have eventually necessitated radical changes in breeding behavior. In particular, increase in size and probably also the fruit diet (comparatively poor in proteins) must have lengthened the period of development of the young. At some point in its evolution the Oilbird must have faced in acute form the "choice" between either making the nest extremely inconspicuous, as do most other caprimulgiform birds and probably its own ancestors, or else making it extremely safe. Its large size, its fruit diet, which involves the accumulation of much regurgitated matter around or under the nest, its awkwardness in trees and lack of complex nest-building behavior (common to all the Caprimulgiformes), must all have favored the choice of a very safe nest site. Of the two main types of safe nest site available, cliffs and hollow trees, there is little doubt that natural selection would favor the former as being safer than tree holes and less sought after by other creatures. Cliff-nesting, as already mentioned, is usually associated with social breeding behavior; hence both for feeding and for nesting natural selection must have favored gregarious as against territorial tendencies. (It is perhaps instructive that one large fruit-eating cotingid, the Cock-of-the-rock, *Rupicola,* has adopted the same type of nest site, on cliffs or in shallow caves, and breeds semi-socially).

Presumably, then, the first step in the evolution of cave-nesting was from the ancestral site (probably the ground or on tree stumps) to cliff-ledges in the open, and it was at this stage that the rudiments of the echo-location faculty were evolved. Pressures of predation probably then led to the seeking of deeper and deeper recesses, until the perfection of echo-location allowed the birds to occupy the deepest caves, and so opened up for them a wealth of nest sites that were completely safe (until the arrival of man) and for which no other creatures competed.

SUMMARY

An account is given of the general behavior and nesting of the Oilbird, based on 3½ years' observations.

Oilbirds are gregarious cave-dwelling birds, almost certainly of caprimulgiform stock. They spend all day in caves and fly out at night to feed on the fruits of forest trees.

The Oilbird's stance is peculiar, the body being tilted forward and the very short legs rotated as far forward as possible. Aerodynamically they are highly specialized for flight within restricted spaces and for load-carrying.

Sight is well developed and is used whenever possible. The sonar method of orientation, discovered by Griffin, is used only when there is not enough light. There is some evidence that the olfactory sense is important in food-finding.

Daily routine and social behavior are described. The birds leave the cave at dusk and return before dawn. They are gregarious while feeding. The pair bond is probably permanent. Aerial displays, probably connected with pair formation, have been seen at night. Courtship behavior on the nest consists of the preening of the female's head by the male.

The nest, eggs and young are described. The breeding cycle is very slow; eggs are laid at in-

tervals of several days, the incubation period is usually 33-34 days, and the fledging period 90-125 days. The young become very fat, reaching a weight half as much again as the adult's weight at about the 70th day. There are two sets of down feathers, followed by the growth of the adult plumage.

The young are fed at long intervals during the night, large young three or four times, smaller young five or six times a night. The food of the young is the same as the adult's. Nestlings eat about one-third or one-quarter of their body weight during each night.

The young acquire temperature control at the age of about three weeks. Both the thick down feathers and the fat deposits are considered to be important in maintaining body temperature.

The ecological aspects of the Oilbird's evolution are discussed. It is argued that the original change from an ancestral insect diet to a fruit diet led to increased gregariousness, increased size, slower development, the adoption of cliff nest-sites, and finally, with the perfection of echo-location, to the colonization of pitch-dark caves.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Typical stance of Oilbird on nest. Note position of feet far forward under breast.

Fig. 2. Oilbird clinging to narrow ledge; showing backward position of inner toe, and tail pressed against rock face. Feet are held much farther back than when perching on level surface (Fig. 1). For explanation see text.

Plate II

Fig. 3. Oilbirds clinging to sloping ledge; showing position of legs and toes. For explanation see text.

Fig. 4. Oilbird in slow flight, at early stage of upstroke; tip of left wing still on the downstroke. To show the extreme width of wing and fully spread tail.

Fig. 5. Oilbird in slow flight; the upstroke. The bend in the wing shows that the wing beat is propulsive.

FIG. 1

FIG. 2

THE NATURAL HISTORY OF THE OILBIRD, STEATORNIS CARIPENSIS, IN TRINIDAD, W.I.

FIG. 3

FIG. 4

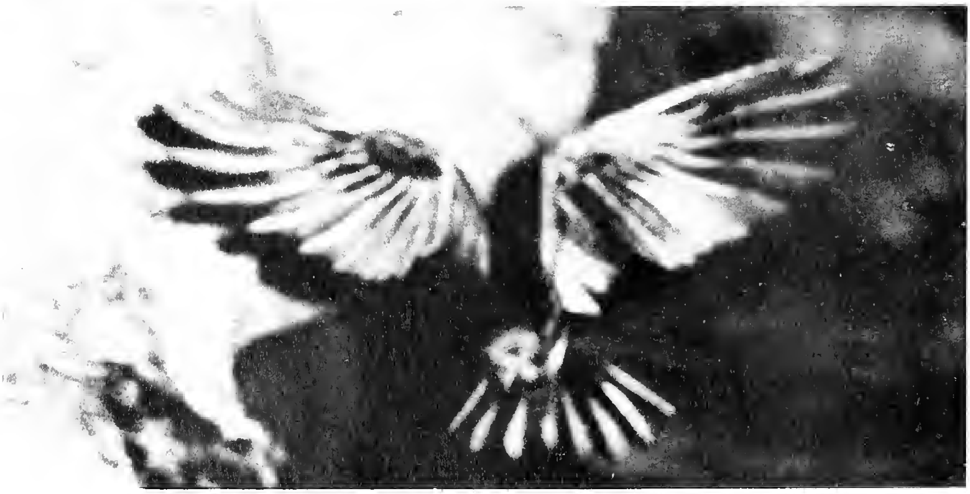

FIG. 5

THE NATURAL HISTORY OF THE OILBIRD,
STEATORNIS CARIPENSIS, IN TRINIDAD, W.I.

4

Fatty Degeneration, Regenerative Hyperplasia and Neoplasia in the Livers of Rainbow Trout, *Salmo gairdneri*

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(Plates I-VI)

Introduction

LIVER tumors in fish were first reported in European brown trout (*Salmo trutta* Linnaeus) by Plehn (1909a, 1924). The first case in rainbow trout (*Salmo gairdneri* Richardson) was described by Haddow & Blake (1933) in England. The disease was also reported in this species in the United States in 1953 by Nigrelli (1954), who indicated that the incidence of hepatomas in such trout might be relatively high. Four tumors were subsequently found in a single lot of 600 3-year-old rainbow trout in a Pennsylvania hatchery, and the histopathology of these was briefly described by Nigrelli & Jakowska (1955). Almost simultaneously, similar tumors that had been reported by Scolari in 1953 in rainbow trout from all the hatcheries along the Alpine Arc and the lake region of northern Italy, were described by Cudkowicz & Scolari (1955). They reported an incidence of 50% or more in some hatcheries and described the histopathology and distribution of the disease and indicated its possible cause.

In the spring of 1960, rainbow trout that were to be introduced into California's waters were examined at the state line by California Fish and Game inspectors and found to be affected with liver tumors. An embargo was immediately placed on all rainbow trout being shipped into the state. This led to the examination of trout in many hatcheries, whereupon it soon became evident that trout with liver diseases were widely distributed in the United States, with an incidence of hepatomas of 50% or more being reported in some hatcheries. In retrospect, some fishery personnel have indicated that the hepatomas were prevalent in rainbow trout as far back as 1937 (Rucker *et al.*, 1961). It is highly probable that the disease went unrecognized for a great number of years, since little or no attention was given to fish pathology by fishery biologists in this country until relatively recently.

Since no critical distinction has been made between regenerative hyperplasia and neoplasia, we cannot accept the numerous unofficial reports that all the "tumors" seen in rainbow trout are hepatomas, especially if the term is used to indicate a neoplastic process. The present paper is therefore principally concerned with a microscopic analysis of the livers of rainbow trout from several hatcheries, mainly in Idaho, Montana and Wyoming, and those of a few specimens of brown and rainbow trout taken from natural waters.

Observations and Discussion

Three hundred and thirty-three livers were examined. They varied considerably in size and appearance, and those with apparent lesions were characterized by grayish or yellowish spots, streaks (Plate I, Fig. 1), numerous petichiae or larger clots, or by one or more encapsulated (Plate I, Fig. 2) and non-encapsulated nodules on the surface or deep in the parenchyma. In a few instances, livers with hob-nail or miliary appearance were also seen.

The so-called normal liver showed a histological picture resembling that consistently found in teleosts kept in captivity and fed artificial diets. The usual pattern consisted of lobules with a central vein in which radiating liver cells, separated by sinusoids, were arranged as a muralium[1], or wall, two cells in thickness but without polar orientation of the nucleus (Plate

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[1] The significance of two-cell-thick muralia in hepatomas of mammals and birds is discussed by Elias (1955).

III, Fig. 6). The over-all picture was one of uneven staining, suggesting physiological differences in the cells of the various lobules. The liver cells were uniform in size and typical in shape, but some relatively larger cells with larger nuclei, suggestive of polyploidy, were also observed. The cytoplasm was usually lightly basophilic, but the degree of basophilia sometimes varied with the lobules, apparently affected by the presence or absence of cytoplasmic particulates. The nucleus was typical, with one or more acidophilic nucleoli. Although binucleate and multinucleate cells were not unusual, mitotic figures were rare.

After appropriate staining, changes indicative of disease were interpreted as fatty infiltration (Plate II, Fig. 3), glycogen infiltration or depletion, ceroid deposition (Plate II, Fig. 4), hematochromatosis (Plate II, Fig. 5), focal necrosis with lymphocytic infiltration (Plate III, Figs. 6, 7), portal cirrhosis (Plate III, Fig. 8), biliary cirrhosis (Plate III, Fig. 9), hyperplasia and neoplasia.

Hyperplasia, which ranged from simple solitary (Plate IV, Fig. 10) or widely diffuse regenerative areas (Plate IV, Fig. 12) to single or multiple nodules, was found in 50 of the livers. The hyperplasia was evident as islands or as extensive growth of liver cells of irregular pattern in which uninucleate and multinucleate elements were densely packed, especially at the periphery of the nodules. Some of the larger nodules were completely necrotic. In some areas, clusters of larger cells with acidophilic cytoplasm and eccentric nuclei were present (Plate IV, Fig. 11). The origin and nature of these cells, some of which contained ceroid, remain to be determined. In close proximity to these, as well as in areas adjacent to regions of apparent active growth, there were considerable morphological changes in the liver cells, and these changes extended even to elements more distantly located. The lobules were often distorted and the cells showed variation in size, shape and staining reactions; in many instances the tissue assumed an adenomatoid appearance (Plate V, Fig. 13). These effects were more pronounced in hyperplastic livers with extensive fatty infiltration and ceroid deposition and with portal and biliary cirrhosis.

Nineteen of the livers were diagnosed as neoplastic and the changes noted in them ranged from adenoma (Plate IV, Fig. 14) to hepatocellular carcinoma. The latter exhibited either a normal cell arrangement (but without distinct lobular pattern), or an anaplastic, highly pleomorphic structure with considerable degeneration and hemorrhage. The hepatocellular lesions were frequently indicated by a solid cord-like cellular arrangement in which the hepatic cells were relatively small, the nuclei pyknotic and vascularization scanty (Plate V, Fig. 15). Cholangiomatous changes (Plate VI, Fig. 16) and cirrhosis (Plate VI, Fig. 17) were found in a few cases. Other details were similar to those reported by Nigrelli & Jakowska (1955) and by Cudkowicz & Scolari (1955).

Some of the abnormal conditions described above, including adenomatous and cholangiomatous growths, were also found in livers of four rainbow trout and Loch Leven (brown) trout taken from natural waters in Idaho.

The histological picture of the atypical cell growths in the livers of hatchery-raised rainbow trout is strikingly similar to that reported in mice and other experimental animals kept for prolonged periods on a low choline diet, and in which fatty livers with little or no cirrhosis develop (Hartroft, 1954, 1955). In general, liver diseases in higher animals involving necrosis, fatty changes and ceroid deposition, with or without hyperplasia or neoplasia, have been attributed to deficiency or imbalance of one or more of the following substances: methionine, cystine, choline, vitamin E, vitamin B_{12} and riboflavin (Kensler *et al.*, 1941; Endicott & Lillie, 1944; Engle *et al.*, 1947; Lee, 1950; Casselmen, 1953; Daft, 1954; Drill, 1954; Schwarz, 1954; Hartroft, 1954, 1955; Salmon & Copeland, 1954; Salmon *et al.*, 1955; see also Tannenbaum, 1953).

Although most of the above essential substances are incorporated into artificial trout diets in the United States, it is generally agreed that the complete nutritional requirements of trout have not yet been fully determined, a fact which is in part responsible for the wide variety of diets still in use in hatcheries here and abroad. The non-tumor and regenerative lesions described in this paper may be indicative of some nutritional faults. Some of these abnormal conditions, especially in relation to fatty degeneration and ceroid deposition, have been previously reported in experimentally- and non-experimentally-fed hatchery trout (Plehn, 1909b, 1915, 1924; Gaschott, 1929; Hewitt, 1937; McLaren *et al.*, 1946 ;Mann 1952; Davis, 1953; Schäperclaus, 1954; Scolari, 1954; Faktorovich, 1956a,b, 1958, 1960). Some striking effects of purified rations on the livers of rainbow trout were shown by McLaren *et al.* (1946). Yearling rainbow trout, fed a ration composed of cerelose 48%, casein 40%, fat 2%, dried liver 5% supplemented with brewers' yeast, were able to maintain, for a period of time, a hemoglobin level and growth rate equal to that produced by feeding 100% dried liver or a standard hatchery meat ration. After 8 weeks, however, the fish suddenly died and autopsies showed that they had developed greatly enlarged, yellow, lobulated livers. In this case, the liver damage was prevented by reducing the carbohydrate level to 20%.

The most important disease in hatchery-raised rainbow trout is generally considered to be fatty degeneration of the liver, but with proper diet recovery is often complete. Thus, Faktorovich (1956b) for 11 months carefully followed the regeneration of hepatic tissue in rainbow trout during recovery from fatty degeneration, a condition which he later (1960) interpreted as taking the form of ceroid deposition. It should be emphasized, however, that ceroid deposition appears to be a general characteristic of teleosts, especially those kept in captivity (Pickford, 1953; Wood & Yasutake, 1956; Nigrelli, 1960), those infected with parasites (Nigrelli, 1954), or those exposed to toxic substances, *e.g.*, copper (Calventi *et al.*, 1960).

The epizootic nature of the liver diseases in rainbow trout suggests that hereditary, viral or carcinogenic agents may also play a role. The possibility that the tumors are hereditary in origin has been indicated by Cudkowicz & Scolari (1955). Evidence for this suggestion is based on the following: (1) rainbow trout in the hatcheries in northern Italy are all the progeny of fish imported from Rocky Mountain streams in 1880, (2) a high incidence of tumors was found in populations derived from a stock inbred for 20 years in one hatchery, (3) the liver tumors were not found in hatcheries in Switzerland and Bavaria, two areas that had had no exchange of fish with the Italian hatcheries, and (4), rainbow trout from Denmark that were raised in the Italian hatcheries under identical conditions were not affected with the growths.[2]

Rainbow trout in the United States have been artificially bred for approximately the same length of time as in Europe. Although inbreeding records are not available to us, hatchery practices, such as discarding runts and selecting highly colored or early maturing fish, inevitably lead to the selection of special strains—possibly with inherent susceptibility to liver dysfunctions, *e.g.*, inability to metabolize fats properly. Whether or not such strains can be associated with specific genetic factors remains to be determined. In certain mouse colonies, mutant strains (CBA and C3H) highly susceptible to hepatomas have spontaneously appeared (Andervont, 1950; Woolley, 1951).

Virus and carcinogens as etiological agents cannot be excluded, but mammal geneticists generally agree that their principal effect is to enhance the existing intrinsic susceptibility to spontaneous tumors (Heston, 1941; Heston & Deringer, 1947, 1949; Duran-Reynals, 1953; Sangvi & Strong, 1958). The following substances, occurring in nature or introduced with feeding, are known to produce liver damage in fish and other animals: zinc chloride, copper sulfate (Calventi, Jakowska & Nigrelli, 1960), carbasone, sulfonamides, antibiotics (Goodman & Gilman, 1955), bentonite (Jakowska & Nigrelli, 1956), radioactive and ionizing substances, and arsenicals (see Hueper, 1953; Wilson, 1954). Their possible role in the etiology of liver diseases in rainbow trout must be considered. In addition, pathological liver changes may be associated with the following well-known diseases of trout: furunculosis, ulcer disease, infectious (viral) pancreatic necrosis, bacterial or viral infections of the kidney, mycosis, helminthiasis, cnidosporidiosis and acute or chronic anemia. Increased pressure in the venous system, resulting from these diseases, may severely affect the liver. The effect on the hepatic cells may result from anoxemia, a condition also associated with acute and chronic anemia.

Whatever the cause of the liver lesions in rainbow trout, most of the damage is degenerative, and any hyperplasia seen represents a compensatory response of parenchymal tissue. The pleomorphic changes noted in surrounding and more distant hepatic cells are the result of pressure from the active hyperplasia. It is highly probable that in certain instances the hyperplasia is supervened by neoplasia, especially if the proliferation of the hyperplastic tissue becomes excessive.

Most of the so-called hepatomas in rainbow trout are hyperplasias rather than neoplasias. It has been pointed out by Hartroft (1955) that the differential diagnosis of hyperplasia and neoplasia in the livers of both experimental animals and man has perplexed and confused pathologists for many years; the close resemblance of small neoplastic foci in a cirrhotic liver to foci of hyperplasia irresistibly suggests that the former may arise regularly from the latter.

The sudden and widespread concern about hepatomas in rainbow trout is reminiscent of a similar situation at the turn of the century with regard to thyroid tumors in trout and other salmonids in Europe and the United States. These growths were originally described as

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[2] Cystic degeneration of the liver has also been described in rainbow trout in an Italian hatchery by Castelnuovo & Rizzo (1938). It was suggested that this disease is congenital.

adenocarcinomas or carcinomas and, as in the present case of the hepatoma, the disease occurred in epizootic proportions (Plehn, 1902; Pick, 1950; Gaylord & Marsh, 1914). Most of the thyroid tumors were later recognized as simple hyperplasia or goitres (Marine & Lenhart, 1911; Marine, 1914). This interpretation has been substantiated by the fact that since iodine has been regularly added to the water or food, the incidence of thyroid tumors has been reduced to a point where they now are extremely rare, even though a relatively large number of the growths originally described were actually neoplastic. A similar interpretation may prove to be true for the liver tumors of the rainbow trout.

SUMMARY

Microscopical findings in livers of more than 300 hatchery-raised rainbow trout from several states and from rainbow and brown trout from natural streams are described. Most of the livers resembled those of fish kept in captivity and fed artificial diets for prolonged periods. The lesions, when present, were mainly degenerative and characterized by fatty infiltration, glycogen depletion or infiltration, ceroid deposition, hematochromatosis, focal necrosis with lymphocytic infiltration, biliary and portal cirrhosis and other histological and cytological changes. These conditions were associated with compensatory non-nodular and nodular hyperplasia of the liver cells, and it is assumed that under certain circumstances hyperplasia is supervened by neoplasia. Hereditary, nutritional, carcinogenic and other possible etiological factors are discussed.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. Female rainbow trout (*Salmo gairdneri* Richardson) showing discolored lesions on the liver. Reduced 2.5 ×.

FIG. 2. Section through an encapsulated tumor mass of rainbow trout from a Pennsylvania hatchery. 5 ×.

PLATE II

FIG. 3. Fatty infiltration in an otherwise normal liver of a rainbow trout. Hematoxylin-eosin. 500 ×.

FIG. 4. Extensive ceroid deposition of liver. Acid-fast stain. 250 ×.

FIG. 5. Accumulation of siderin in areas surrounding biliary duct. Hematoxylin-eosin. 1000 ×.

PLATE III

FIG.. 6. "Normal" lobule of liver of rainbow trout with two-cell-thick muralia radiating from a central vein. Note necrotic areas adjacent to the lobule. Hematoxylin-eosin. 250 ×.

FIG. 7. Typical lymphocytic infiltration in a necrotic area. Hematoxylin-eosin. 500 ×.

FIG. 8. Section of otherwise "normal" liver showing portal cirrhosis and a small necrotic area. Hematoxylin-eosin. 250 ×.

FIG. 9. Biliary cirrhosis and disorganization of adjacent hepatic elements. Masson's stain. 400 ×.

PLATE IV

FIG. 10. Focal necrosis and localized hyperplasia of liver cells from rainbow trout. Hematoxylin-eosin. 250 ×.

FIG. 11. Cluster of acidophilic cells with eccentric nuclei. Some cells contain ceroid. The nature and origin of these cells are unknown. Hematoxylin-eosin. 500 ×.

FIG. 12. Diffuse regenerative hyperplasia associated with extensive degeneration of the liver. Hematoxylin-eosin. 400 ×.

PLATE V

FIG. 13. Adenomatoid appearance of hepatic tissue associated with hyperplastic foci in more distantly located areas of the same section. Hematoxylin-eosin. 400 ×.

FIG. 14. Details of adenomatous liver. Note large ceroid globule. Masson's stain. 2000 ×.

FIG. 15. Cord-like arrangement of hepatocellular tumor. Hematoxylin-eosin. 600 ×.

PLATE VI

FIG. 16. Cholangiomatous changes in hepatoma of rainbow trout. Hematoxylin-eosin. 500 ×.

FIG. 17. Extensive cirrhosis seen in hepatoma of Pennsylvania rainbow trout. Hematoxylin-eosin. 250 ×.

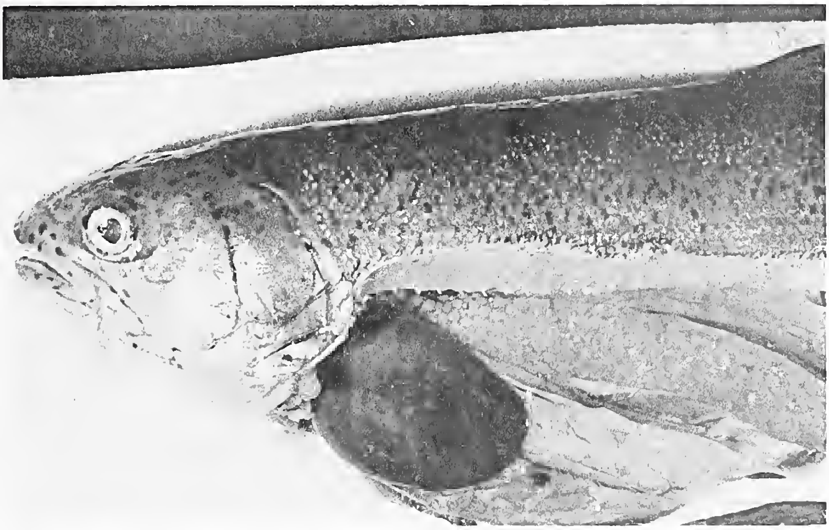

FIG. 1

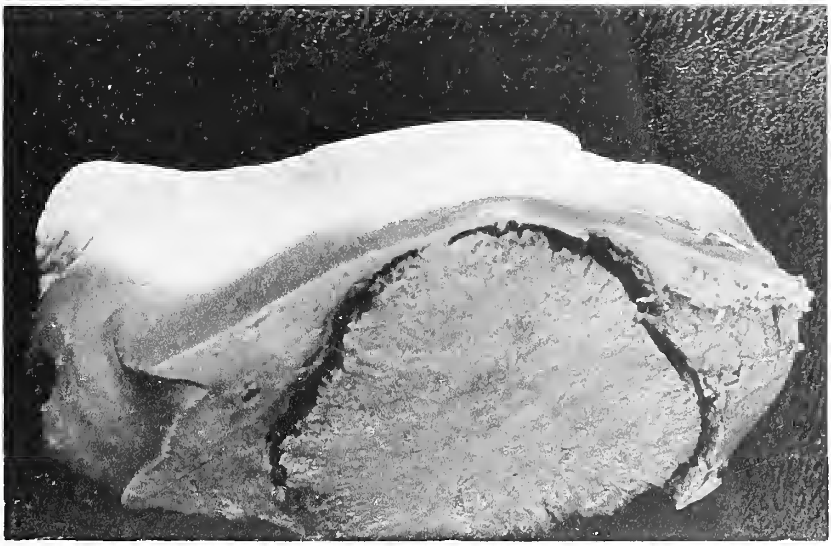

FIG. 2

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

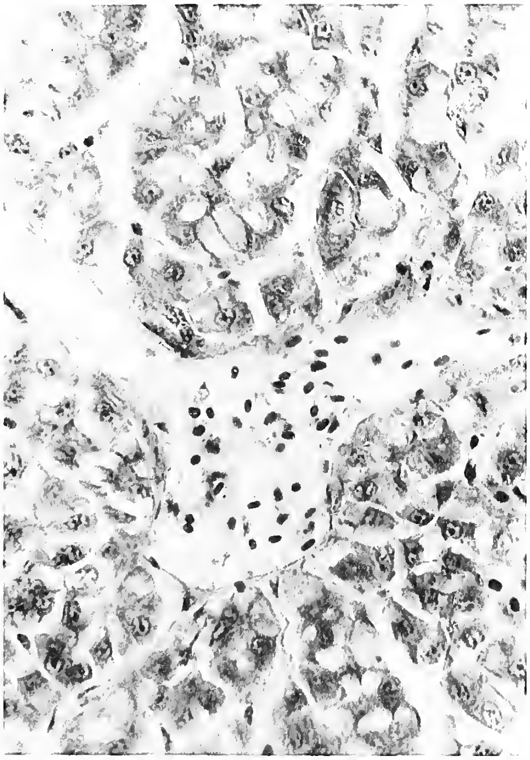

FIG. 3

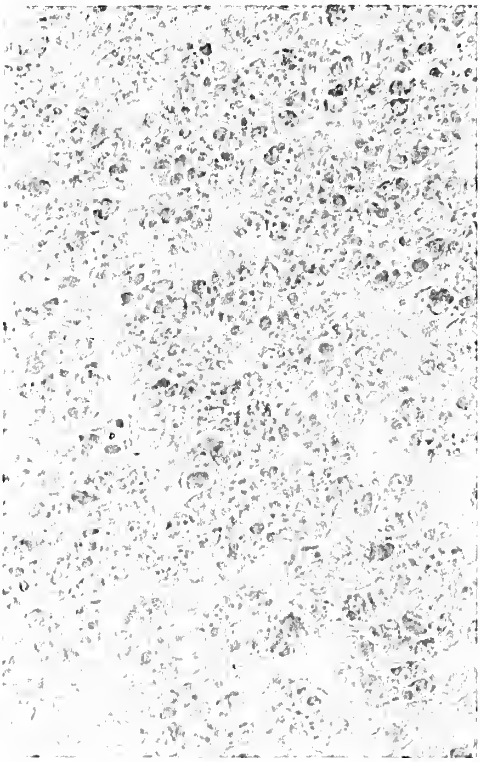

FIG. 4

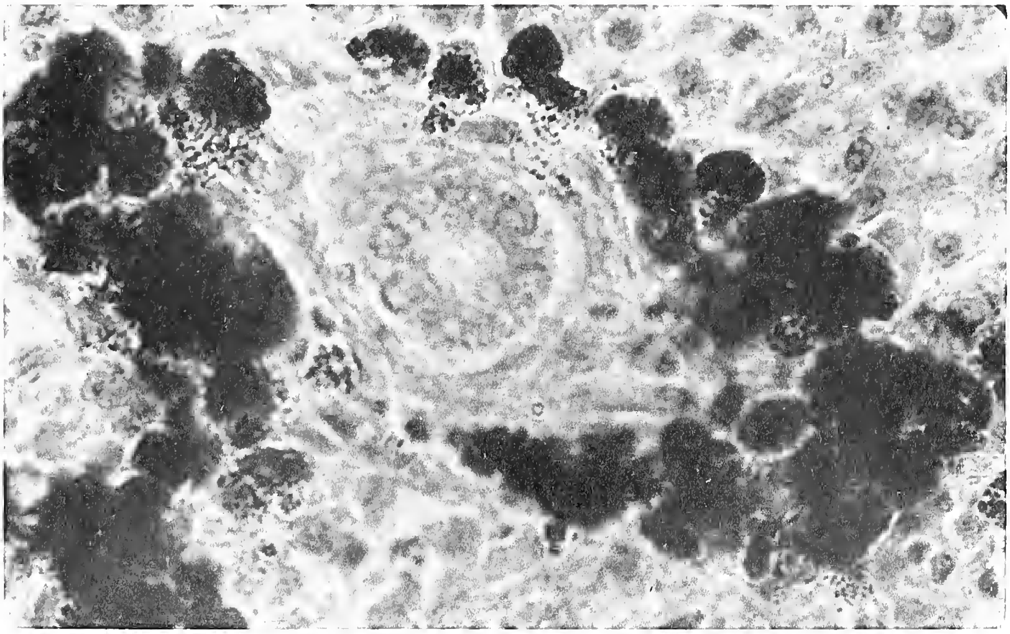

FIG. 5

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA
IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

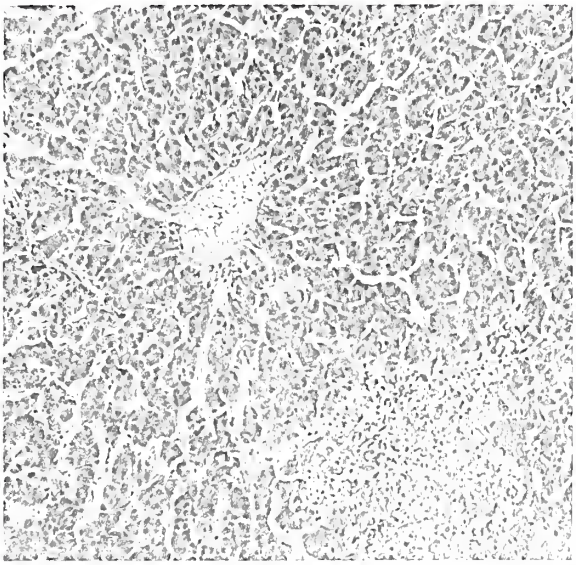

FIG. 6

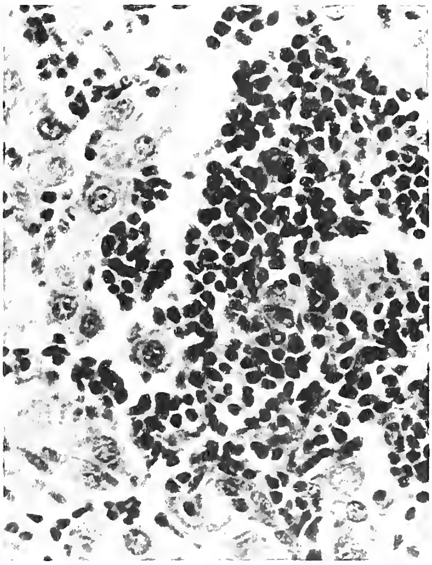

FIG. 7

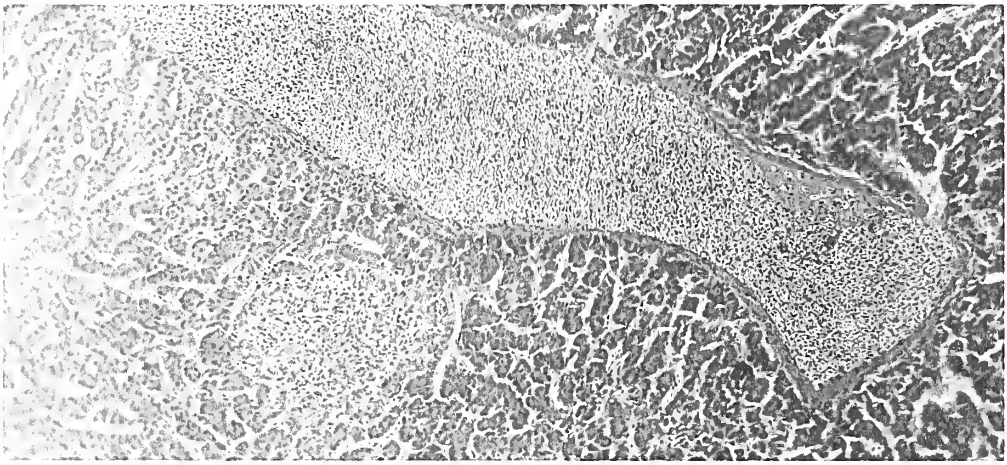

FIG. 8

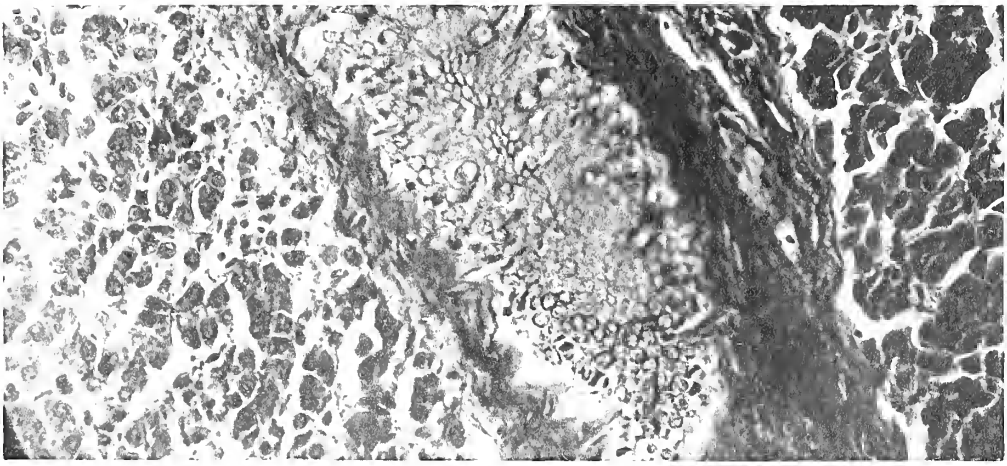

FIG. 9

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA
IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

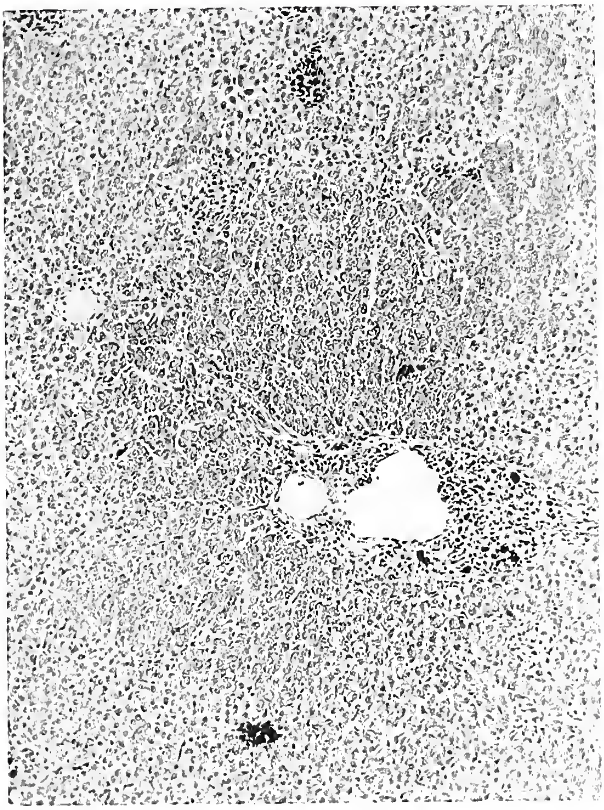

FIG. 10

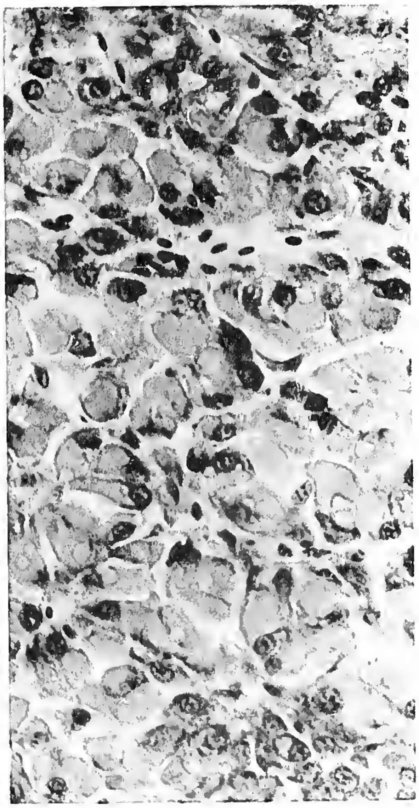

FIG. 11

FIG. 12

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

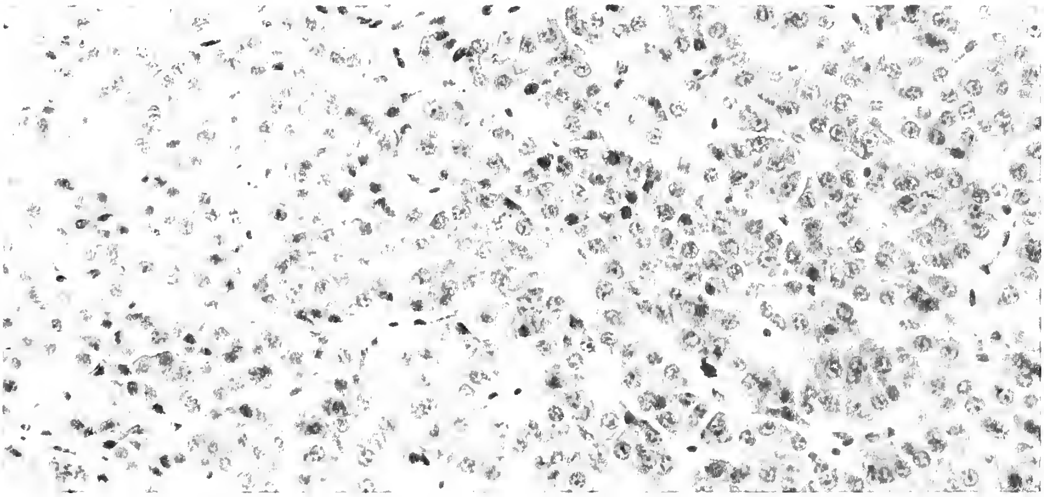

FIG. 13

FIG. 14

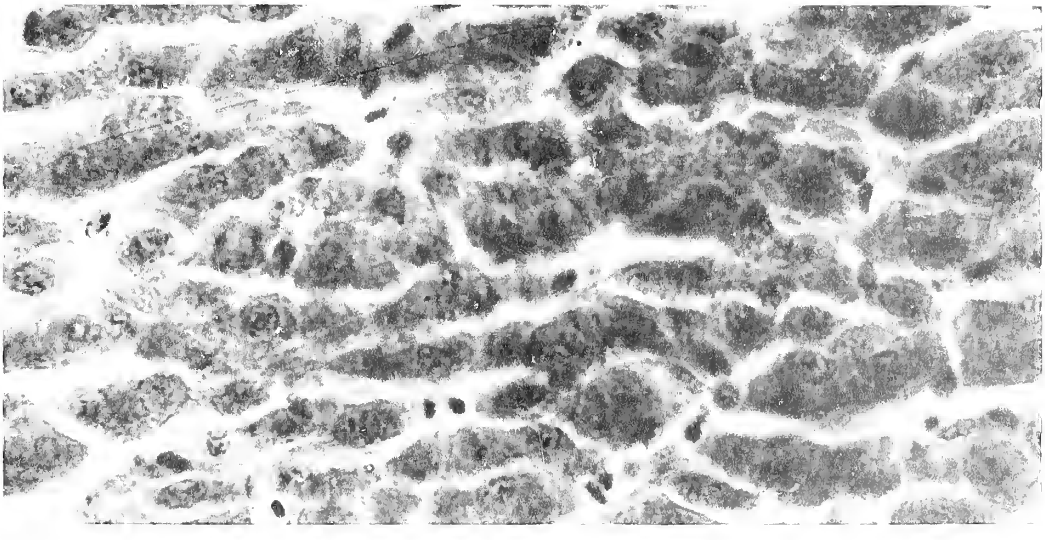

FIG. 15

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA
IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

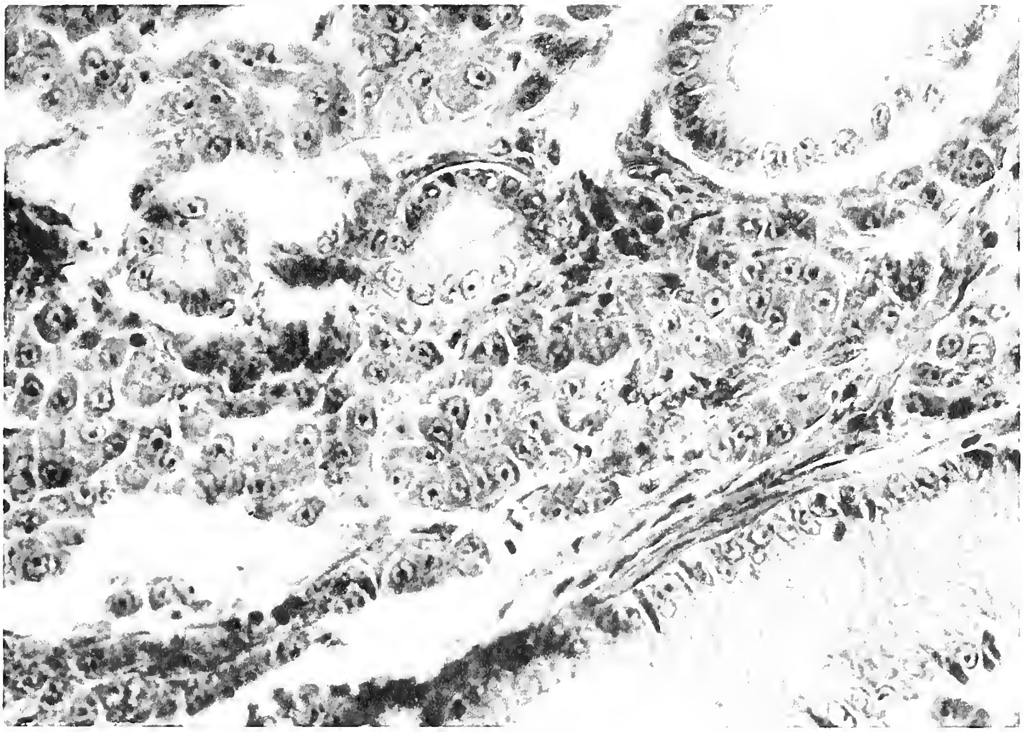

FIG. 16

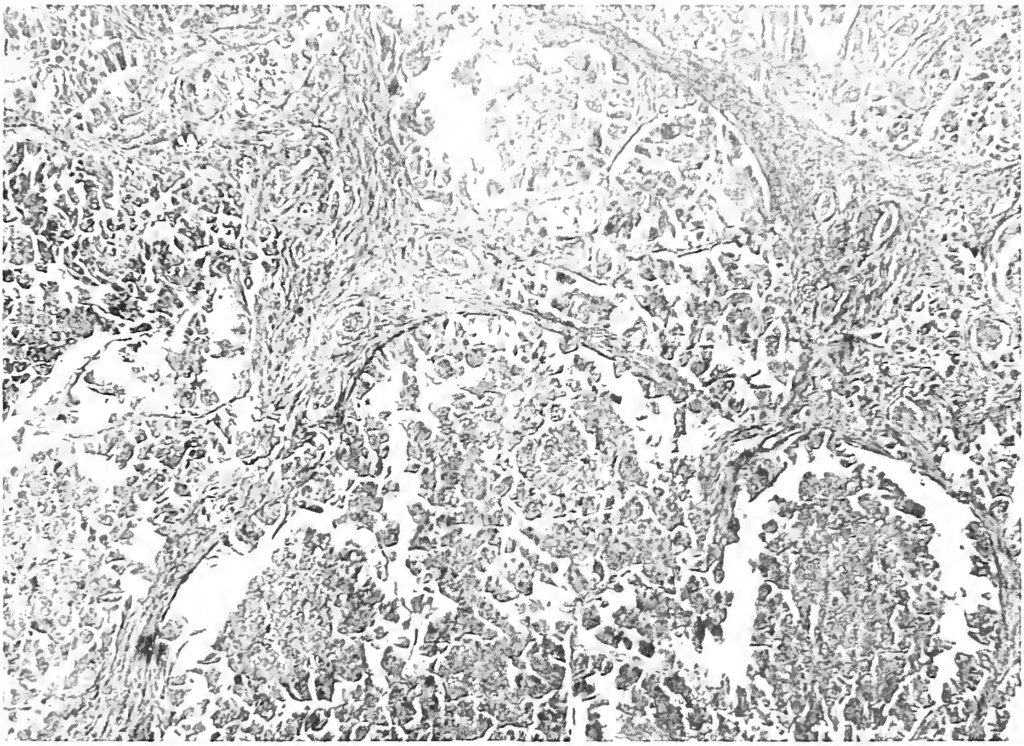

FIG. 17

FATTY DEGENERATION, REGENERATIVE HYPERPLASIA AND NEOPLASIA
IN THE LIVERS OF RAINBOW TROUT, SALMO GAIRDNERI

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Contents

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Morphological Effects of Low Temperatures during the Embryonic Development of the Garter Snake, *Thamnophis elegans*[1]

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Louisiana State University, School of Medicine and Department of Biology, New Orleans

(Text-figures 1-4)

SINCE SCUTE PATTERNS and scute ("scale") counts are among the principal taxonomic characteristics of reptiles, any experimental evidence suggesting that scutellation can be altered by environmental factors is of considerable interest to herpetologists. One of us (Fox, 1948a) reported one such experiment in which exposure to low temperatures during embryonic development resulted in decreased numbers and modified patterns of scutes in garter snakes. If such accidents of development can happen in the laboratory, conceivably they can also occur under natural conditions.

Although the data presented in 1948 were statistically significant, they were not altogether convincing because of the small number of surviving experimental litters and the small size of these litters. Hence, the experiment was repeated in 1948 under essentially the same laboratory conditions as in 1947 (reported in 1948).

METHODS

The subspecies of garter snake used in this experiment was the same as was used in the previous report. This was referred to as *Thamnophis elegans atratus* according to the designated classification at that time (Fox, 1948b). However, this population of garter snakes was of the terrestrial form which later (Fox, 1951) became known as *Thamnophis elegans terrestris.* None of the snakes used was of the semi-aquatic type which Fox (1951) recognized as a distinct although sympatric race of garter snake identical to the type specimens of *Thamnophis elegans atratus* (Kennicott).

The garter snakes, *Thamnophis elegans terrestris,* were collected from a 25-mile stretch of the Pacific coast which included the 1947 collecting site on Skyline Blvd. of the San Francisco Peninsula in San Mateo County, California. Since the Skyline Blvd. area had been collected heavily during the previous three years, a more extensive collecting area proved necessary in order to find adequate numbers of gravid female snakes. All animals were collected during the last week of April and the first week of May as it had been determined from previous collecting that gravid females obtained during this period would already be inseminated and would either have ovulated recently or would probably ovulate within a few days following capture.

Immediately upon capture 18 gravid females were placed in a cool room (experimentals) in which the temperature ranged from 65° to 85° F, and four gravid females were placed in a warm room (controls) in which the temperature ranged from 75° to 95°F, the same temperature ranges utilized in 1947. These rooms were located in a small, incompletely insulated greenhouse and the temperature variations reflected variations in the outside environmental temperatures. The temperature rose and fell simultaneously in both rooms but a marked differential was maintained between them. The small control sample seemed permissible in view of the very large control sample available from the previous experiment (1947) and the desirability of making the experimental sample as large as possible.

The four control females littered 46 young in early August. By September the experimental mothers had not littered and it was decided to terminate the experiment. The mothers were killed and fetuses were obtained from 15 of them. The others contained partially reabsorbed

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[1] Experiment conducted at the University of California, Berkeley.

[2] Partially supported by a grant from the Louisiana Heart Association.

TABLE I

Samples	Sample Size	Ventrals	P[3]	Subcaudals	P[3]
1 ♀ 1948 Experimental	63	150.3 ± 10.0[1] 138 – 160[2]		65.6 ± 9.3[1] 48 – 77[2]	
2 Mothers of 1948 Experimental	12	154.3 ± 3.9 149 – 160	.03	71.2 ± 4.0 67 – 80	.005
3 ♀ 1948 Control	20	154.5 ± 3.5 150 – 158	.008	73.7 ± 3.8 68 – 76	<.001
4 ♀ 1947 Control	50	154.9 ± 4.1 147 – 167	.005	70.4 ± 2.9 62 – 77	.001
5 ♂ 1948 Experimental	65	154.6 ± 11.0 134 – 163		74.7 ± 11.2 48 – 86	
6 ♂ 1948 Control	26	160.8 ± 3.4 154 – 164	<.001	82.7 ± 6.5 71 – 89	<.001
7 ♂ 1947 Control	58	158.7 ± 3.6 152 – 168	.007	80.5 ± 3.7 71 – 88	<.001

[1] Mean and standard deviation.
[2] Range.
[3] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples.

embryos and hard masses of yolk in their oviducts. The experimental fetuses were in various stages of maturity but all had fully completed the development of scute patterns. The young snakes and their mothers were preserved in alcohol or formalin and stored in jars until 1958. During the interval three of the litters dried out so badly that reliable scale counts could not be made. Therefore the experimental sample is based on 12 litters yielding 128 fetuses. In a few other instances the number of scale rows around the body was questionable due to dehydration of the specimens. These were also omitted from the calculations.

The number and condition of the following scutes were observed on each specimen: ventrals, subcaudals, longitudinal rows around the body, upper labials, lower labials, preoculars, postoculars, temporals, loreals, nasals, chin shields and anal plate. Special abnormalities were noted wherever they appeared.

The significance of differences between the experimental litters and (1) their mothers, (2) the 1948 controls, and (3) the 1947 controls were calculated (Tables I, II, III, and IV). Males were tested against males and females against females for all scute counts. Within the experimental samples and the control samples the litters were pooled and no allowance was made for variation between litters. In cases of bilateral characteristics, left and right sides were tested independently. Student's *t* test was used in calculating the significance of the differences in numbers of ventral and subcaudal scutes.[3] All other characteristics were tested by formula for chi-square (including Yates' correction factor). Probability values were taken from Fisher's tables.

RESULTS

Ventrals and Subcaudals.—The female experimental fetuses (Table I, Row 1) had significantly fewer ventral and subcaudal scutes than their mothers (Row 2), the females of the 1948 controls (Row 3) and the females of the 1947 controls (Row 4). The mean numbers of ventrals and subcaudals of the male experimental sample (Row 5) were very significantly smaller than those of the 1948 (Row 6) and 1947 (Row 7) controls.

Seven litters averaged significantly lower in numbers of ventrals and/or subcaudals than the averages of the control samples or the natural population (Text-figs. 1 and 2). Two litters averaged approximately the same as the latter averages, and three litters averaged slightly high-

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[3] A comment is in order concerning the assumption of homogeneity of variance in the groups under study. The hypothesis that the variances were equal in the two populations of experimentals was accepted; likewise, the hypothesis that the variances were equal in the five control groups was accepted. The hypothesis that the variances were equal in all seven groups was rejected. Therefore, when comparisons are made between the experimental and control groups by means of the *t*-test the assumptions of the *t*-test are not met. In spite of the above, the approximate *t*-test was used and the appropriate number of degrees of freedom estimated.

er than the control averages. Extreme reductions in scute numbers occurred in six of the experimental litters. In one of these the four males averaged 137.8 ventrals and 57.3 subcaudals, whereas the lowest averages for males in a single control litter were 155.4 and 75.6 respectively. The lowest litter averages for female experimental fetuses were 143.7 ventrals and 54.3 subcaudals, whereas the lowest averages for a control litter were 153.0 and 68.1 respectively.

It is of interest to compare each litter with its respective mother. Ventrals (Text-fig. 1) in the female fetuses of eight experimental litters and subcaudals (Text-fig. 2) in six were markedly lower than the numbers of these scutes on their respective mothers. In four litters the averages of the females were more or less equal to those of their respective mothers, but in no case was the litter average markedly higher than that of the mother. Additionally, in litter No. 10 (Text-fig. 1) the two surviving female fetuses were normal but three of the four males possessed the smallest number of ventrals encountered.

Scale Rows.— The numbers of longitudinal scale rows on the body were counted at the neck, thoracic region and at the caudal end of the body just in front of the vent. In using chi-square to test the differences between samples, frequencies of occurrence of 20 scale rows or less were tested against frequencies of 21 rows or more in the neck and thoracic regions; for the caudal end of the body frequencies of 16 rows or less were tested against 17 or more.

This race of garter snake usually has 19 scale rows at the neck. No wild populations in the range of the subspecies were found with more than 21 rows and only in the northernmost populations were a few individuals found with fewer than 19 rows (Fox, 1948a). The majority of experimental males and females possessed 19 scale rows at the neck (Table II, Rows 1 and 5) but three had only 17 rows, one had 22 and two had 23. Hence, animals exposed to cool temperatures had a wider range of variation than did the control samples. The number of scale rows at the neck of the mothers of the experimentals did not differ significantly from those of their female offspring (Table II). The six individuals with extremely low (17) or extremely high (22, 23) numbers of scale rows were all born to mothers with 19 scale rows.

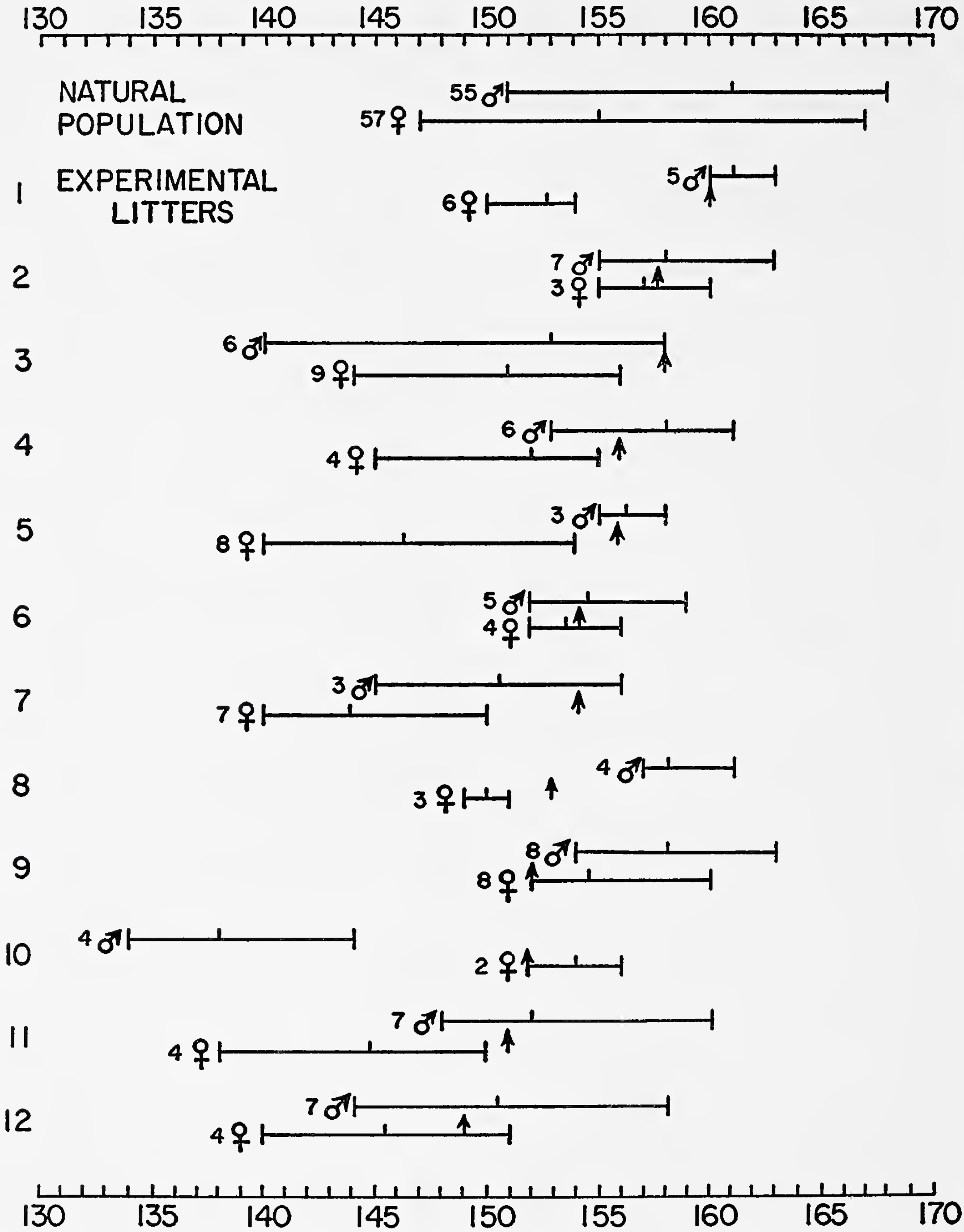
A peculiar inconsistency appeared in the 1948 study. The controls, both males and females, showed a high tendency for 21 scale rows at the neck, whereas nearly all the controls of the 1947 experiment had 19 rows. This resulted in a very significant P value when comparing the 1948 experimentals with the 1948 controls. However, when comparing the 1948 experimentals with 1947 controls no significant differences were found between the females, and the experimental males revealed an unexpected significantly greater tendency towards an increased number of scale rows (Table II).

There are 21 scale rows in the thoracic region (the usual position of the maximum number) in about 75 per cent. of the wild population (Fox, 1948a). The 1948 experimentals showed a considerably greater tendency toward a reduction to 19 scale rows than did their mothers, the 1947 or 1948 controls (Table II), or the wild population. A surprisingly large number of the experimental males had only 17 scale rows in the thoracic region, whereas no count this low was found among the controls or wild population. Nearly all individuals with 17 scale rows were born to mothers with 21 rather than 19 scale rows. This suggested that the experimental conditions played a more significant role in determining fetal scutellation than did the maternal pattern. The 1948 male controls displayed a greater tendency toward 21 scale rows in the thoracic region than did the 1947 male controls.

As in almost all specimens from the natural population in the area of collection, 17 rows of scales were present at the caudal end of the body of nearly all controls. Although there was a greater tendency toward reduction of this number in the experimentals, this trend did not test to be significant (Table II). Nearly all of the individuals showing a posterior reduction of scale rows were from four litters. One of these litters included one individual with 14 rows, seven individuals with 15, one with 16 and two with 17. The mother of this litter had a typical scale formula: 19-21-17. An unexpectedly large number of male experimentals with 18 or 19 scale rows occurred in three experimental litters.

Labials.—In *T. e. terrestris the* customary number of upper labials is 8 (Text-fig. 3A), but a tendency for this number to be reduced to 7 (Text-fig. 3B) has been found in populations from cooler climates (Fox, 1948a). Male and female experimental fetuses were considerably more variable (Table III) in this characteristic than the wild sample. In spite of the occasional occurrence of nine upper labials, the male and female experimental samples had significantly fewer upper labials (Text-fig. 3C, D, E, F) on both left and right sides than either their mothers, control fetuses or the natural population from the area of capture.

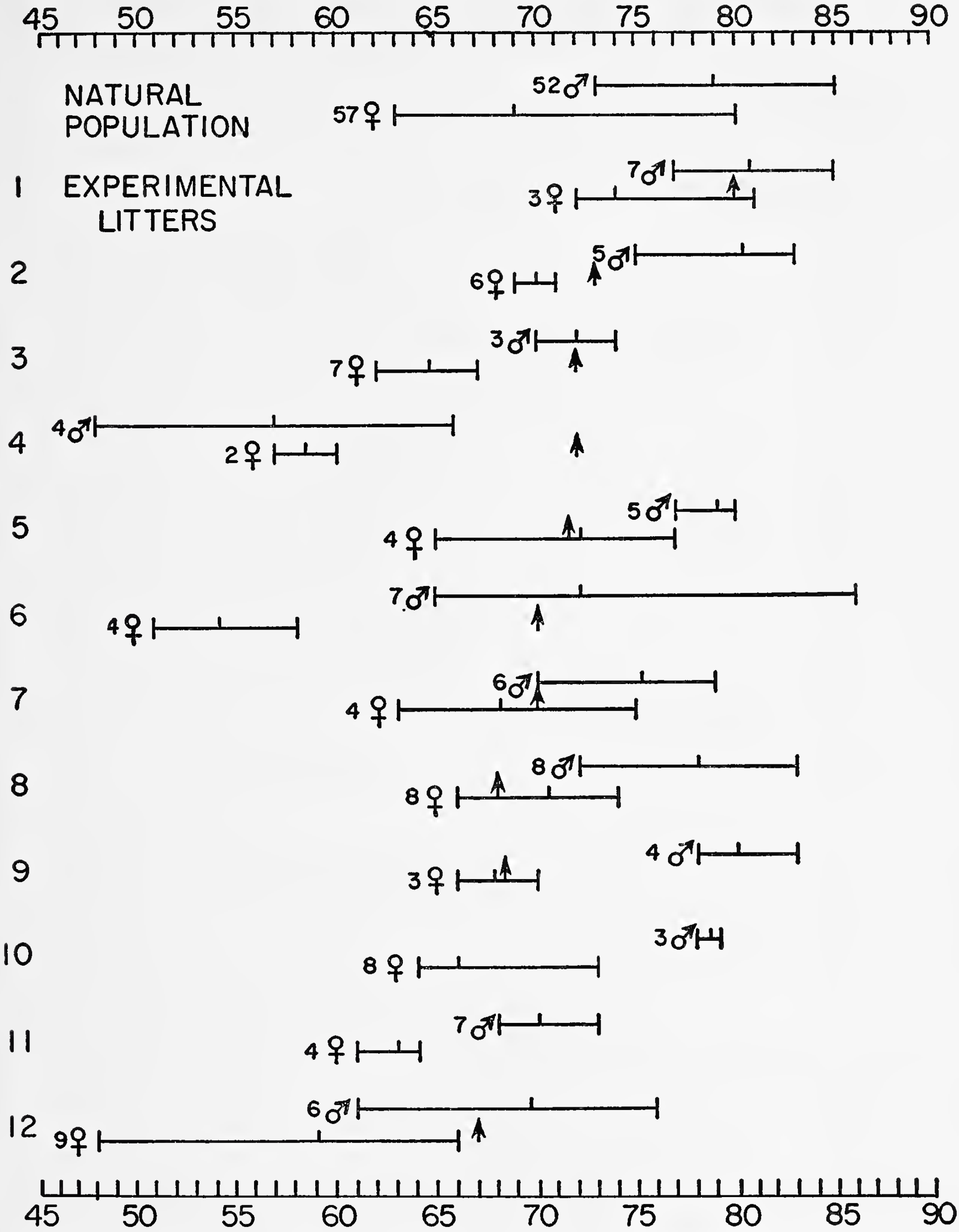
Ten lower labials were present in 82 per cent. of the natural population. As with the upper labials, although this normal scale number was



TEXT-FIG. 1. Range and mean number of ventral scutes found in the 12 experimental litters and compared with the natural population from the San Francisco Peninsula. The number of ventrals of each mother is indicated by the vertical arrows which are arranged in increasing order from bottom to top.

occasionally exceeded by a few experimental fetuses, the strongest trend was towards a significant reduction (Table III; Text-fig. 3B, C, F; Text-fig. 4B).

Most individuals of all but three litters possessed a reduced number of upper and lower labials. Of these three litters, one contained an unusually high number of individuals with 11



Text-fig. 2. Range and mean number of caudal scutes found in the 12 experimental litters and compared with the natural population. The number of caudals of each mother is arranged in increasing order from bottom to top. Mothers of litters of 10 and 11 had incomplete tails.

lower labials (Text-fig. 1E). The other two litters possessed the characteristic number of labials, in spite of the fact that the mother of one of these litters had a partially reduced number of labials, *i.e.* 8-7 upper labials and 10-9 lower labials. Among the litters with reduced numbers of labials was one whose mother possessed a bilateral reduction of lower labials (9-9). Eighty-

TABLE II. FREQUENCIES OF NUMBER OF SCALE ROWS

Samples		14	15	16	17	18	19	20	21	22	23	P[1]
1 ♀ 1948 Experimental	Neck				2		33	3	16		1	
2 Mothers 1948							9		3			.90
3 ♀ 1948 Control								1	16	3		<.001
4 ♀ 1947 Control							37	5	8			.90
5 ♂ 1948 Experimental					1		38	3	14	1	1	
6 ♂ 1948 Control								1	22	3		<.001
7 ♂ 1947 Control							53	3	2			.005
1 ♀ 1948 Experimental	Thoracic Region				1		16	7	27	3		
2 Mothers 1948							3		8	1		.30
3 ♀ 1948 Control								1	19			.007
4 ♀ 1947 Control								1	49			<.001
5 ♂ 1948 Experimental					8	1	19	5	24	1		
6 ♂ 1948 Control									25	1		<.001
7 ♂ 1947 Control							11	3	44			.001
1 ♀ 1948 Experimental	Caudal end of Body		3	5	44	2						
2 Mothers 1948					11	1						.15
3 ♀ 1948 Control					20							.12
4 ♀ 1947 Control			1	3	46							.56
5 ♂ 1948 Experimental		1	7	7	35	7	1					
6 ♂ 1948 Control					26							.22
7 ♂ 1947 Control			3	4	51							.15

[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples.

five per cent. of the lower labial scale counts of fetuses in this litter were found to be nine. This number was found on either right or left side or bilaterally. The remaining 15 per cent. of the scale counts showed a reduction to eight lower labials or the normal number of ten. Eight labials occurred either bilaterally or unilaterally, but ten occurred only unilaterally. No example of extreme reduction occurred in this litter. All of the cases of extreme reduction in number of upper and lower labials occurred in litters of mothers with the characteristic number of labials.

Preoculars and Postoculars.—Although a single preocular scute is characteristic of the race (Text-fig. 3A), even in natural populations an occasional specimen is found in which this scute

TABLE III

Samples		Upper Labials							Lower Labials						Preoculars				Postoculars				
		4	5	6	7	8	9	P[1]	7	8	9	10	11	P[1]	1	2	3	P[1]	1	2	3	4	P[1]
1 ♀ 1948 Experimental	L		8	10	14	30	1			20	26	15	2		44	18	1		3	40	16	4	
	R		4	13	21	23	2			23	24	16			40	22	1		6	29	24	4	
2 Mothers 1948	L				1	11		.01			2	10		<.001	11	1		.20		2	10		.001
	R				1	11		.002			2	10		<.001	11	1		.10		1	11		.005
3 ♀ 1948 Control	L				3	17		.01			1	19		<.001	20			.017		3	17		<.001
	R				1	19		<.001			2	18		<.001	20			.006		2	17	1	<.001
4 ♀ 1947 Control	L				5	45		<.001			1	49		<.001	47	3		.006		5	45		<.001
	R				5	44	1	<.001			3	44	3	<.001	49	1		<.001		2	48		<.001
5 ♂ 1948 Experimental	L		6	16	10	28	5		2	17	25	20	1		38	26	1		1	33	29	2	
	R	2	4	9	19	28	3		1	18	28	16	2		41	24			1	34	28	2	
6 ♂ 1948 Control	L				1	25		<.001			2	24		<.001	26			<.001		1	24	1	<.001
	R				1	24	1	<.001			2	24		<.001	26			.001		2	22	2	.001
7 ♂ 1947 Control	L				10	48		<.001			3	55		<.001	51	7		<.001		5	53		<.001
	R				5	53		<.001			2	56		<.001	55	3		<.001		3	55		<.001

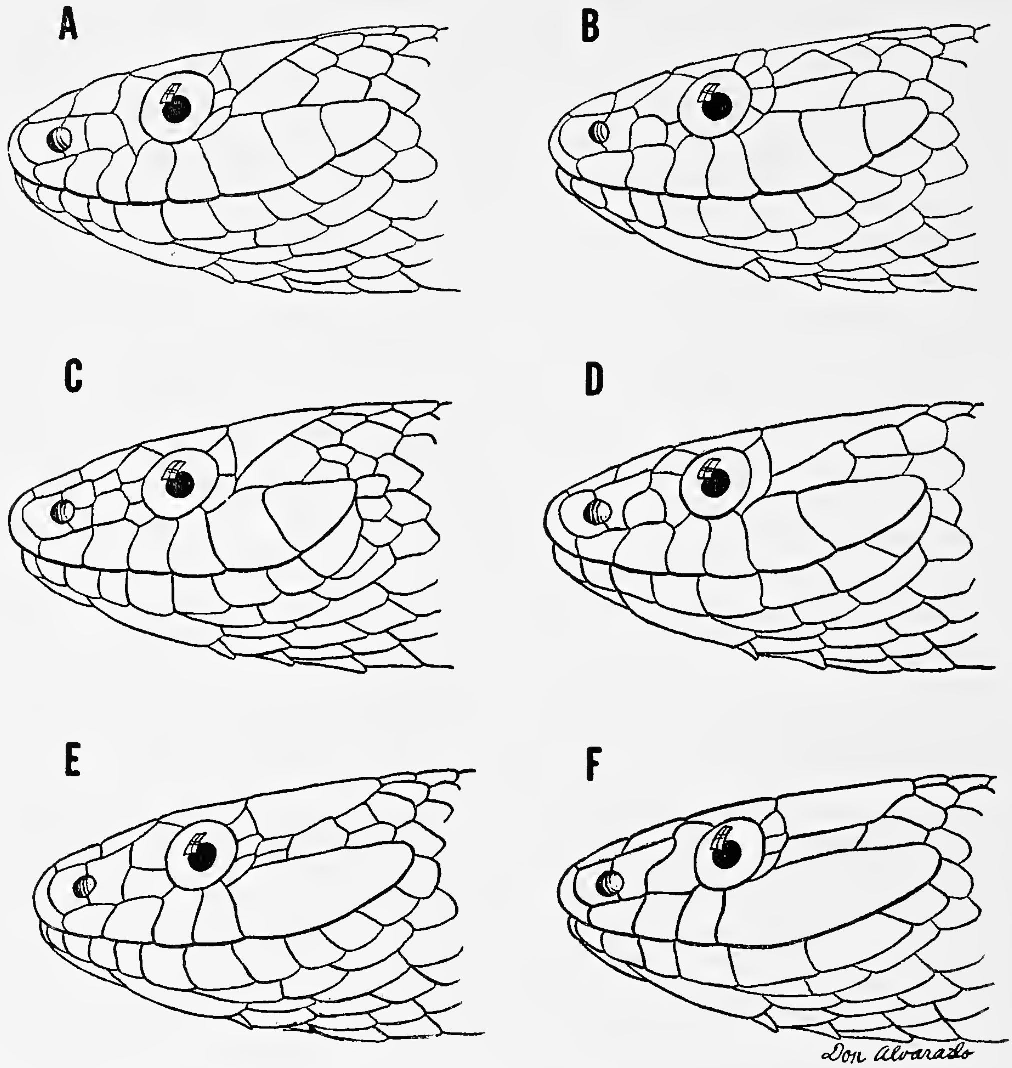
[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples. L (left) and R (right) sides tested separately.

is horizontally divided about one-third of the distance from the bottom. The cool room treatment resulted in a high proportion of preocular scutes being divided into two scutes (Text-fig. 3B, and D) and rarely into three scutes (Table III, Text-fig. 3C).

In natural populations postoculars usually appear as three separate scutes (Text-fig. 3A). However, the skin bordering the eye posteriorly is occasionally fused into only two separate scutes or rarely further divided to form four separate scutes. This trend towards deviation from the typical pattern of three scutes was very pronounced in the experimental litters. Four postoculars (Text-fig. 3B) occurred more frequently than in natural populations and the tendency towards reduction of the number of scutes to two (Text-fig. 3C) or even one (Text-fig. 3D) was far greater than was found in the mothers, the controls (Table III) or natural populations. In two specimens the uppermost postocular was fused with an upper first temporal.

Several of the experimental mothers exhibited atypical patterns of preoculars and postoculars (Table III). Their offspring did not appear to display a significantly greater tendency to deviate from the characteristic pattern of these scutes than did the offspring of mothers with typical patterns. Of the 12 litter mothers, six had an atypical number of preoculars or postoculars on one side and six had typical numbers on both sides. It is difficult to demonstrate that the ocular pattern of the mothers determined the pattern of the fetuses. However, the litter with the largest proportion of fetuses with characteristic ocular scute patterns (13 out of 17) was born to a mother with the characteristic pattern. On the other hand, a litter of nine whose mother possessed two preoculars on both sides contained five fetuses with one preocular on both sides, four with one on one side and two on the other, but none with the pattern of the mother.

Temporals.—The arrangement of temporal scutes also proved to be very labile. A number of different patterns are indicated in Table IV and Text-fig. 3. The arrangement 1-2-3 (Text-fig. 3A) is the most common pattern in this species. Much deviation was seen in wild populations, in the mothers of the experimentals and in the control fetuses (Table IV). In order to use the method of chi-square to test the significance of the differences, the frequencies of the pattern 1-2-3 were treated as the expected and all other variations were pooled as deviations from the expected. As can be seen from the P values in Table IV, the experimental samples proved to have a significantly greater deviation



Text-fig. 3. Variations in scute patterns on side of head. **A.** Typical pattern: 1 preocular, 3 postoculars, 1 postnasal, 1 loreal, 8 upper labials, 10 lower labials, temporals 1-2-3. **B.** Postnasal and upper preocular meet, preocular divided below, 4 postoculars, 7 upper labials, 9 lower labials, temporals 2-2-3. **C.** Postnasal divided, 2 loreals (upper and lower), 3 preoculars, 2 postoculars, 6 upper labials, 8 lower labials, temporals 1-3-3. **D.** Postnasal fused to upper loreal, preocular divided above, 1 postocular, 6 upper labials, 7 lower labials (first fused to mental), temporals 1-2-2. **E.** Lower loreal fused to lower preocular, 3 postoculars, 5 upper labials, 11 lower labials, temporals 2-1-2. **F.** Upper loreal fused to postnasal, lower loreal fused to lower part of preocular, 4 upper labials, 7 lower labials, temporals 1-1-2.

from the expected than did the controls. When the left and right sides of the experimental female fetuses were compared to the respective sides of the mothers, the level of significance was border line (Table IV, Row 2); however, when left and right sides of the samples were pooled the difference proved to be very significant (P = .007). Variation of this characteristic was so great among the experimental fetuses that there was no clear correlation between the patterns of the fetuses in each individual litter and the pattern of the respective mothers.

Anal Plate.—The anal plate, which is normally undivided in the genus *Thamnophis,* was found to be divided in ten male and nine female experimental fetuses and grooved or notched in seven others. All of these fetuses came from five litters. Divided or grooved anal plates were not observed in the controls, the mothers or the natural populations of this race. However, Tanner

TABLE IV

Samples		Temporals														Anal Plate	
		1-1-2	1-1-3	1-2-2	1-2-3	1-2-4	1-3-2	1-3-3	1-3-4	2-1-2	2-1-3	2-2-2	2-2-3	Others	P[1]	Divided	Undivided
1 ♀ 1948 Experimental	L		2	8	23	1		3	1	1	1	1	5	17		9	54
	R	3	4	9	20	1	1	2	1			3	3	16			
2 Mothers 1948	L			3	9										.02	0	13
	R	1		2	8			1							.05		
3 ♀ 1948 Control	L				15			4					1		.008	0	20
	R				19			1							<.001		
4 ♀ 1947 Control	L			3	40	3		1					1	2	<.001	0	50
	R			4	39	2	1	1						3	<.001		
5 ♂ 1948 Experimental	L	4	2	8	27			3		2	1			18		10	55
	R	3	3	8	20	1		5	2				1	22			
6 ♂ 1948 Control	L				19	3		4							.009	0	26
	R				16	4	1	4	1						.015		
7 ♂ 1947 Control	L	1	1	12	42			2							.001	0	58
	R			4	51	1								2	<.001		

[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples. L (left) and R (right) sides tested separately.

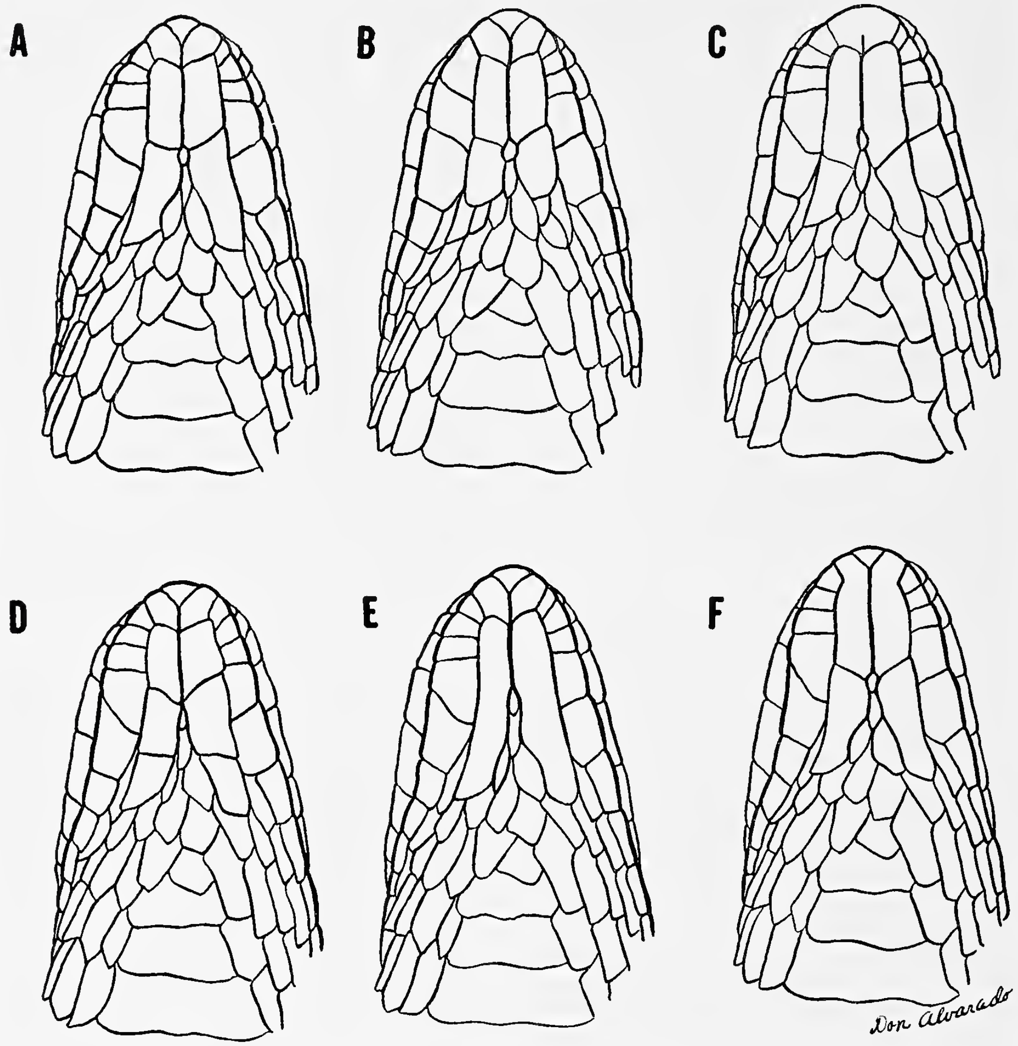
(1950) reported the occurrence of several specimens of another race of this species (*Thamnophis elegans vagrans*) from several populations in Utah with divided or grooved anal plates.

Chin Shields.—The same litters in which divided anal plates occurred showed considerable variation in the chin shields (Text-fig. 4). The posterior chin shields were frequently unusually short (Text-fig. 4B, C), whereas the anterior chin shields were occasionally exceptionally long (Text-fig. 4C). In three individuals the chin shields were divided into three pairs instead of the normal two pairs (Text-fig. 4D). In two others the anterior and posterior chin shields were fused (Text-fig. 4E), resulting in a single chin shield on one side in one instance and paired single chin shields in the other. Anterior chin shields were fused to the first labials in another specimen (Text-fig. 4F).

Loreals, Postnasals and Postrostrals.— Typically a single loreal scute separates the postnasal and preocular scutes (Text-fig. 3A). Only three experimental litters displayed variations from this condition. However, out of 31 individuals in these litters only one was completely normal in this respect and only two were merely unilaterally malformed. The remaining 28 fetuses had bilateral abnormalities of various types. In four individuals the preocular and postnasal made contact above the middle of a single loreal (Text-fig. 3B). In most others there were two loreals, one on top of the other (Text-fig. 3C). The upper loreal appeared to be derived from the lateral portion of the prefrontal. In five individuals this upper loreal was fused to the postnasal (Text-fig. 3D) on one or both sides of the head. In two individuals the lower loreal was fused to the lower preocular on one side (Text-fig. 3E). Peculiar fusions in one individual left the lower loreal fused to the lower preocular and the upper loreal fused to the postnasal (Text-fig. 3F). In one individual two lower loreals were present, but no upper. Postnasals were occasionally divided (Text-fig. 3C).

In one experimental litter three individuals out of ten possessed a single small median postrostral scute. This characteristic was not noted in any other garter snakes. In three individuals from two litters the mental scale was fused to the first lower labial: two on one side of the jaw only and one on both sides (Text-fig. 4C).

Harelip.— Three experimental litters contained individuals in which the naso-labial groove had failed to close during embryonic development. This condition resembled the harelip of mammals. In one litter of 11, two individuals retained open naso-labial grooves on both sides and



TEXT-FIG. 4. Variations in scute patterns on ventral surface of head. **A.** Typical pattern: 2 pair chin shields, 1 mental, 10 lower labials. **B.** Posterior chin shields short, 9 lower labials. **C.** Anterior chin shields unusually long, first lower labials fused to mental. **D.** Three pair of chin shields. **E.** One pair of chin shields. **F.** First lower labials fused to anterior chin shields.

three on one side only. In a second litter of six the cleft was present on both sides in two and one side in one. In the third litter of 11 one fetus retained a cleft on one side. Of the nine individuals with this malformation, seven were males and two were females.

DISCUSSION

On the basis of admittedly limited data, Fox (1948a) suggested that scutellation patterns of garter snakes could be influenced by lowering the environmental temperature during the gestation period. The larger number of significantly affected experimental litters found in the current study completely confirms this hypothesis and leaves little doubt that cool temperatures during embryonic development do result in an alteration of scute patterns. The principal effect observed was a reduction in the number of ventrals, subcaudals, labials and scale rows around the body; but all scute patterns underwent a marked increase in variability. There was no indication that ventrals or subcaudals were increased in any of the experimental litters; those experimental litters with higher numbers of these scutes fell well within the range of the controls (Table I) or the natural population (Text-fig. 1). There was a slight suggestion of increase in numbers of scale rows around the body. However, only in the case of the number around the caudal end of the body does there appear to be a possibly significant increase (Table II), and this would be difficult to prove statistically.

A few experimental animals appeared to have an extra upper or lower labial. This extra labial was less frequently present in the wild population or the controls. However, since the evaluation of the presence of an extra labial is very subjective, it does not appear wise to emphasize strongly the few cases where the number of labials were increased. Clearly, the major effect of cool temperatures was a reduction in the number of labials. Scale characters such as preoculars, postoculars, temporals, loreals and chin shields are probably best described as showing a great deal of variation in their response to chilling. Many patterns resulting from atypical fusions or divisions of scutes appeared among the experimentals.

Of the twelve experimental litters reported on in this paper, eight showed significant modifications of most scale patterns analyzed, while the remaining four showed more variations in certain scale patterns than did the control litters. As can be seen in Text-figs. 1 and 2 and the Tables, not only individual litters but the means and the ranges of the pooled experimental litters differed very significantly from those of the control samples, the natural population and the litter mothers in most characteristics measured.

In addition to the fact that the temperature differential between the cold and warm rooms clearly influenced the pattern of fetal scutellation, temperature fluctuations within the rooms themselves may have played a significant role in influencing the outcome of the experiment. Fluctuations in the cool room may have been responsible for the survival of the experimental fetuses. We have been informed by Dr. James A. Oliver of the American Museum of Natural History (personal communication) of similar experiments in which snake eggs were maintained at a constant low temperature. These experiments resulted in the death of all cold-exposed embryos. He suggests that our success may have been due to the fact that the temperature fluctuations in our cool room (65°-85°F) resulted in the animals being exposed to lethal temperatures for only part of the day, the remainder of the time being spent at temperatures compatible with morphogenesis and survival. His explanation seems plausible, for even with our fluctuating temperatures there was a high mortality in the 1947 experimental series and in the more successful 1948 series three entire litters died.

Although it is clear that environmental temperature can influence the scutellation pattern of developing garter snakes, the actual mechanism by which it controls scute development is unknown. The data indicate that the fluctuating temperatures of our experimental rooms did not influence all scale characters in the same way. Ventrals, subcaudals and scale rows in the thoracic region, if affected at all, were invariably reduced in number, whereas preoculars and anal plates were subdivided, resulting in increased scale counts. Further, individual characters were not uniformly affected in different embryos. Fetuses maintained in the cool room usually showed a marked reduction in the number of labials and scale rows around the body in the neck region, yet there were more fetuses than usual in which these scale numbers were increased. Individual responses were particularly marked in such scutes as postoculars, temporals, loreals and chin shields where lowered temperatures lead to a wide range of patterns resulting from subdivision, lack of division, abnormal growth or fusion of scutes.

This multiplicity of reactions to lowered temperatures might be anticipated because studies of developing turtle (Yntema, 1960) and fish (see review by Tåning, 1952) embryos indicate that in poikilothermic vertebrates the morphogenetic effects of temperature variations depend on a number of factors. In the most comprehensive investigation of this type, Tåning (1952) demonstrated that modifications of individual meristic characters of embryos of the sea-trout, *Salmo trutta trutta,* varied independently with the exact degree of temperature applied. At constant temperatures between 2.7°C and 14°C the number of vertebrae was lowest at 6°C and rose at higher or lower temperatures. Curves for the number of fin rays, however, were almost the reverse of that for the vertebrae since the former reached their maxima between 6°C to 10°C and fell off at higher or lower temperatures. Tåning further demonstrated that temperature variations affected the morphogenesis of the vertebrae differently at different stages of development. During an early sensitive period from fertilization to gastrulation the temperature effect was as stated above, namely a decrease in vertebral number with a temperature drop from 10°C to 4°C. During a later "supersensitive" period, just before the last vertebrae were preformed as posterior mesodermal segments, the temperature effects were reversed. At this time a drop from 10°C to 4°C resulted in a marked increase in the number of vertebrae. Lastly, this study indicated that while genetical differences were important in determining vertebral modifications, fin ray variations appeared to be almost purely phenotypic.

Tåning's findings are of particular interest because if the thermal reactions of developing garter snakes are similar to those of the sea-trout, many puzzling aspects of our data can be clarified. The consistent ratio of 1 vertebra: 1 pair of ribs: 1 ventral scute which pertains in snakes made us suspect that modifications in the number of these scutes represented a basic vertebral and somite disturbance. Examination of eight stained and cleared experimental fetuses with reduced numbers of ventral scutes confirmed our suspicions. With very few exceptions, one ventral scute was associated with one pair of ribs and one vertebra. With the possible exception of the terminal scute, the subcaudals and vertebrae also appeared to maintain a 1:1 ratio. The consistent reduction of ventrals and subcaudals in our experimental samples is therefore in keeping with the data of Tåning (1952), Schmidt (1921), and Lindsey (1954), who demonstrated that, down to a certain critical point, low temperatures usually resulted in a reduction of vertebrae in fish embryos and with those of Yntema (1960) who found that there was a progressive drop in the rate of somite formation in snapping turtle embryos developing at temperatures decreasing from 30° to 10°C. Our data would contrast, however, with those of Gabriel (1944) and Dannevig (1950) who found, respectively, that the vertebral numbers of *Fundulus* and plaice (*Pleuronectes platessa*) embryos increased at lower temperatures. Possibly, in the case of these latter authors, investigations were carried out below the critical temperature point of the species.

The inconsistent response of some characteristics to lowered temperatures and the wide range of scale patterns found in our experimental series are readily understandable in view of Tåning's findings that the thermo-sensitive periods of different characters occur at different stages of embryonic development and that temperature responses of individual characters can vary during different thermo-sensitive periods. Since examination of several hundred specimens of *T. e. terrestris* over a period of three years has indicated that nearly all members of the San Francisco peninsula population ovulate within a period of two to three weeks, we are reasonably sure that most of our embryos were exposed to experimental treatment during or immediately subsequent to the first week of development. There was undoubtedly, however, a considerable range in the exact developmental stage achieved by individual litters and embryos at any one specific time during the experiment. Sudden extreme temperature changes or "shocks," which have been shown to be particularly effective in influencing morphogenesis (Tåning, 1952; Lindsey, 1954), could have occurred sporadically at night in our experimental rooms. These would have been experienced simultaneously both by embryos in a relatively thermo-resistant stage and by embryos which were thermo-sensitive for one character or another at that particular time. As Goldschmidt (1945) points out, both unfavorable temperature changes and most mutant genes affect morphogenesis by changing the rate of reaction velocities and altering the sequence of normal developmental processes. Hence phenotypic characteristics which are produced in one species by mutant genes can frequently be experimentally produced in another by temperature shock. Regardless of whether the cause is genetical or environmental, interference with the normally integrated reaction systems necessarily has different morphogenetic effects at different times. At one period of development it may inhibit the production of a determining substance, at another it may delay the production of such a substance until the normally reacting tissues have lost their capacity for response, at still another time it may cause a critical substance to be present in excessive amounts during a particularly sensitive period.

Although, as outlined above, it is possible to offer a plausible explanation of our data solely on the basis of an embryonic sensitivity to thermal variations, one can not entirely ignore the fact that the physiology of the mothers was disturbed by the experimental treatment. Snakes maintained in the cool room did not feed as readily as those in the control room and the gestation period of the former was considerably prolonged. Conceivably, these alterations of the maternal physiology could have secondary teratogenic effects on the embryos. Although it seems unlikely that such secondary effects played an important role in determining the outcome of our experiment, they must be considered as an unknown variable in any analysis of the data. We have considered the possibility that repetition of our experiment with an oviparous species might eliminate the problem of secondary teratogenic maternal influences and thus yield more conclusive data on embryonic responses to chilling. We have come to the conclusion, however, that the results of such a study might be even more difficult to interpret. First, when snake eggs are laid the embryos have usually reached a relatively advanced state of development. Our own data and those of Dr. G. W. D. Hamlett (personal communication) indicate that freshly laid eggs of several genera of colubrid snakes contain embryos with at least 3½ to 4 coils. We have ascertained by personal examination of stained and cleared specimens that *Heterodon platyrhinos* embryos at the time of egg laying have attained the adult number of body somites although the tail somites are not completely formed. Apparently even in oviparous snakes the exposure of fetuses to teratogenic influences during the earliest stages of development necessitates a simultaneous exposure of the mother. Since such a procedure would allow secondary maternal teratogenic influences to operate during the earliest and, to the belief of most teratologists, the most sensitive stages of development, data obtained on oviparous species would be no more conclusive than those obtained on viviparous species. Secondly, oviparous eggs must be maintained in a mold-free environment under suitable moisture conditions. Inequalities in the amount of moisture in the medium surrounding the eggs or in the humidity of the air in the cool or warm rooms would introduce variables into the experiment which would be difficult to control or evaluate. In a viviparous species, however, presumably moisture conditions would be relatively constant and equivalent in both experimentals and controls.

Since several investigators (see review by Tåning, 1952) have demonstrated that intraspecific genetic differences as well as the thermal environment determine some meristic characteristics of fish embryos, it is unfortunate that the phenotypes of our male parents are unknown. While we were aware from the onset that our experiment might be subject to criticism because of this deficiency, several factors convinced us that it would be impractical to attempt to obtain a series of garter snakes embryos of known parentage for experimental study.

First, we were confronted with the possibility of prolonged sperm survival in the female oviduct. This can be a critical problem in genetical studies on snakes, particularly in some species of Colubrinae and Boiginae where records of survival up to several years have been reported (see review by Fox, 1956). Although the senior author subsequently failed to find sperm in the oviducts or seminal receptacles of either *T. sirtalis* or *T. elegans* at the end of the summer following parturition (Fox, 1956), Blanchard (1942) had demonstrated that sperm of *T. sirtalis* could survive through the winter following fall copulation. Blanchard's data convinced us that if reasonably positive knowledge of the male parentage was to be obtained, female garter snakes would have to be captured during the summer and maintained in isolation in the laboratory until the following spring. This procedure would prevent fall copulations and afford time for sperm from previous copulations to die off.

Several years' study of the reproductive systems of wild and captive garter snakes by the senior author had indicated, however, that unless large outdoor pits or cages similar to those used by Blanchard (1942) were available, such a long period of captivity would prevent successful breeding. He found that although males apparently underwent normal spermatogenic cycles in captivity, the larger follicles of the female ovaries nearly always underwent atresia shortly after being brought into the laboratory. Lack of ovulation and follicular atresia occurred even in many females captured during the peak of the breeding season, at a time when they were swollen with large, palpable follicles. Only two of about 100 female garter snakes he had maintained in the laboratory over winter or for one to two years had been known to ovulate.

Because of the above difficulties, we decided that the present study could only be carried out with expediency if already inseminated females, captured as close to the time of ovulation as possible, were utilized. Although this meant that the exact phenotypes of the male parents were unknown, we had at our disposal data on a large sample of males of the wild population from which the mothers were collected. The chance that the phenotypes of the fathers of our experimental litters differed significantly from those of control litters or males of the natural population is exceedingly small.

In general, thermal variations rather than heredity appeared to play the major role in determining the phenotype of our experimental fetuses. When the female fetuses of the 12 experimental litters were compared to their respective mothers, eight litters were found to deviate markedly from the maternal scale pattern. The remaining four litters showed a closer resemblance to their mothers. Even among the latter litters, however, a few characteristics showed more variation than was present in the mothers. These data reinforce the findings of the previous experiment (Fox, 1948a).

In respect to ventrals and subcaudals there was slight evidence that the maternal phenotype influenced that of her offspring. In calculating correlation coefficients between mothers and male and female fetuses we found that male ventrals and female subcaudals tested to be significant at the .05 level, whereas female ventrals and male subcaudals did not test to be significantly correlated. Since the number of these scutes closely parallels the vertebral number, it is interesting that Ege (1942) and Tåning (1952) found that heredity played an important role in determining the vertebral number of sea-trout embryos although other meristic characters seemed to be determined almost wholly by the thermal environment. Little or no correlation between the snake mothers and fetuses was found when other scale patterns were compared.

Most scute variations seemed to result from the direct teratogenic action of low temperatures on the embryo, and similar action probably produced the harelip occurring in three experimental litters. This malformation also occurred in fetuses of hamsters frozen for short periods during the first week of development (Smith, 1957). In interpreting his data, Smith discussed the possibilities that his results may have been due to maternal hypoxia, freezing of body water with a consequent increased concentration of solutes in the medium surrounding the embryo, placental damage, or direct embryonic arrest. Our data lend support to his hypothesis of direct embryonic arrest.

Examination of the wild population of *Thamnophis elegans terrestris* from our collecting grounds in San Mateo County, California, indicates that even under natural thermal conditions the experimental species exhibits an unusually wide range of scutellation patterns. When compared to another species, *Thamnophis sirtalis tetrataenia,* which inhabits the same area, it was found to be much less constant in respect to a number of scale counts. In *T. e. terrestris,* for example, the most common scale row formula (19-21-17) is present in only 65.5 per cent. of the population whereas in *T. s. tetrataenia* the characteristic pattern (19-19-17) occurs in 94.5 per cent. of the specimens. Similarly, while a lower labial formula of 10-10 occurs in 85.3 per cent. of *T. s. tetrataenia* and an undivided preocular in 100 per cent., in *T. e. terrestris* only 75.0 per cent. have a lower labial formula of 10-10 and the preocular is divided in 11.3 per cent. of the specimens.

This natural variation which occurs in the experimental species, coupled with the fact that there was a very high mortality of experimental fetuses in the first experiment (Fox, 1948a), has led one geneticist to suggest to the senior author that the lowered scale counts he obtained in the 1947 series were due to selection rather than to direct thermal influences. It was postulated that given a wide range of genetic variation within a litter, only those fetuses with low scale counts might have been able to survive the experimental treatment. We find this hypothesis untenable for several reasons. First, the mortality of experimental fetuses in the present experiment was not high enough to allow for the selective removal of an appreciable number of fetuses with normal or higher scale counts. Secondly, many of the extreme scale counts and patterns which appeared in our experimental series have never been seen either in the control litters, in the wild population or in litters of recently captured females. One must conclude, therefore, that they do not normally occur or are all selectively eliminated before birth under normal temperature conditions. The fact that our data indicate that there is no appreciable prenatal mortality in the litters of control or wild females argues strongly against the latter possibility. Lastly, the hypothesis is based on the assumption that the variable scale counts of *T. e. terrestris* are based solely on genetical differences. Since our experiments have demonstrated that one of the chief effects of cold exposure during gestation is the production of a wide range of scutellation patterns, it seems possible that much of the natural variation which occurs in the population from which the experimental snakes were taken may be due to the thermal conditions of its habitat. Certainly, in the cool, fog-bound coastal hills which it inhabits there is ample opportunity for periodic chilling during embryonic development. The fact that *T. sirtalis tetrataenia,* which is exposed to identical thermal variations, does not exhibit a similiar variability, does not necessarily negate this possibility. A genetic difference in susceptibility to thermal variations could explain this difference between the two species.

Lest taxonomists, as a result of our experiment, become unduly concerned over the possibility that non-genetical variations of usually reliable taxonomic characteristics may have resulted in erroneous recognition of subspecies or species of reptiles, we should like to state that we doubt whether many such errors have occurred. In spite of all the induced modifications of scute patterns in *T. e. terrestris,* the experimental young were still recognizable as to subspecies on the basis of color and pattern although many of them would not fit "keys" to the genus *Thamnophis* and a few would even "key out" to different genera. The limitations of the artificial "keys" are not important, however, when a complete description of a "kind" of animal is concerned. Perhaps the main thing that this study might emphasize for the taxonomist is the desirability of large samples when describing a new form. It might further lead one to anticipate a fair amount of variation in poikilothermic animals, particularly in those populations from areas that are fog-bound or cool where there is an opportunity for chilling during embryonic development.

SUMMARY

1. Gravid garter snakes of the subspecies *Thamnophis elegans terrestris* were maintained at temperatures fluctuating from 65°-85°F (cool room, experimentals) and 75°-95°F (warm room, controls) throughout most of the gestation period.

2. The experimental fetuses showed a very wide range of variation in scutellation patterns. The principal effect of lower temperatures on ventrals, subcaudals, labials and scale rows around the body was a reduction in number. Other scutes divided or fused irregularly. Of special interest was an unusual incidence of divided anal plate in five litters and harelip in three litters.

3. Only in respect to the ventrals and subcaudals was there a possible correlation between the scale patterns of the fetuses and their mothers. The data show that considerable phenotypic variation is possible within the limits of the genotype of a population.

4. In all probability, the nightly chilling of the embryos during early critical periods of morphogenesis was the most important teratogenic factor to which they were exposed. Since thermal variations behave like most mutant genes in that they interfere with the normal sequence of development processes (Goldschmidt, 1945), lowered temperatures produced scale patterns which are not only atypical for the subspecies but atypical for the genus.

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6

The Orang-utan in Sarawak

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(Text-figure 1)

ALTHOUGH numerous naturalists and collectors have visited Borneo and Sumatra in the past hundred years, the orang-utan (*Pongo pygmaeus*) remains the least known of the great apes. No detailed reports on the status and distribution of this animal have ever been published for Borneo as a whole. Stott & Selsor (1961) present the only recent information for the British colony of North Borneo. For Sumatra, Carpenter (1938) furnishes the sole account. Data on the ecology and behavior of free-living orang-utans are based primarily on the sporadic observations of Schlegel & Müller (1839-44), quoted in Yerkes & Yerkes (1929), Wallace (1869), Hornaday (1885), and Beccari (1904). Yerkes & Yerkes (1929) summarize the available information and no major references can be added.

The reported rapid decline in the orang-utan population of Sarawak in recent years has focused the attention of conservationists on this small British colony in the northwestern corner of Borneo. In order to determine the present status of the orang-utan, or *maias* as the animal is called locally, I visited Sarawak from November 7, 1960, until January 2, 1961. This report presents my findings as well as pertinent notes on the ecology and behavior.

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Valuable comments on the manuscript were made by Dr. John T. Emlen, Mr. Harold J. Coolidge and Mr. B. E. Smythies.

Procedure and Methods

The data on the distribution and status are based on personal observations, and on information in the files of the Sarawak Forest Department. Extensive foot and boat transects were made in two of the three accessible major forest tracts still inhabited by orang-utans, and the forest areas immediately surrounding these tracts were also visited. Transects were conducted in all major types of orang-utan habitat and the status of the animal evaluated by means of a crude index based on the number of nests seen and the number of hours walked.

In 1959 all section forest officers were asked by the Forest Department to collect information about the orang-utan in their area. In addition, Mr. Gaun anak Sureng made a survey from May 3 to June 22, 1959, in a region later visited by myself. The governor appointed in 1959 a Maias Protection Commission headed by Mr. D. L. Bruen to ascertain the status of the animal in the colony. The distributional data of the various inquiries are incorporated in this report. Information was also collected from Dayak hunters by asking them about local conditions. Their information, whenever checked, was correct.

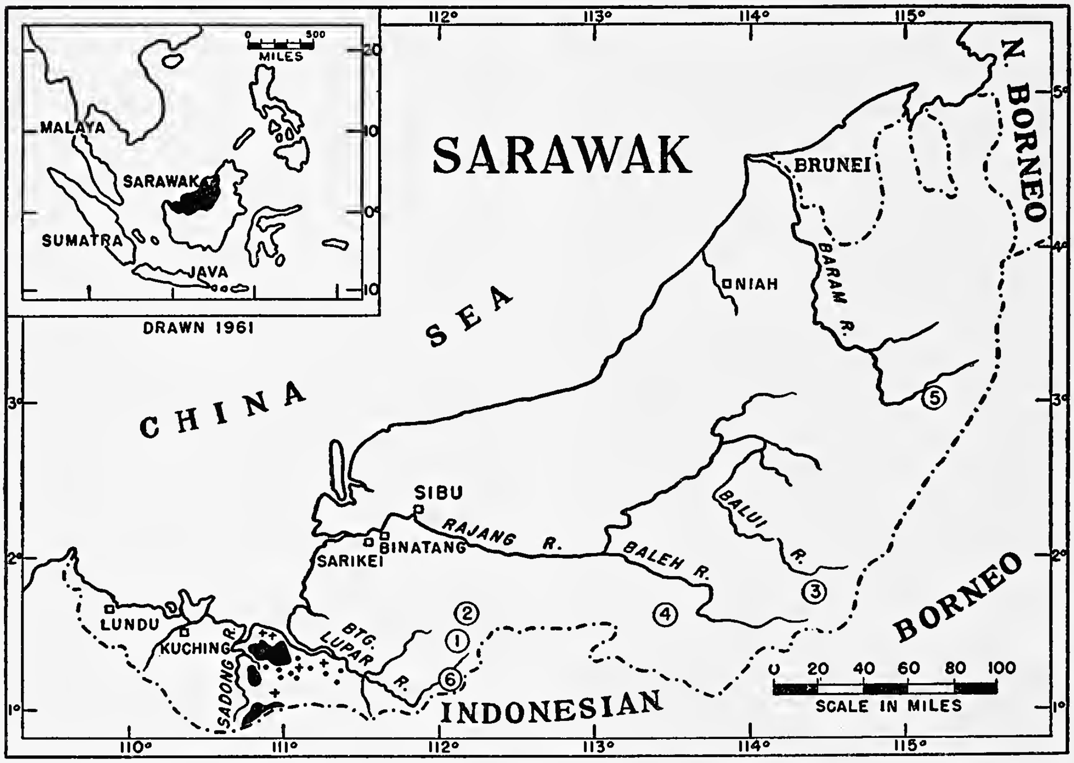
Most behavior data are based on personal observation; information from natives is not necessarily reliable. Orang-utans were watched quietly and alone until they detected me.

Past Distribution

Detailed knowledge about the interior of Sarawak is not available prior to the landing of James Brooke in 1839. There is, however, little doubt that in prehistoric and more recent times the orang-utan was distributed more extensively than at present. At the Niah caves, for example, excavations revealed numerous orang-utan remains in all layers below the Neolithic into the middle Paleolithic (von Koenigswald, 1958). Today no orang-utans occur within about 130 miles of the caves. Beccari (1904) mentions that rare stragglers appear at times in the Sarawak River valley southwest of Kuching. A few animals are supposed to exist in the headwaters of the Batang Undup River, a tributary to the Batang Lupar, according to Forestry Department files. In view of the extensive cultivation and almost complete destruction of primary forest it is doubtful if animals survive in the area today. Until 100 years ago a population is said to have existed near Binatang on the lower Rajang River, but the area was heavily settled by Dayaks and Chinese between 1861 and 1870 (Harrisson, 1949). A few orang-utans may, however, have persisted until recently. In 1956 a local forester received a report of two animals at Ulu Sungei Daro near Binatang. The Maias Protection Commission notes records from Ulu Sungei Pasin in the Sarikei district and from Sungei Assan and Sungei Naman southeast of Sibu near the Rajang River. Today the area has become so heavily populated that the records probably belong in the category of past distribution.

Present Geographical Distribution

Today orang-utans inhabit only a fraction of the 47,500 square miles that comprise Sarawak. The only known concentration occurs in some forests of western Sarawak between the Sadong and Batang Lupar rivers, an area of about 1,600 square miles bordered on the south by Indonesian Borneo. Cultivation, mainly along the larger rivers and in the well-drained uplands, has elim-



Text-fig. 1. Geographic distribution of the orang-utan in Sarawak. The three black areas indicate forest reserves which are known to harbor orang-utans. Dots and crosses indicate definite records outside the reserves, the former noted by the Forest Department and the latter by myself. Numbers within a circle represent records of stragglers or local populations of unknown size.

TABLE I. DISTRIBUTION OF ORANG-UTANS OUTSIDE THE MAIN AREA OF CONCENTRATION

Location	Source	Estimate of Numbers
1. Upper Ulu Ai area	Forest Dept.	About 100
2. Sungei Katibas area	Forest Dept.	
Between Sungai Knowit and Katibas	Forest Dept.	1 infant collected (1956)
3. Between Ulu Batang Balui and Ulu Batang Baleh near border	Forest Dept.	10-20
4. Sungei Pila, Mengiong Forest Reserve (Sungei Mengiong south of Batang Baleh)	Forest Dept.	Less than 20
5. Sungei Baram near Long Moh	Forest Dept.	1 male shot (1958)
6. Border region near Lubok Antu	Maias Protection Commission	—

inated over 600 square miles of forest in that area. Two hundred and ninety square miles are set aside as forest reserves. The map (Text-fig. 1) indicates the forest reserves, which harbor a sparse but continuous population of orang-utans. Records from within the past two years outside the reserves are indicated by dots and crosses. Reports of isolated populations and stragglers from areas not visited during the study are listed in Table I.[1] The numbers correspond to those on the map. Locations 3 and 4 appear to represent populations of unknown size in the border hills between Sarawak and Indonesian Borneo. Location 5 is a straggler, for orang-utans are unknown to the natives of the area (Smythies, pers. comm.). But the whole border region is nearly uninhabited and seldom penetrated, and it is possible that orang-utans occur sparsely along the whole range between locations 4 and 5. Locations 1 and 2 are included in the 625 square mile Lanjak-Entimau forest reserve, which is surrounded by shifting cultivation. Location 6 apparently represents a scattered remnant in an area greatly disturbed by agriculture.

The only other distributional data come from infant orang-utans captured by Dayaks and confiscated by the government. Some of these records are unreliable, because infants are smuggled over from Indonesian Borneo, and confiscations are usually made in or near towns. Thus, there are records from Lundu, Sibu and Bau near Kuching, regions from which there are no recent records of free-living animals.

NUMERICAL STATUS

Although no population estimates are available prior to 1959, it is apparent from the earlier literature that orang-utans have declined drastically in the past hundred years. Hornaday (1885) not infrequently encountered animals twice in the same day while traveling along rivers on which I saw only scattered nests during the present study. Beccari (1904) noted a total of eight orang-utans in three separate encounters in one day while walking in the forest near the Batang Lupar River. In 1960 I observed animals on the average of only once a week.

This population decline is not surprising in view of the hunting pressure to which the orang-utan has been subjected. James Brooke (1848) in 1840, for example, collected four animals during one days' outing. Wallace (1869) shot or captured 17, Hornaday (1885) 43, and Beccari (1904) 24. All these animals were collected between the Sadong and Batang Lupar rivers. In addition, numerous zoo collectors left with one or more infants, only obtained by shooting the mother. This slaughter continued until 1947 when the orang-utan became legally protected in Sarawak.

Data on comparison of population levels in different parts of the range were essayed by indices based on the number of nests encountered divided by the number of hours walked. The most intensive transects were conducted in the lowland and hill forests of the 106.5 square miles of the Sabal and Balai Ringin reserves adjoining

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[1] It was not possible, in the short time available, to check the accuracy of the various reports about isolated populations and stragglers. Sarawak lacks roads and most travel is by river, a time-consuming and expensive undertaking if uninhabited country is to be visited. Unknown territory along the Indonesian border is so extensive that censuses would require at least 6 months. Thus, the only records available are the scanty ones by forestry officers, which undoubtedly are second-hand for the most part.

the Indonesian border. In the former 4.3 (1.4-8.0) nests per hour were recorded; in the latter 1.7 (0-3.2) nests. Going on the assumptions that my rate of walking is one mile per hour, that orang-utans build one nest per day, that all nests are counted in a 200-foot wide strip, and that nests remain clearly visible for six months, a total population of 36 independent animals was derived for the two reserves. This figure could perhaps be raised to 40 or 45 if infants are included. Assuming that a rough density of one animal per two square miles is typical, a population of only 150 orang-utans would appear to inhabit the 290 square miles of forest reserves between the Sadong and Batang Lupar rivers. The remaining forests surrounding the reserves have been extensively disturbed and patches have been isolated by clearing and logging. Small, scattered populations persist, however, and if transect data in three locations are representative, an additional 200 animals could perhaps be added, bringing the number between the Sadong and Batang Lupar rivers to 350. This estimate agrees closely with that of Smythies (1960) who writes: "The only concentration of *maias* appears to be in the swamps between the Sadong and Batang Lupar (even here it is doubtful whether there are more than 200-300 left), and south of the Serian-Simanggang Road in the Balai Ringin and Sabal Forest Reserves."

A population estimate for the whole of Sarawak is entirely conjectural in view of the paucity of distributional records in the unknown border regions. It can, however, be safely said that the total population is not less than 450 animals and probably not more than 700.

ECOLOGICAL DISTRIBUTION

Orang-utans are animals of primary equatorial rain forest and as such their habitat shows little ecological diversity. Much of their habitat in Sarawak lies below an altitude of 500 feet in the swampy forests bordering the coast and the major streams. To the south the land rises and becomes gently rolling, interrupted here and there by prominent hills. Along the Indonesian border of western Sarawak mountains reach a height of 3,000 feet. Above that altitude the trees become more stunted. Banks (1931) notes that the upper limit of orang-utan distribution over most of their range appears to be 3,000 feet, although he mentions a record from as high as 6,000 feet on Mount Kinabalu in North Borneo.

Climatically Sarawak is characterized by rainfall of 120 to 160 inches per year, and uniform high temperatures (72 to 88°F at mean sea level) and humidity (98% mean relative humidity at 0600). Rain falls during all months of the year, but is heaviest from October to February (Seal, 1958). In keeping with the climate, the forests consist almost entirely of evergreen trees, probably exceeding 2,500 species in number, and covering some 75 per cent. of Sarawak. Several types of forest can be distinguished. Those inhabited by orang-utans are described below. Terminology and classification follows that of Smythies (1960). No obvious habitat preference for certain types of primary forest could be detected in the orang-utan.

Peatswamp Forest—Five vegetation types are recognized by the Forest Department in the peatswamp forest of Sarawak (Smythies, in litt.), but only the mixed swamp forest type, which is by far the most prevalent, is of importance to orang-utans. Three of the forest reserves containing orang-utan (Sedilu-Sebuyau, Simunjan) are primarily of the mixed swamp forest type. Structurally this forest is typical rain forest with an upper canopy of big trees 120 to 150 feet high and several layers of poorly-defined lower-story trees. Groundcover is essentially absent and the peat is covered by water ankle to chest deep over much of the area. Except along the margins of the rivers where alluvium is present, the soil consists of over 95% organic matter in a semi-liquid, decomposed state. Drainage and irrigation are generally impracticable, and the soil is therefore of little use for agricultural purposes, other than the growing of sago palms. Although there are only about 200 big tree species, some of these are valuable for timber and are being extensively logged.

Heath Forest—Small stands of heath forest occur over most of Sarawak on very poor, acid podsol soils. The trees are of relatively small size, lacking for the most part the upper story trees with big trunks. This habitat is of little importance to orang-utans, for only small patches exist in their present range; one visited supported a sparse population of animals.

Riparian Forest—This type of forest, rarely half a mile wide, occurs on riverine alluvial soils. Where rivers flow rapidly *Shorea* spp. is especially prominent; along slow-moving streams the valuable timber tree *Eusideroxylon zwageri* becomes important. Because the soil is rich and the forest easily logged, this habitat has for all practical purposes disappeared.

Dipterocarp Forest—This type is divided altitudinally into (a) Lowland Dipterocarp forest, (b) Hill Dipterocarp forest and (c) Moss forest. Together they still cover 28,000 square miles (59 per cent.) of Sarawak. Structurally the lowland and hill forests resemble the mixed swamp forest. Both contain some valuable timber species, and large areas, mostly of the lowland type, are being logged. (a) The lowland forest comprises many hundred tree species dominated by such Dipterocarps as *Shorea, Dipterocarpus* and *Dryobalanops.* The topography is rolling to hilly and the ground well-drained. The soil is non-podsolic, and much is probably suitable for agriculture. Orang-utans inhabit lowland forest in the adjoining Balai Ringin and Sabal reserves, and most likely in the Lanjak-Entimau reserve and in other border forests. (b) The hill Dipterocarp forest is confined to steeply sloping, often rocky, ground at altitudes above 1,000 feet. It is similar to the lowland forest except that the number of Dipterocarp species is fewer and the amount of big timber has decreased. Some of the slopes might be suitable for agriculture. Orang-utans inhabit this type in the Sabal reserve. (c) Moss forest occurs on the mountain ridges at about 3,000 feet in the Sabal reserve. Most of the big trees are lacking; others are of small girth and rarely exceed 80 feet in height. A few nests were noted in this habitat, but on the whole it is probably too restricted to be of much importance to orang-utans.

DISTRIBUTIONAL DYNAMICS

Although the major portion of Sarawak is still covered by the types of forests used by orang-utans, only a small fraction of the available habitat is frequented by the animals today. This, coupled with the fact that in the past they inhabited areas not occupied now, suggests that neither topography nor habitat had been limiting factors to dispersal and to numbers before the advent of agriculture. The high mountains (to over 5,000 feet) along sections of the Sarawak-Indonesian Borneo border act undoubtedly as partial barriers to a spread to and from the west, but numerous low gaps exist which appear to have been penetrated in the past all the way to the coast. Large rivers, like the Sadong and Batang Lupar, are definite barriers to direct movement perpendicular to their flow, but most rivers follow a course which does not hinder access to and from Indonesian Borneo.

It is not improbable that hunting by man has, at least in some parts, been the major limiting factor before the expansion of agriculture. That primitive man hunted and ate orang-utans extensively is shown by the remains in the Niah caves.

In the past hundred years habitat destruction has been added to hunting as a limiting factor to dispersal in some areas, and the former has reached a stage where the orang-utan is effectively prevented from extending its range in the major area of concentration. The Sadong-Batang Lupar population is hemmed in by two large rivers and agriculture; the population in the Lanjak-Entimau forest is surrounded by shifting cultivation.

It is doubtful if the orang-utan will ever adapt to habitat other than primary forest and old secondary forest. Although the animal can survive in very lightly logged forest where the canopy is only partially broken, no sign was ever noted or records obtained from very young secondary forest or from forest so heavily disturbed by logging that large sections of the canopy have been removed. Unfortunately the area with the greatest present-day orang-utan concentration supports also a heavy human population. All marginal agricultural land will undoubtedly have been put to use within the next few years, and the remaining patches of forest will be so logged and dissected with fields as to make them unsuitable for orang-utan.

The only hope for the remaining orang-utans in Sarawak lies in the preservation of the forest reserves which still harbor animals. These reserves are at present being logged in the accessible portions. In the peatswamp forest a 60-year cycle is used; in the lowland forest a 70-year cycle. The available undisturbed habitat in the reserves appears to be still adequate to support the orang-utan population. But logging has damaged some sectors so heavily that expansion of this activity can only be detrimental to the remaining animals.

All hunting, too, must cease if the orang-utan is expected to survive. Illegal hunting for meat and infants appears to continue on a limited scale. Between 1956 and 1960 a total of 13 infants were confiscated by the government, some of which were, however, smuggled over from Indonesian Borneo.

BEHAVIOR

Orang-utans were seen four times and observed directly for 5¾ hours. Indirect data from nests were also collected.

Locomotion — The orang-utan is basically arboreal, a habitat to which it is eminently adapted, for it possesses in effect four hands with which to clasp. It apparently frequents the ground only rarely. Wallace (1869) and Shelford (1916) received information of Dayaks injured by males which were surprised on the ground, and I was several times informed that orang-utans descend to eat the fruits and shoots of the palm *Zalacca conferta* in times of food shortage.

My observations confirm those of Wallace (1869) and Hornaday (1885) that the orang-utan brachiates on occasion but that its usual mode of progression is by climbing. One subadult was observed as it fled through the tree tops over a distance of ¼ mile. On large horizontal limbs it moved on all fours. It carefully but rapidly climbed into smaller branches, reached for and pulled in the foliage of the adjoining tree, secured a firm hold, and, with legs briefly free, swung across. Once it jumped three feet down to another branch, the only such instance noted. It also brachiated along a branch for about eight feet. The animal was greatly disturbed by my presence, and moved along at a speed of about three miles per hour, making it barely possible for me to keep up on the uneven ground.

Similar observations were made on two other orang-utans. A female with infant swung her legs free several times while holding onto a branch with her hands, primarily to reach another limb beneath her; but only once did she brachiate along a branch for about ten feet. A medium-sized male, who was followed by me for two hours, brachiated several times for distances of less than ten feet each. The rest of the time he climbed, always holding on to as many branches as possible. Twice he came to a gap in the canopy which prevented him from reaching the branches of the adjoining tree. In the first instance he inched out on a small horizontal limb until it bent under his weight, bringing him to within grasping distance of another branch. This he clutched and only when his hands were anchored securely did he release the first branch which had been retained with the feet. Another time he climbed into the crown of a vertical branch. Suddenly he threw his weight to one side, continuing his hold with one hand and foot only. The bending branch and extended arm enabled him to grasp the foliage of the next tree.

The grasping power in the feet enables the animal to hang with ease in practically any position. Hanging by one arm and foot while reaching for food is not unusual, and once a subadult hung two to three seconds by its feet alone.

Group Size and Composition—The orang-utan is the least gregarious of the apes. Whereas chimpanzees and gorillas sometimes go in groups of ten or more and gibbons and siamangs occur most frequently in groups of three to six, the orang-utan is rarely encountered in aggregations of more than two or three. Most of the records by early collectors are of single animals or groups of two. The largest aggregation noted by Brooke (1845), Wallace (1869) and Hornaday (1885) was three; by Beccari (1904) four; by Mjöberg (1930) six. Carpenter (1958) noted four, with a fifth animal in the vicinity. During the present study no single animals were seen. Three of the four encounters were of groups with two animals each, and only once did direct and indirect evidence point to a group of four animals.

Group composition varies. Lone adult males have been encountered by nearly all explorers and collectors. Although Wallace (1869) writes: "I never saw two full grown animals together . . .," other investigators have done so. Table II presents the composition of four groups as determined during this study as well as other compositions mentioned in the literature. The data show that the most frequent combinations are(1) lone males, (2) females with one or more subadults of various ages, (3) pairs, and (4) small groups of subadults.

Nothing is known about the stability of groups. Data from a group of four animals which had just moved into a section of forest suggests that groups may be quite plastic. The group consisted of a female and a small infant, which were seen, and a subadult and an adult male judging by the size of the nests and by information from natives who had encountered them. One night all four animals nested in adjoining trees; another night the female with infant and juvenile nested together, but the male slept about 400 feet away; a third night the female with infant and juvenile again nested together but there was no sign of the male in the vicinity. The female was observed that morning, but the juvenile was not with her and searching for about two hours did not reveal it.

Mother-Infant Relationship—Personal observations were made on (1) a female with small infant, and (2) a female with a semi-independent infant of about two years.

(1) A female fed in a *Durio* tree at a height of about 70 feet with the baby clinging to her side, its head just below her armpit. The arms and legs of the infant were "spidery" and with but little hair, yet it was alert and looked around. The infant began to advance across the mother's chest, and she cradled it in one arm. Restless, it clambered upward toward her shoulder, but she pulled it down. It moved up again, so the female pushed it over to its original position at her side. When it continued its wanderings, she laid it across her arm and groomed the back, using the long fingers of the same arm that held the infant. Finally she grasped a branch above her head with both hands, and the baby attached itself to her side, a place it occupied for the next hour while the female first fed and later climbed away through the trees.

(2) A female fed in one tree and her large offspring in another 50 feet away. They joined

TABLE II. COMPOSITION OF ORANG-UTAN GROUPS

Group Size	Composition	Source
1	1 young male	Wallace (1869)
1	1 adult male	Wallace (1869) Hornaday (1885) Beccari (1904)
1	1 female	Wallace (1869)
2	1 male, 1 female	Beccari (1904)
2	1 medium-sized male, 1 female	This study
2	1 female, 1 infant	Wallace (1869) Hornaday (1885)
2	1 female, 1 semi-independent infant	This study
2	2 subadults (of undetermined sex)	This study
3	3 subadults	Wallace (1869)
3	1 female, 1 subadult, 1 infant	Brooke (1848) Hornaday (1885)
3+	1 female, 2 or more subadults	Wallace (1869)
4	1 male, 1 female, 1 subadult, 1 small infant	This study
5	2 females, 2 juveniles, 1 male in the vicinity	Carpenter (1958)

and the infant sat beside its mother, one arm across her back. The infant was about five feet from her when she spotted me. Immediately she reached over and snatched the infant to her chest. As the female climbed into the next tree, it moved and held on to her back, one arm over her shoulder, the other grasping her side. The female traveled about 500 feet, stopping intermittently to look down at me. While moving the infant clung either to her back or to the side; while sitting it always stayed at the side. Although fully capable of climbing independently, the infant not once released its hold on the mother and stayed with her as she built the night nest an hour later.

Foods and Food Habits—Orang-utans are primarily frugivorous. They apparently also eat the leaves of certain trees, according to Hornaday (1885) and others, but no direct evidence for this was obtained. The shoots and fruits of the palm *Zalacca conferta* are eaten, according to the natives. One instance was noted in which an orang-utan had gnawed the inner lining of bark from a tree (probably *Ganua motleyana*) and spit out the chewed pulp. At times the animals raid cultivated fruits at the edge of the forest. Perhaps insects and other animal foods are sometimes eaten. Ryhiner (1958) observed an orang-utan "occasionally catching an insect on the bark." Mrs. Harrisson (pers. comm.) maintains that they eat the eggs of birds in the wild.

Table III presents a list of forest trees which produce fruits eaten by orang-utans. Information on food habits was supplied by natives and was reliable in the three cases when I was able to check it. Many of the fruits listed are eaten also by man, *Hylobates moloch* and various monkeys. Hornaday (1885) confirms that orang-utans eat the cultivated *Durio* and *Nephelium*.

Animals were feeding when observed at 0845, 1100, and 1625. A male and female fed quietly on the small olive-sized fruits of *Calophyllum*. The only sound was the steady patter as seeds rained on the leaves below—a sound which first attracted attention to the animals. They plucked several fruits in a row by pulling them off between thumb and index finger; they then popped them all into the mouth, chewed, and ejected the seeds through pursed lips. Similar behavior was noted in a female and large infant, except that the whole of the small fruit of *Grewia* was eaten. Feeding ceased as soon as the animals saw me.

A female with small infant, on the other hand, continued to feed in spite of my presence from 0845 to 1005 on the muskmelon-sized fruit of the durian (*Durio carinatus*). She had nested during the night in the adjoining tree, and was feeding when first found. The spiny durian fruits were plucked by hand, usually one at a time. Once she put her mouth close to a durian, tapped the back of the fruit with one hand and at the same time grabbed it between her teeth and detached it by jerking her head back. Three times fruit were transported to another branch as far as 20 feet away. Twice one was carried in the mouth, and a third time one was held in the mouth and another with one foot. When eating she held them lengthwise in the long hand and rapidly bit off chunks of tough outer skin. The soft pulp was also discarded. Apparently only

TABLE III. FRUITS EATEN BY ORANG-UTAN

Forest Type	Species
Cultivated fruits	*Durio zibethinus* *Nephelium lappaceum* *Artocarpus* spp.
Peatswamp forest	*Pongamia*, probably *P. pinnata* *Polyalthia* sp. *Quercus* sp. *Pometia pinnata* **Durio carinatus* *Nephelium melanomisoum* (?) *Copaifera palustris*
Heath forest	*Sandoricum*, probably *S. emarginatum* *Mangifera*, probably *M. havilandii*
Lowland Dipterocarp forest	*Diospyros maingayi* *Garcinia* sp. *Quercus* sp. **Grewia* sp. *Sandoricum* sp. *Nephelium* sp. *Durio* sp.
Hill Dipterocarp forest	**Calophyllum* cf. *retusum*

* Indicates direct observation of feeding.

the pecan-sized seeds were eaten, of which each durian contains about ten. During the time of observation the female consumed the seeds of at least 15 durian, wasting only half of one seed.

Nests and Nesting Habits—Orang-utans build nests when wounded (Brooke, 1848; Wallace, 1869), in the evening before going to sleep and perhaps during the day when resting. St. John (1862) and Wallace (1869) mention that orang-utans hide in nests when pursued. No detailed accounts of orang-utan nests have been published. Hornaday (1885) briefly and erroneously writes: "The nest of the orang-utan is simply a lot of small green boughs and twigs broken off by the animal, and piled loosely in the fork of a tree . . .," and Wallace (1869) and Beccari (1904) make essentially similar statements.

Nests may be placed anywhere in a tree as long as there is sufficient support and a suitable quantity of small branches from which to construct a stable platform. The 228 nests observed in this study were found in forks made by the main trunk and smaller limbs, in the forks at the extremity of the larger limbs, along one or more horizontal branches, in the crowns and even between two close trees. Thirteen nests were examined in detail. The basic platform was constructed of three to nine (mean 4.7) major branches broken in toward the animal. They were laid across each other without pattern or interweaving. Of 62 branches used in nest construction, only seven were completely detached.

Most nests have a lining of small twigs about one foot in length, which the animal breaks off around the nest. In one instance the twigs were collected seven feet below the nest. The broken ends usually face outward, but in some nests they appear to be placed at random. The number of twigs in each nest varied from 0 to 34 (mean 10.0).

The height of nests above ground is given by Wallace (1869) as 20 to 50 feet; Hornaday (1885) 15 to 40 feet; Banks (1931) 30 feet; and other authors quote similar heights. The heights of 228 nests in mixed swamp, lowland and hill forests was either estimated or determined with a camera range finder. The results are summarized in Table IV.

From Table IV it is evident that the majority of nests (82.3 per cent.) occur between 31 and 70 feet; in other words, in the middle of the lower story trees. However, a few nests are located in the lowest story and in the tops of the emergent trees. There are no records of nests on the ground.

TABLE IV. HEIGHT OF ORANG-UTAN NESTS ABOVE GROUND

Height of nest (in feet)	Number of nests	Percent
0-10	0	.0
11-20	2	.8
21-30	5	2.2
31-40	35	15.3
41-50	57	25.0
51-60	56	24.5
61-70	39	17.5
71-80	14	6.1
81-90	6	2.6
91-100	7	3.0
101-110	1	.4
111-120	5	2.2
121-130	1	.4
Total	228	100.0

Wallace (1869), Hornaday (1885) and others have described the building of nests in free-living orang-utans. The amount of time spent in construction and the elaborateness of the nest depends probably on the weather conditions and general disposition of the animal. Nest building was observed once when an agitated female built a nest up 120 feet during a downpour at 1730. She squatted in a fork among the smaller branches. With one hand she broke in a limb, pushed it under foot while reaching for the next one with the other hand. The latter as well as a third branch were also placed beneath her feet. She paused briefly, then tore off a final branch and laid it across the others. The time required for building this crude nest was approximately 10 seconds.

Reaction to Man—Orang-utans usually avoid contact with man, but in areas where primary forest adjoins cultivation and the animals are not hunted, they may become used to the noisy presence of Dayaks working in their fields.

The reactions of orang-utans to me standing beneath their tree varied. On the one end of the scale were two subadults who fled soundlessly and as rapidly as possible; on the other a female with small infant who, except for brief glances, ignored me while feeding and finally moved off at a leisurely pace, once descending to 45 feet to urinate directly above me. This same female showed no response to natives talking at a distance. During the other two encounters the animals fled slowly, stopping intermittently to produce vocalizations and other sounds, and to throw branches to the ground.

Sounds—Sounds were emitted only when the animal was aware of my presence and presumably annoyed. Under similar circumstances Hornaday (1885) notes deep, guttural growls, and Wallace (1869) howling. Three types of sound were made interchangeably but continuously for 45 minutes by a female with large infant: (1) A smacking or kissing sound was made by tipping the head back, pursing the lips with the lower one protruding beyond the upper one and drawing the air in. Three times the female put the knuckles of one hand to her mouth and kissed them loudly. The kissing sound was usually followed by (2) a gluck-gluck-gluck that resembled loud gulping of liquid. The lips were pursed as above, and the throat moved as if she swallowed air. After the gulps the female produced several times a sound that can be best described as (3) a loud two-toned burp, starting on a long, low key to end on an abrupt higher note. These sounds were heard again during another encounter with a female and a male. Both gave the smacking sound, the male continuing it at the rate of once every few seconds for two hours. But only once did the male "gulp" and "burp."

Throwing of Branches—The fact that orang-utans may drop or throw branches if an observer stands beneath the tree has been recorded by Wallace (1869) and Attenborough (1957), although strangely neither Hornaday (1885) nor Beccari (1904) noted this behavior—probably because the animal was always shot before it had a chance to do so. I observed throwing of branches twice: (1) After fleeing from me for 40 minutes, a male stopped in a tree. He moved about and stepped on dry branches which broke off. Once he broke one off with the palm of his hand and watched the limb as it crashed to the ground. He also reached for branches around him, bent them until they snapped, wrenched them from the tree with a jerk, then threw them downward. Half an hour later he hurled branches for 10 more minutes. (2) A female with a large infant spent 15 minutes throwing a total of about 30 branches varying in size from twigs to limbs ten feet long and three inches in diameter. Considerable effort was expended at times in tearing off the larger branches. Limbs were thrown in three ways: (a) she merely held the branch at her side and dropped it limply; (b) she looked down at me and swung the branch like a large pendulum, and at the peak of the arc closest to me she released it; (c) she lifted branches either as high as her chest or above her head with one hand and hurled them down forcefully. Whatever interpretation is given this behavior, there is no doubt that it induced me to jump nimbly at times and that it kept me effectively away from beneath the tree.

Summary

The distribution of the orang-utan encompasses the northern tip of Sumatra and extensive sections of Borneo. A two-month study in Sarawak, which comprises about ⅙ of Borneo, in all types of primary forest habitat, revealed that the orang-utan is limited in distribution to about 2,000 square miles and in number to not less than 450 and probably not more than 700 animals. Hunting has been the major cause for their decline in the past and habitat destruction threatens them at present in their major area of concentration. Preservation of the continuous tracts of primary forest still inhabited by orangutans is essential if the small population of this ape is expected to survive. All hunting for meat and specimens, including live animals for zoos, must remain prohibited.

Behavior data is based on observations of 228 nests and four visual encounters for a total of 5¾ hours on eight animals. Orang-utans seldom descend to the ground. In the trees they are climbers, but brachiate on occasion. Groups number rarely more than four, and lone males and females are not infrequently encountered. Subadults appear to form their own group at times. There seems to be little group stability. Observations on a female with a small infant and a female with a large infant are described. Orang-utans are primarily frugivorous. Nests may be built nearly anywhere in a tree, but the majority are found 31 to 70 feet above ground. Basically the nest consists of three to nine branches broken in to form a platform and a lining of 0 to 34 twigs. A female built a crude nest in ten seconds. Reaction to man varies from immediate flight to seeming indifference. Sounds made by agitated animals can best be described as smacking, gulping, and burping. Throwing of branches while the observer stood near the tree was noted in two animals.

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7

Observations on the Feeding, Shedding and Growth Rates of Captive Snakes (Boidae)

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INTRODUCTION

OPPORTUNITIES for recording the growth rates and observing certain activities of the large boids are exceedingly limited. Field studies are non-existent because of the difficulties of restraining and measuring in the wild creatures of such great size and strength, and among captive specimens, those responsible for them are usually reluctant to subject them to the necessary handling for even so worthy an objective as the collecting of new data. Few observations of even the feeding schedules and shedding rates of these great serpents have been recorded. Thus our knowledge of many aspects of their life histories is very meager.

The Highland Park Zoological Gardens in Pittsburgh has had in its care several well-adjusted pythons and boas, snakes with which certain liberties may be taken. The senior author initiated a series of observations during his tenure as Zoo Herpetologist from 1946 to 1952, observations which his successor, the junior author, resumed and expanded, beginning in 1953 and continuing through the present. A summary of their findings is presented in this paper.

REPTILIAN GROWTH RATES

The misconception that reptiles generally grow slowly has long been widely accepted. It is surprising that this fallacy has been so tenacious when one considers the volume of data refuting it, data that are based on several reptilian orders.

Heller (1902) reported that a juvenile Galapagos tortoise (*Testudo vicina*) increased in weight from 29 pounds to 130 pounds in three years. Ditmars (1933) observed the growth rate of captive alligators (*Alligator mississippiensis*) and found that newly hatched young having a length of eight inches and a weight of only 1.75 ounces could in six years attain a length of 72 inches and a weight of 72 pounds. Breeding adults continued this rapid growth for a time, so that a six-foot eleven-inch specimen grew 55 inches in six years to a length of eleven feet six inches. McIlhenny (1935) validated Ditmars' figures with a field study that yielded essentially identical information.

Pope (1957) has reported that an Indian Rock Python (*Python molurus*) in his custody was "about 24 inches" long on hatching. At the age of one year its length had reached 60 inches and its weight 3.5 pounds; at two years, 100 inches and 21 pounds; at three years, 125 inches and 38 pounds. Oliver (1952), reporting the first year's growth of three *molurus* hatched in the New York Zoological Park, stated, "One year ago on this date . . . our three young Indian Rock Pythons emerged from their eggs. They were then only a little more than one foot in length and weighed under four ounces . . . On their first anniversary the largest measured four feet two inches in over-all length and weighed a pound and a half . . . The other two individuals are only slightly smaller than their brother." Wall (1921) mentions one brood of this species in which the hatchlings were "about 2 feet." In a second brood, the young upon hatching averaged two feet five inches. Some of these grew eleven inches in only four months. It is clear from all these observations that young reptiles may indeed grow rapidly.

There is, however, less information available regarding the growth rate of pythons and boas once they have reached maturity. Loveridge (1945) recorded a *Python reticulatus* at the London Zoo that grew from a length of ten feet to twenty-one feet in ten years. "Another took fourteen years in which to grow from 19 to 24 feet. On this basis we can roughly estimate growth as proceeding at the rate of a foot per year in the period between 10 and 20 feet, but at only about half that rate subsequently." Hence, even among the initiated, the notion persists that these giant snakes grow rapidly at first, but can increase their dimensions only very slowly after they have achieved adult proportions. The following data lead us to reconsider this hypothesis.

Python sebae

Our own observations agree with earlier conclusions that young boas and pythons grow rapidly. When an African Rock Python was received on October 3, 1951, its length of slightly over two feet indicated an age of less than one year. Thirteen months later it measured between three and three and one-half feet and had a greatest diameter of three-fourths of an inch. Since the snake shed its skin seven times during this first year, there was a length increment of some two inches per shedding. No periodic measurements were taken subsequently, but on May 9, 1957, the python was found to be nine feet three inches in length and 37 pounds in weight. With only a few months of the first year unknown, the average annual increase in length for this snake's first six years was 14 inches. On June 18, 1960, its length was 11 feet 2 inches and its weight 56 pounds. Thus during 37 months of its seventh, eighth and ninth years, it had an average annual increment of only 7.6 inches and 6.3 pounds. Its food intake during this period totaled 148 pounds, or 7.8 pounds of food for every pound of weight gained. The animal has been kept in a temperature between 73° and 78° Fahrenheit and supplied with excess food all 12 months of the year. A record of the food it consumed is presented in Table I.

Python molurus

The Indian Rock Python, a light-colored female, was within a few inches of nine feet when received on December 18, 1947. Because the snake had a tendency toward rectal prolapse, as well as for other reasons, it was measured but twice during its ten years' tenancy in the Pittsburgh Zoo's reptile wing. On January 21, 1955, it measured just 13 feet in total length. On January 7, 1958, it was found dead and at this time measured 13 feet 8 inches in over-all length. Its weight a few weeks earlier had been found to be 39 pounds. The slow growth during its last three years and the decidedly low ratio of weight to length are clearly not normal, and thus limit the value of these data. Pope's observations (1957) on the same species, already noted, should be compared. The present specimen increased its length by only 56 inches in ten years, despite a total food intake of 654 pounds. Forty-eight inches of this total was gained during its first seven years, or an average of seven inches per annum. Records of this specimen's food intake and shedding frequency are presented in Table II.

Python reticulatus

Perhaps the most spectacular snake now on display in the United States is "Colossus," the male Reticulated Python received at the Pittsburgh Zoo on August 10, 1949. The locality from which it was taken is unknown, but the fact that it was shipped to the United States from Singapore suggests that it was probably of Malayan origin. On arrival, its length was just 22 feet. After having rejected the fowl and rabbits offered it during its first two months, it ac-

TABLE I. FOOD INGESTED BY A CAPTIVE *Python sebae,* 1951-1960

Year	Feedings	Food Taken				Total Weight of Food (lbs.)
		Mice	Rats	Rabbits	Other	
1952*	17	32	8 juv.	—	—	2.5
1953	33	110	11 juv.	—	2 English sparrows	11.0
1954	20	91	4 juv.	—	2 pigeons	8.3
1955	26	46	1	19	2 starlings, 1 guinea pig	47.7
1956	10	—	2	8	2 guinea pigs	27.5
1957	12	—	—	11	3 ducks	48
1958	13	—	—	12	3 squirrels	52
1959	8	—	—	11	—	48
1960	9	—	—	6	2 chickens, 1 duck	44
8¾ yrs.	148					289

*First eight months.

TABLE II. FOOD CONSUMPTION AND SHEDDING FREQUENCY IN A CAPTIVE *Python molurus*, 1948-1957

Year	Feedings	Food Taken			Total Weight of Food (lbs.)	Dates of Shedding
		Rats	Rabbits	Other		
1948	14	45	—	1 pigeon, 1 jungle fowl, 3 ducks	59	Apr. 16, Aug. 5
1949	13	69	–	———	67	May 4, Aug. 3
1950	8	26	4	———	36	Mar. 7, May 19, Sep. 23
1951	12	–	18	1 duck	80	Mar. 4, Aug. 7, Oct. 26
1952*	6	1	11	———	59	Apr. 5, June 10, Aug. 7
1953	12	–	7	6 ducks, 2 guinea pigs	68	Mar. 29, Oct. 5
1954	6	–	1	8 ducks	52	Mar. 12, Sep. 27
1955	4	–	5	———	34	No record
1956	11	–	12	3 ducks	85	”
1957	13	–	15	7 ducks, 3 pigeons	114	”
9¾ yrs.	99				654	

*First eight months.

cepted the first pig offered. This was on October 14 when it took a 15-pound suckling. Thus began a pattern of regular feeding that has continued until the present time (Table III).

This Reticulated Python has accepted nothing but pigs; rabbits, chickens and ducks have been refused on several occasions. When hungry, the snake strikes and seizes the prey with its mouth immediately upon the animal's introduction into the cage. It is instantly enmeshed in one or one and a half coils. This grasp is usually retained for about 15 to 25 minutes although the prey rarely survives longer than three minutes. Stunned food-animals receive the same treatment as active ones. After the long waiting period, the mouth grip is freed and the coils are relaxed without being released, as the snake begins a thorough investigation of its food, the tongue flicking leisurely in and out throughout the process. If the snout and ears of the prey chance to be buried in the gravel of the cage floor, the snake prolongs its search and finally grasps the

TABLE III. FOOD CONSUMPTION AND SHEDDING FREQUENCY IN A CAPTIVE *Python reticulatus*, 1949-1960

Year	Feedings	Pigs	Total Weight of Food (lbs.)	Dates of Shedding
1949*	4	4	98	Dec. 2
1950	9	9	287	Feb. 11, Apr. 29, July 1, Sep. 12, Nov. 10
1951	6	6	203	Feb. 11, Apr. 18, July 10, Oct. 6, Dec. 20
1952†	7	7	202	Mar. 30, July 6
1953	6	6	169	Feb. 10, Aug. 25
1954	5	5	161	Feb. 13, Dec. 22
1955	5	5	154	Apr. 16, Oct. 23
1956	4	4	108	Mar. 8, July 3, Sep. 30, Dec. 20
1957	5	5	136	Mar. 10, May 13, Aug. 5, Oct. 8
1958	5	5	128	Apr. 1, June 8, Nov. 4
1959	6	6	184	July 8, Aug. 31, Nov. 19
1960	6	6	161	Feb. 11, Aug. 2, Nov. 1
11 years	68		1991	

*Last five months.
†First nine months.

animal again, nearly always by the shoulder, and drags it a foot or two before resuming its investigation. Should this procedure fail to disclose either the snout or an ear, the snake may lose interest and abandon the attempt to feed, or it may repeat its effort to locate one of these crucial points by again moving the prey. Apparently this snake will secure its mouth grip preparatory to swallowing at only one of two points: the snout or an ear. Indeed, the identification of one of these two points by the snake's tongue may possibly be thought of as a releaser mechanism which triggers the engulfing behavior.

The largest pig offered to this python weighed 54 pounds. It in no way taxed the snake's capacity and was swallowed in 64 minutes.

This Reticulated Python was 22 feet long when received on August 10, 1949. On June 4, 1951, it was approximately 23 feet 3 inches long, having increased about 15 inches in 22 months. Perhaps our most accurate size data for this specimen were obtained on February 24, 1954, when it was found to weigh 295 pounds. The junior author noted the weight in his daily journal on that date and stated: "This was shortly after its winter fast of 4½ months. The snake was then returned to its transfer cage. I opened the transfer door, which is 14 × 18 inches, and the python began to crawl back into its main cage. By placing a measuring tape on its back and working along hand over hand so that it would not slip, I got a measurement of 27 feet, two inches, which I believe will be as accurate a measurement as we shall get until the snake dies." The specimen had grown 47 inches in 32½ months. On November 15, 1956, it was found to measure 28 feet 6 inches, having grown 16 inches in the intervening 33 months. The snake has shed from two to five times a year.

We cannot offer these length data as exact measurements, because of the way in which they had to be collected, but we are certain that they are accurate within a few inches. They show that large snakes can continue to grow at an appreciable rate after they have achieved adult dimensions. Having attained 85 percent. of the maximum length known for its species, this specimen is still growing at an average rate of 10.75 inches per year. This figure is in good agreement with a recent report from the New York Zoological Park of a 19-foot 4-inch, 170-pound Reticulated Python that gained 10 inches and 26 pounds in a single year.

A. C. Stimson's observations (*in litt.*) on a male Reticulated Python in the Houston Museum of Natural History show how great may be the range of variation in growth rates under different conditions: "We have had it for 22 years and it was approximately 18 feet long when received. It now measures 24 feet, five inches and weighs 247 pounds." Hagenbeck's prize Regal Python was 28 feet in length, and weighed 250 pounds (Pope, 1937). This was the heaviest snake for which we find a definite record, so that the Pittsburgh specimen may be the heaviest snake ever weighed under reliable conditions. It would be unreasonable to suppose, however, that this captive would outweigh the record 32-foot specimen, for which no weight was recorded. We may assume that a snake of the latter dimensions greatly exceeds 300 pounds in weight.

Eunectes murinus

Our larger Anaconda measured 16 feet 4 inches and weighed 108 pounds when it was received from a shipper in Belem, Brazil, on June 13, 1950. After shedding on June 24, it refused living pigs, rabbits, ducks and carp through an eight-week period until, on August 10, it accepted a small mallard duck. Since that date only waterfowl—mallard ducks, white pekin ducks and a snow goose—have been accepted (Table IV).

The Anaconda has grown more slowly than the pythons. On February 26, 1954, it was found to be 18 feet 7 inches long and to weigh 160 pounds. This represented a gain of 27 inches and of 52 pounds in 44 months. By March 21, 1957, it had attained a length of 19 feet 6 inches and a weight of 200 pounds, having increased its length by 11 inches and its weight by 40 pounds in 36 months. Most recently, measurements were recorded July 10, 1960, when the snake's total length was found to be 20 feet 7 inches. This represents an increment of 13 inches in 40 months. It was not weighed.

The average annual length increment for this specimen during nearly eleven years in captivity was only five inches. It may be that this growth rate would have been increased if more food had been accepted, but the snake refused to increase its intake despite every opportunity to do so. During the 81 months between the earliest and most recent weighings, a net gain of 92 pounds has been recorded. During this same period it consumed 539 pounds of waterfowl, or 5.86 pounds for every pound of weight gained. Despite our Anaconda's very lethargic behavior, it cannot compare in efficiency with Pope's (1957) phenomenal young *Python molurus* which reportedly gained a pound for every 1.76 pounds of food consumed. Pope (1955) mentions a gravid female Anaconda having a length of 19 feet which weighed 236 pounds. Soon thereafter she gave birth to 72 young, each of

Table IV. Food Consumption and Shedding Frequency in a Captive *Eunectes murinus*, 1950-1960

Year	Feedings	Ducks	Total Weight of Food (lbs.)	Dates of Shedding
1950	8	20	54	June 24
1951	14	24	114	Feb. 7, May 30, Sep. 13
1952*	8	17	88	Jan. 25, June 13
1953	8	17	83	Mar. 3, Sep. 17
1954	7	13	65	Jan. 8, July 1
1955	7	16	80	Jan. 8, July 18
1956	6	11	55	May 8, July 14, Dec. 16
1957	7	12	72	May 16, Nov. 29
1958	9	12	68	May 2
1959	8	11	65	No record
1960	7	8	52	May 1, Aug. 11
—	—		—	
10½ years	89		796	

*First eight months.

which was about 38 inches. Assuming that each of these young weighed about a pound, the mother's residual weight would have approximated that recorded for our specimen of comparable length.

The average growth per ecdysis for the *murinus* is again seen to be a little more than two inches. This figure is notably constant in the four snakes reported herein.

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8

The Feeding Mechanism of Fiddler Crabs, with Ecological Considerations of Feeding Adaptations[1,2]

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(Plate I; Text-figure 1)

INTRODUCTION

THE ability of an organism to obtain nutrition from its environment is one of the basic requisites for survival and is thus a factor governing the distribution of animals. Should an animal become uniquely adapted to obtain food in a specific manner, the variety of habitats in which it can live becomes accordingly limited. This study of the feeding mechanism of three species of fiddler crabs, *Uca pugilator* (Bosc), *U. pugnax* (Smith) and *U. minax* (Le Conte), was undertaken to observe feeding adaptations which may contribute to limiting their distribution.

Ecological studies have elucidated the grosser conditions prevailing in typical habitats of *Uca*. Pearse (1914), working at Woods Hole, Massachusetts, and Schwartz & Safir (1915) at Cold Spring Harbor, New York, described the habitat of *U. pugilator* and *U. pugnax*. Both species live centrally between the tide marks, where the substrate consistency permits burrowing. However, *U. pugnax* is limited mainly to the mud or clay areas, while *U. pugilator* lives on a sandy substratum. Gray (1942) studied *U. minax* at Solomons Island, Maryland, and noted that the substratum may vary from sand and mud, to clay, though this crab is mainly limited to habitats of lower salinity. The more comprehensive study of distribution of *Uca*, done in the Georgia salt marshes by Teal (1958), has generally supported these observations.

The same workers have also considered the food material of *Uca*. Both Pearse (1912), then working in the Philippines, and Schwartz & Safir found algae and vascular plant tissue to make up the bulk of the stomach contents in the *Uca* examined, though decayed animal matter and inorganic material were also present. By experimental feeding, Gray found *U. minax* to ingest a wide variety of foods, with the notable exception of putrified material. In controlled experiments, Teal found *U. pugilator* and *U. pugnax* able to live on cultured marsh bacteria.

In regard to the manner in which *Uca* feeds, Pearse (1912) described rather completely the action by which the minor chela passes material into the buccal cavity where the food to be ingested is selected by the mouth parts. Working in Brazil, Matthews (1930) observed *U. leptodactyla*, a small species, to take single grains of sand into the buccal cavity, where adhering organic matter is scoured off and the cleaned grain rejected. This scouring action is achieved by the movement of the second maxilliped endopodites across the bristled endite lobes of the first maxilliped protopodite. Matthews also observed spoon-shaped tips on some medianly-projecting bristles of the second maxillipeds. These bristles have become known as spoon-tipped hairs, mainly through the descriptive work of Crane (1941, 1943). Examining tropical *Uca*, as well as the three species of the northern temperate region, she observed a great number of spoon-tipped hairs in the sand-inhabiting species, while crabs living in muddy habitats have an abundance of woolly hairs on the second maxilliped. She suggested the con-

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nection of such hair modifications with feeding. Altevogt (1957) has compiled a list of crabs which show a similar type of hair modification, which supports Crane's generalization correlating the modification of the second maxilliped hairs with habitat. He observed that the spoon-tipped hairs aided in the ridding of coarse particles from the buccal cavity. He has also contributed to the understanding of the feeding mechanism with his description of the washing of coarse material from the buccal cavity by water from the branchial cavity, while the lighter detritus is suspended in the fluid and adheres to the mouth parts for eventual passage to the mouth.

In this study of the feeding mechanism in *Uca*, the morphology of the mouth parts is described, with composite figures showing their relative positions within the buccal cavity. The various aspects of the sorting process within the cavity are considered in detail. Habitat limitation created by the mode of feeding is examined and species differences in mouth-part structure are analyzed, so that the ecological consequences of such specific adaptation may be considered.

I wish to express my appreciation to Dr. F. John Vernberg, who directed this study, and to Dr. R. H. Siepmann for his translation of the German paper by Altevogt.

METHODS OF STUDY

While the grosser aspects of the feeding mechanism were observed in the field with the aid of binoculars, or in aquaria in the laboratory, the more detailed processes occurring within the buccal cavity were observed with a dissecting microscope. Details of the mouth parts and associated hairs were most easily discerned when the crabs were submerged in a pan of water. Since *Uca* is very hardy, the third maxilliped could be excised to permit observation of the actions of the other mouth parts. Careful removal of one or both of the first maxilliped endite lobes permitted similar observations of the appendages more immediate to the mouth. The manner in which the mouth appendages manipulate particles was seen by placing sand grains or pieces of modeling clay on the appendages, while the direction of action of the mouth parts was observed by stimulating them with a probe.

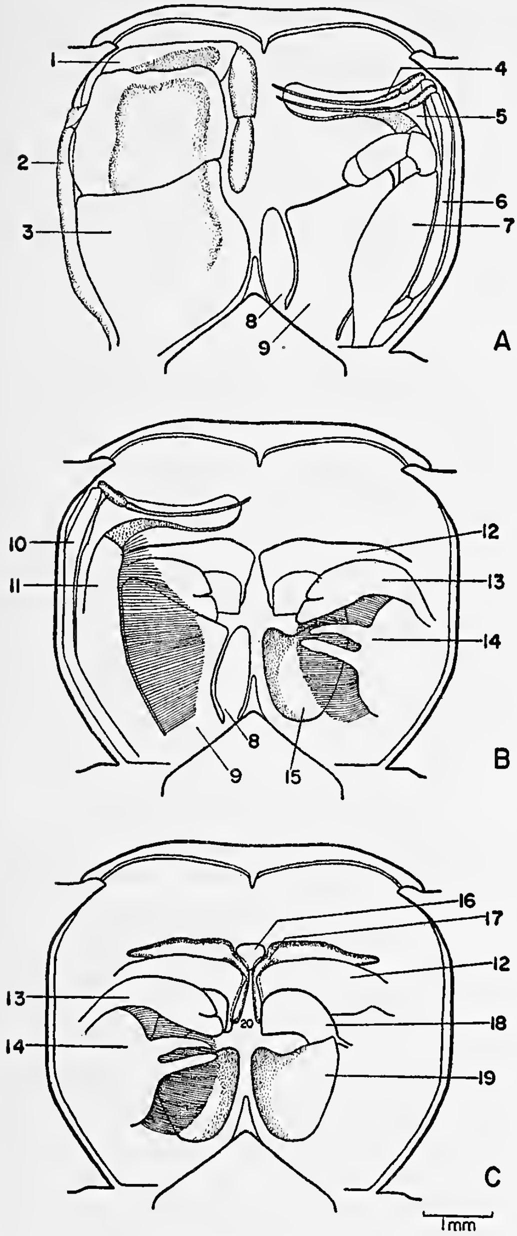
Semidiagrammatic figures of the mouth parts, drawn to show their relative position within the buccal cavity, are included to aid in the understanding of the interrelationship of the appendages. Though the hairs which cover the surfaces of the mouth parts have been generally deleted from the figures for simplicity, these hairs are described in detail in the text.

Observations of the habitats in which the crabs live, as well as the studies of their general distribution, were made during the summers of 1957 and 1958 at Beaufort, North Carolina. The habitats have been characterized by the condition of the substratum, and by the topography and vegetation. Percentage of sand content of the substratum was determined by the hydrometer method as outlined by Bouyoucos (1928).

APPENDAGES AND MOUTH STRUCTURES OF THE BUCCAL CAVITY

The terminology adapted for description of the mouth parts follows that used by Snodgrass (1950, 1952). However, reference was also made to the general descriptions of crustacean appendages by Siebold (1874), of the Brachyura by T. H. Huxley (1878), and of *Callinectes sapidus* by Lochhead (1950). The appendages are described here with reference to their relative position within the buccal cavity while the crab is in a typical feeding position. At this time the buccal cavity is at a 45° angle in respect to the horizontal, which means that the upper edge of the third maxillipeds can also be termed the anterior edge, and the lower edge, the posterior. These terms are used interchangeably in this discussion.

The third maxillipeds enclose the buccal cavity from beneath. The basal portion of the maxilliped, termed the protopodite, is composed of a coxopodite and a basipodite. An epipodite arises laterally from the coxopodite and extends along the base of the branchiostegite, where it functions as a valve to regulate the intake of respiratory water before it turns into the gill cavity proper. The endopodite and exopodite extend from the basipodite. On the endopodite, the ischiopodite and meropodite are greatly enlarged and join to form a broad plate. The endopodite is bent medially at the fourth joint, to fold the three terminal segments across the meropodite, and finally to extend downward along its median edge as an endognathal palp. The palp is flexible and is used to clean the eyes, antennae and antennules, as well as functioning in the feeding process. The exopodite of the third maxilliped borders the endopodite as a long segment, which terminates as a slender, many-jointed flagellum which extends medially across the buccal cavity. The peduncle of the exopodite shares with the broad plate of each endopodite and their terminal segments to form an operculum across the buccal cavity when the third maxillipeds are closed (Text-fig. 1A). Plumose hairs (setae) fringe the edges of the third maxillipeds, making the appendage more effective as an operculum.



TEXT-FIG. 1. The buccal cavity of *Uca*, ventral view. **A.** The maxillipeds, with the left third maxilliped removed. **B.** Right first maxilliped, maxillae and mandibles. **C.** Right second maxilla, first maxillae, with mandibles in detail. **1**, third maxilliped endognathal palp; **2**, third maxilliped exopodite; **3**, third maxilliped endopodite; **4**, first maxilliped flagellum; **5**, first maxilliped endopodite "flap"; **6**, second maxilliped exopodite; **7**, second maxilliped endopodite (meropodite segment); **8**, first maxilliped coxal endite; **9**, first maxilliped basal endite; **10**, first maxilliped exopodite; **11**, first maxilliped endopodite; **12**, mandible; **13**, second maxilla basal endite; **14**, second maxilla coxal endite; **15**, first maxilla; **16**, anterior lobe of labrum; **17**, mandibular palp (endopodite); **18**, first maxilla basal endite; **19**, first maxilla coxal endite; **20**, month region.

The segmentation of the second maxilliped is similar to the third, though the two basal segments are now united. The endopodite is composed of the five typical segments, but in contrast to the third maxilliped the ischiopodite is fused with the basipodite, and the meropodite is greatly enlongated (Text-fig. 1A). The terminal segments of the endopodite extend medianly from the meropodite, with flexibility similar to those forming the palp of the third maxilliped. The exopodite of the second maxilliped also corresponds with that of the third maxilliped.

The first maxilliped deviates from the general form of the other two maxillipeds as large inward-projecting lobes, or endites, extend from both the coxopodite and basipodite (Text-fig. 1A, B). Short bristles completely cover the outer surfaces of the endites to form a continuous brush-like surface across the lower half of the buccal cavity. The segments of the endopodite extend more along the sides of the cavity, with the meropodite elongated, as in the second maxillipeds, but narrower. A comb, made up of closely set, stout hairs, extends from this segment to lie beneath the bristled endite lobes. The hairs achieve the appearance of a comb due to their close and regular spacing, which is maintained along much of their length by numerous setules along each hair that serve to hold them together on one plane. The fourth joint of the endopodite is again bent, making the terminal segments extend across the buccal cavity. However, in the first maxillipeds, the terminal segments of the endopodites are broad and flattened, so that they appear as flaps. Defining the top of the buccal cavity, the flaps serve to concentrate mineral and food material in the vicinity of the mouth parts. Also, being heavily fringed with plumose hairs, they prevent particulate matter from entering the gill cavities by way of the excurrent openings, which are located immediately above. The exopodite of the first maxilliped is parallel to the endopodite, as described for the other maxillipeds. The terminal flagellum lies, along with the other flagella, across the upper surface of the wide distal portion of the endopodite (Text-fig. 1B). Together, the three pairs of exopodite flagella function to prevent material suspended in the water from entering the excurrent respiratory opening, for by rapid, upward-flicking motions, the flagella can set up currents in the water which carry away such suspended material.

The second maxilla is distinct in form in that a pair of bifid endites extends medianly from the coxopodite and basipodite. The lower, or coxal, endite takes the form of narrow finger-like projections which are relatively devoid of hairs, while the upper, or basal, endite appears as one large lobe, though its primitive bifid condition is noted by a short slit which extends into the endite from its median edge (Text-fig. 1C). A comb, very similar to that of the first maxilliped, arises from the arched base of the coxal endite and extends beneath the bifid endite. The comb, with the endite, underlaps a portion of the first maxilla, while the upper endite lies under the first maxilla basal endite. The endopodite of the second maxilla is present only as a tiny spiked remnant extending from the proximal part of the upper endite. The exopodite has combined with the epipodite to form the long, flattened lobe called the scaphognathite. The lobe extends laterally into the gill chamber, where it acts as a pump, moving respiratory water as the second maxilla oscillates laterally.

On the first maxilla, a relatively large lobe extends medianly from an arm of the coxopodite (Text-fig. 1C). The lobe is covered with fine, short hairs, except at its median edge where the lobe curves strongly upwards toward the mouth; there it is fringed with a few rows of stubby, stout bristles. These bristles are set so that they point upwards at a slight angle towards the mouth. Another arm extends from the coxopodite of the first maxilla, which is regarded as the basipodite. This segment also bears an endite, which underlaps the mandible. The endite is slightly curved to fit the contour of the mandible, and its median edge is armed with stubby, stout bristles similar to those of the coxal endite. The small endopodite appears to be rudimentary here, while the exopodite and epipodite are absent from the first maxilla.

The mandibles extend across the upper portion of the buccal cavity with broad quadrate basal parts carrying the large gnathal lobes which project underneath the mouth. In *Uca*, the biting edges are toothless, giving the gnathal lobes a blunt appearance. However, the lobes narrow down to thin, sharp edges as they curve inwards and the upper side becomes somewhat hollowed. Thus it is possible that the mandibles may be utilized for mastication. A three-segmented palp, made up of the terminal joints of the endopodite, extends along the anterior edge of the gnathal lobe and then turns downward to lie in the hollow above the biting edges of the mandible (Text-fig. 1C). Siebold has suggested that the palps serve as tactile organs for the mandibles. Neither the exopodite nor the epipidite are represented in this appendage. A pair of paragnaths lies above and close to the mandibles and immediately over the mouth. They are flat elongated lobes which project forward from the posterior ends of the lateral mouth folds. They have no musculature, according to Snodgrass (1950), though with their fringe of short plumose hairs they appear as rudimentary mouth parts.

The mouth is a distensible opening above the lower portion of the mandibles. From the top of the mouth arises a triangular - shaped fleshy upper lip, called the labrum, which comes to lie beneath the mouth. A portion of the labrum also projects anteriorly above the mandibles as a small triangular lobe. The two lower sides of the lobe are grooved in such a fashion that the mandibular palps fit into it as they curve around the anterior edge of the mandibles (Text-fig. 1C). Thus, any vertical movement of the palps will cause a corresponding movement of the labrum, an upward movement lifting the labrum away from the mouth opening, while a downward movement serves to partially close the opening. Otherwise the mouth is without modification, but the elastic tissue quickly coalesces to form the tubular esophagus.

THE FEEDING MECHANISM

In feeding, the fiddler crab scrapes the surface of the substratum with the minor chela. Among the mineral particles scooped up are detritus, algae, bacteria and perhaps nematodes. The minor chela carries this material up to the buccal cavity and passes it between the slightly gaping third maxillipeds. Within the buccal cavity, the food material is selected for ingestion and then passed on to the mouth. Unsuitable material is passed to the bottom of the buccal cavity and permitted to fall out between the third maxillipeds. This is mainly large inorganic particles. Ingestion of a small amount of coarse mineral material is apparently not harmful to the crab, for it can be rejected by the cardiac stomach if need be. However, the presence of a large quantity of coarse material, such as sand grains, may interfere with the preparation of food material for digestion by the small, closely-set teeth of the gastric mill. Such a condition does not appear to be present in crabs feeding on a muddy substratum, for it is unlikely that fine, ingested silt particles would interfere with the functioning of the gastric mill. Thus, although there is little selection against such fine inorganic particles during feeding, as evidenced by the large percentage of silt in the fecal pellets of mud-feeding crabs, selection does occur among crabs inhabiting areas having a coarse substrate.

One method by which food material is sorted from the coarse mineral fraction is described by Altevogt and termed the flotation process in the present discussion. It involves introduction of water into the buccal cavity to float light food and silt particles free from the heavier material. The lighter fraction is suspended in water and held between the mouthparts, especially between the maxillipeds, by capillary action, while the heavier particles are washed to the lower part of the buccal cavity. At the base of the third maxillipeds, outside of the buccal cavity, the rejected material forms a fluid ball which is subsequently removed by the minor chela.

The water required for the flotation process is pumped from the gill cavity out through the excurrent opening by action of the scaphognathite. Flooding the buccal cavity, the water flows by gravity around the first maxilliped endopodite flaps and over the mouth parts. The reverse beat of the scaphognathite pulls a portion of the water back from the buccal cavity, while the remainder can return by way of the incurrent opening at the base of the branchiostegite. As the water is withdrawn, many of the fine, suspended particles adhere by capillary action to the mouth part surfaces, particularly to those covered with plumose hairs. Also, fine particles adhere to the two pairs of comb surfaces within the buccal cavity. The remainder of the suspended particles will be retained either by the plumose hairs fringing the distal flaps of the first maxilliped endopodites as the respiratory water is further withdrawn anteriorly, or by a similar fringe at the base of the third maxillipeds if the water passes out by that route. Experimentally, the flow of respiratory water into the buccal cavity can be stimulated by placing a flake of pablum on the first maxilliped endites. This reaction may be a result of weight on the bristles of the endites, or perhaps the bristles have gustative sensory properties.

This sorting process, which utilizes the difference in weight between the fine food material and coarse mineral matter as a criterion of selection, is supplemented by the coordinated actions of the mouth parts. These appendages hasten sorting by passing the food accumulated on their various combs and hair-covered surfaces on to the mouth, as well as by aiding to rid the buccal cavity of the larger and heavier material. The mouth parts also appear to be capable of freeing food material from the coarse particles, which increases the efficiency of the sorting process.

Food material is passed between the third maxillipeds and into the buccal cavity by the minor chela at a point near the tips of the endopodite palps. The tips of the palps lift outward and the endopodite plates swing open and lower slightly to make room for the food behind their broad, curved surfaces. The palps then return to their normal position and the endopodite plates partially flex closed, pressing the material up toward the first maxillipeds. The palps, aided by long hairs projecting from their tips, may also assist in pushing the material upwards.

Sorting by the mouth parts begins as the material is moistened by respiratory water. The particular role of the first and second maxillipeds in the feeding process varies with the type of substratum on which the crab feeds. *Uca pugilator* typically inhabits protected sandy tidal areas. When the crab feeds on the sandy substrate, relatively little food material is placed in the buccal cavity by the minor chela in proportion to the number of sand particles. Also, much of this food material clings to the sand grains, rather than being freely suspended in the interstitial water. Thus the feeding process involves more than merely a separation of the heavy mineral fraction from the lighter material, as is achieved by the flotation process. Rather, if the crab is to obtain a very large amount of food, it must be capable of removing from the mineral particles any food material which clings to them. The efficiency of the feeding process depends on the thoroughness and rapidity with which this is achieved.

The cleaning of adhering food material from sand grains is achieved in part, in *U. pugilator,* by modification of the bristles on the basal endites of the first maxillipeds. In general, the bristles are considerably enlarged, appearing quite stout and stubby. Toward the more median half of the endite they are broadened and flattened, with two edges of the bristles lobed from the base. The lobes curve inwards slightly to make the bristles cupped. Thus, the first maxillipeds of *U. pugilator* present an irregular surface against which sand grains may be scraped and food consequently removed as the second maxillipeds sweep the grains toward the sides of the buccal cavity. The bristles on the upper portion of the endites are set with their broadened surfaces perpendicular to the arc circumscribed by the second maxillipeds, permitting food material to accumulate on the broad, cupped surfaces as it is freed from the sand. The bristles on the lower portion of the endites have their cupped surface turned upwards, which enables them to catch any fine material washed down by the flotation process. In addition the modified bristles also serve to brush fine material from the first maxilliped endopodite combs which underlie them.

The cleaning process is also facilitated by similar modifications of the tips of some of the hairs on the meropodite of the second maxillipeds. These hairs are generally referred to as spoon-tipped. A spoon shape is achieved by the tips broadening and becoming shallowly cupped as the lobes near the tip arch inwards. There are five or six large lobes fringing two sides of the hair near the tip, in addition to a wide terminal lobe which is turned down over the tip to complete the cup. As the hair narrows proximally, it is fringed by several small lobes which grade into a series of serrate projections that soon become a mere fringe continuing along two sides of the shank for approximately half its length. These modifications make the hairs capable of effectively drawing large particles across the first maxilliped endites.

Though some spoon-tipped hairs are present on the second maxilliped meropodites in all species of *Uca* considered here, it is in *U. pugilator* that the highest degree of hair-tip modification is found. This is true both in the number of hairs which are spoon-tipped, and in the size of the modified tip. Almost all the medianly-projecting hairs of the meropodite in *U. pugilator* are modified (Plate I, Fig. 1). The short hairs covering the surface of the segment create a surface of stout, cupped bristles very similar to that described on the first maxilliped basal endites. This surface extends beyond the inner edge of the segment as the hairs become longer, with the more median hairs of the meropodite projecting well into the buccal cavity. As the hairs increase in length, the depth of the spooned portion and the extent of lateral lobing increases slightly. Proceeding posteriorly along the length of the meropodite, the hairs in each row decrease in length so that the spoon-tips are arranged in diagonal rows, which places the broadened hair-tips closely together to produce a continuous surface. While the spoon-tipped hairs extending from the second maxilliped meropodite of *U. pugilator* vary in number among individuals, it may be generally stated that there are close to one hundred spoon-tipped hairs which project beyond the inner edge of the meropodite in the smaller crabs. In the larger individuals over two hundred such hairs may be present on the meropodites. The shorter modified hairs, which do not project beyond the inner edge of the meropodite, range in number from seventy to one hundred in this species.

The spoon-tipped hairs on the second maxilliped of *U. pugilator* are believed to function primarily in the feeding process. Although some of the sand taken into the buccal cavity will fall to the base of the third maxillipeds because of its weight, as the cavity is flooded with respiratory water, many of the grains become lodged among the bristles of the first maxilliped endites, particularly the long bristles fringing the coxal endite. It is this sand which is picked up between the modified hair-tips of the meropodite and swept across the bristled endites of the first maxillipeds, resulting in rapid and efficient removal of adhering food material. The sand grains are usually too large to be carried individually by a spoon-tip; instead, a grain is caught between neighboring spoon-tips, where it is firmly held as the close mat of broadened tips exerts pressure from the sides, and the stiff underlying hairs prevent the grain from falling through the mat. Thus, the spoon-tipped hairs, when placed closely together, appear to contribute to the feeding process by providing an enlarged surface of stout hair-tips which can prevent sand grains from being forced up into or through the meropodite hairs when the second maxilliped moves across the bristles of the first maxilliped endites. Such a surface appears necessary in crabs which feed on a sandy habitat, for with loss of sand grains from the surface of the bristled endites only a small percentage of sand would be cleaned and little food material would be recovered. Food freed by the cleaning process is of a very light and filmy texture, which readily adheres to the modified bristles of the first maxilliped endites and the fringes and spoon-tips of the second maxilliped meropodite hairs. Little food accumulates in the spooned portion of the second maxilliped hairs, but it is apparently passed directly to the first maxilliped endites as the meropodite moves over them.

In addition to facilitating the cleaning of organic material sand grains, the sweeping action of the second maxillipeds across the buccal cavity also serves to carry coarse particles away from the central portion of the cavity. This can be considered as another general role of the second maxillipeds and of the spoon-tipped hairs in feeding. Once the particles have been carried to the sides of the buccal cavity, the meropodites lift away from the first maxilliped endites, which permits the sand to fall from the appendages as a vibratory action of the second maxillipeds and the flooding of the buccal cavity with respiratory water provides impetus to free the grains from the hair surfaces. The utilization of water to remove coarse particles from the buccal cavity is suggested by the fluid consistency of the ball of sand which accumulates at the base of the third maxillipeds for discard.

On the maxilliped meropodite of the marsh fiddler crab, *U. pugnax,* fewer of the medianly-projecting hairs are spoon-tipped, as compared to the sand-inhabiting species, *U. pugilator*. Also, in *U. pugnax* the modified hairs are limited to the upper portion of the meropodite (Plate I, Fig. 2). There are several rows of shorter hairs which do not project beyond the edge of the meropodite and which are well-lobed and broadened, but they are of a soft texture, which makes their effectiveness in manipulation of material questionable. However, there are five or six additional diagonal rows of stout, spoon-tipped hairs which extend beyond the meropodite, of a size and shape very similar to the spoon-tips in *U. pugilator*. The thirty to forty modified hairs in *U. pugnax* are not as closely grouped as in *U. pugilator*, somewhat decreasing the effectiveness of the narrow band of spoon-tips in picking up material. The band of spoon-tipped hairs does coincide in position with the upper edges of the first maxilliped endites in *U. pugnax*. Thus, the second maxillipeds appear to aid the feeding process primarily by picking up coarse particles from those edges of the endites and sweeping them away from the central portion of the buccal cavity. It is doubted whether as much food material is scoured from the coarser particles as a consequence of this action as in *U. pugilator*, for the bristles covering the first maxilliped endites of *U. pugnax* are not stout, but have a fine, downy texture.

The thoroughness with which the crabs are able to sort food from the mineral fraction of the substratum corresponds to the availability of food material. On the sandy beach inhabited by *U. pugilator*, while some detritus and nematodes are present in the interstitial water, much bacteria and algae is on the surface of the grains. This crab appears to ingest a minimal amount of sand and the food must therefore be separated from the mineral fraction. For the marsh-inhabiting *U. pugnax*, on the other hand, food is more readily available because the silt with which it is generally associated is of sufficiently fine texture to be ingested.

In *U. minax*, modification of the second maxilliped hairs occurs to a much lesser extent than that observed in either of the other two species. There are very few short hairs present along the upper surface of the meropodite and they are mostly limited to its edges. The modification observed in many of the medianly-projecting hairs consists of a feathering of two sides of the hair, the increase in surface adapting it to handle fine particle matter. In addition, in the larger crabs, many of the longer medianly-projecting hairs are hooked at their tip. In the more medium-sized crabs (of approximately 13.0 mm. to 19.0 mm. carapace width) these hair tips are microscopically flattened, with delicate lobes fringing the two sides of the flat surface near the tips, which then blend proximally into the serrulate projections that feather the shaft. Also, among the medium-sized crabs, several of the longer hairs of the meropodite are spoon-tipped, although in *U. minax* the spooned portion is much smaller and accordingly more shallow than those previously described (Plate I, Fig. 3). The spoon-tipped hairs extend medianly from the upper portion of the meropodite as three or four diagonal rows, each consisting of five modified hairs. These fifteen to twenty-four spoon-tipped hairs form a narrow band in the same position under the first maxilliped endites as in *U. pugnax*. However, in *U. minax*, the spoon-tips are widely spaced and are believed capable of doing little more to supplement the manipulation of material than what is typically achieved by the medianly-extending rows of unmodified hairs.

The presence of only a few modified hairs in *U. minax* is significant in view of the greater availability of food in the high marsh. Since *U. minax* can readily obtain the food associated with the finely sorted muddy substratum or on partially decayed marsh vegetation, there is neither a need for a highly efficient method of sorting food nor a systematic means of ridding inorganic material from the buccal cavity. It is probable that the medianly-projecting hairs contribute to the feeding process merely by dispersing material across the endites of the first maxillipeds, whereby a larger percentage of the particles within the buccal cavity are exposed to washing and respiratory water and food material is more readily separated for ingestion.

In all three species of *Uca* considered here, the last two segments of the second maxilliped palps are fringed with stout spoon-tipped hairs along their lower edge. The modified hairs of the terminal segment have their cupped surfaces facing downwards, while the spoon-tipped hairs of the propodite have the cupped surfaces turned inwards. So placed, the short, well-lobed hairs make the flexible palps very effective in manipulating material within the buccal cavity. In addition to facilitating removal of material from among the mouth parts, these hairs also enable the palps to collect food material which is accumulated on the hairy surfaces of the mouth parts, such as on the first maxilliped endites, or the fringes of plumose hair on the outer edges of the second maxillipeds, where it is deposited after the flotation process. The palps then carry the food to the maxillae, where it is closer to the mouth. The spoon-tipped hairs are conspicuously abundant on the palps of *U. pugilator*, while in the other two species they are somewhat fewer in number and less deeply curved. Yet the basic arrangement of these spoon-tipped hairs remains fairly consistent among the three species, indicating the importance of the palps in performing these functions in the feeding process, regardless of the character of the substratum on which the crab normally feeds.

The second and first maxillae work together to accumulate the finer material for ingestion. The upper pairs of endite lobes of the second maxilla appear to serve primarily to retain food material as it is sorted from the coarse particles, for they are abundantly covered with plumose hairs of moderate length. Underlapping these second maxillae endite lobes are several spoon-tipped hairs which project from the anterior edge of each first maxilliped basal endite. It appears that the hairs are modified primarily to function as surfaces against which the plumose surfaces of the second maxilla can rub, for the lateral movements made by the maxilla as the scaphognathite moves within the gill cavity do move the basal endites against these first maxilliped hairs. With this action, any fine material caught in the plumose hairs of the basal endite of the second maxilla will be carried toward the median edge of the segment, where it is in a position to be passed on toward the mouth. There are also a few hairs which project anteriorly from the second maxilla, which are similarly hooked. Likewise, these hairs would move fine material to the median edges of the first maxilla basal endites which lie above them. There is little species difference in the form or arrangement of the hair modifications discussed here.

Food material is removed from the median edge of the second maxilla basal endite as the segment moves posteriorly and upward, scraping its plumose surface against the anterior edge of the coxal endite of the first maxilliped. This action places the fine material above the first maxillipeds and in a position to be carried to the maxillary combs by respiratory water. In addition, food can be passed from the first maxilla basal endites to the tufted edges of the mandibular palps, as the mandibles move laterally above the endites. This material also appears to be carried from the palps to the maxillary combs as the mandibles are washed with respiratory water.

The combs of the second maxillae curve medianly from the base of the coxal endites, closely adhering to the bristled, convex surface of the first maxilla coxal endites. The combs appear to serve as surfaces to retain material carried into the upper portions of the buccal cavity by the flotation process. The maxillary comb and brush surface of the coxal endite of the first maxilla appear to perform the same function as that described for the first maxillipeds; however the comb and brush surfaces of the maxillae appear to be capable of handling finer material since the hairs of these surfaces are set closer together. The fine food material and silt on the combs are transferred to the median edges of the first maxilla coxal endites as the endites brush the convex combs beneath them. As food material accumulates near the median edge of the bristled surface, the first maxilla can move forward a short distance, placing the inner edge of the coxal endite, and the food material, in the mouth.

DISSCUSSION

Certain aspects of the feeding mechanism of *Uca* help to explain the ecological limitations imposed upon the crabs by their mode of feeding. Analysis of the feeding mechanism shows two general processes to be involved: flotation, and coordinated action of the mouth parts. Considering the flotation process, it is instructive to examine the copious use of water from the gill cavities of the crabs in light of water conditions prevailing in their respective habitats. After the flooding of the buccal cavity with respiratory water, some water is returned to the gill cavities, either via the excurrent openings as the scaphognathite reverses its direction of movement, or by way of incurrent canals, located at the base of the branchiostegite above the coxa of the chelae. The latter route was suggested by Altevogt. However, some water is lost as food is ingested, as well as when material is discarded from the buccal cavity. Additional water is lost by evaporation while the mouth parts are exposed to the air. Thus, to continue feeding, the crab must have access to an external supply of water in order to replenish its respiratory water. This factor is important in limiting the areas where the crabs may feed, and is reflected by the moist condition of the material from which they prefer to feed. Of more general significance, the inclusion of the flotation process as an integral part of the feeding mechanism is one major factor preventing *Uca* from living in a terrestrial habitat.

Among the marsh-inhabiting fiddler crabs, the requisite of standing water for the flotation process affects its distribution little, other than limiting the crab to the intertidal areas, for within that area the substratum remains moist and water is available even during low tide in drainage depressions and in the depression surrounding the burrow entrance. *Uca* can readily replenish its respiratory water from such small pools by lowering the thorax into the water, submerging the incurrent canals at the base of the branchiostegite. *U. pugnax* has been observed to stray from its burrow while feeding and to lower its thorax to take up additional water, only to find the moisture insufficient. Immediately the crab returned to its burrow, where it could take up water, and then once again it began feeding. This relationship between the burrow and a source of respiratory water may be a key factor contributing to the high sense of burrow-centered territoriality which has been observed in *U. pugnax*. That such territoriality has not been observed with *U. minax* is in agreement with its preference for feeding in the muddier portions of the marsh, away from the burrowing area.

In the sandy areas inhabited by *U. pugilator,* the need of water for the flotation process has a more pronounced effect on the movements of the crab. As well as being limited to the moist intertidal portion of the protected beach, the crab may also be required to move to the water's edge to feed, should the beach elevation at the burrowing area be too great for water to remain in the burrow during low tide. Both the tidal magnitude, as influenced by the phase of the moon and the season of the year, and the contour of the beach, will affect the water level, and thus be factors contributing to vertical movements of the crab during feeding.

Ecological considerations may be deduced by examining the role of the mouth parts in feeding. Generally speaking, the mouthparts manipulate material within the buccal cavity by two coordinated actions: that which passes food material toward the mouth and one which removes coarse material from the central portion of the buccal cavity. Species differences are observed in the hairs covering the mouth parts in *Uca*, which reflect the relative importance of the several mouth part actions in the feeding process of each species. Since the majority of these mouth part differences can be correlated with the type of substrate on which the crabs typically live, they can be looked upon as modifications which enable the crabs to feed in that habitat.

Of the three fiddler crabs considered here, the mouth part hairs of *U. minax* appear to be the least modified, it being assumed that the undifferentiated hairs are the more primitive. This species typically lives in the mature *Spartina* marshes, well up the estuary where lower salinities are experienced. Field studies indicate that *U. minax* prefers to feed in low areas where the mud is very fluid. The crab also feeds extensively on bacterial slime on decaying plant material, which is abundant throughout the marsh. The material carried to the buccal cavity by the minor chela is generally of such a fine texture that little sorting would be required by the mouth parts before ingestion could take place. Respiratory water is still pumped into the buccal cavity, however, where it doubtless serves as a solvent aiding in the dispersal of the fine material to the various mouth parts. Once on these hair-covered surfaces the mouth parts pass the fine silt and food material upwards to the mouth.

Should material be rejected from the buccal cavity during feeding in *U.minax,* rejection occurs immediately after the material is placed within the cavity, and all is discarded. Since rejection takes place without sorting, the palps of the second maxillipeds, rather than respiratory water, would serve to direct the material from the buccal cavity. The palps also aid in the manipulation of particles within the cavity, for which they are particularly adapted due to the arrangement of the spoon-tipped hairs on the terminal segments. A similar modification occurs with the longer, medianly-extending hairs of the meropodite, although here it is limited to a minute hooking or flattening of the tip, which is fringed by delicate lobes and setules. These long hairs may increase the efficiency of the sorting process by spreading the material across the first maxilliped endites, where it will be more accessible for washing by respiratory water, as well as providing another surface on which fine material may accumulate.

The presence of flat-tipped hairs on the second maxilliped meropodites in *U. minax* is felt to be a modification serving to increase the efficiency of the sorting process and to enable crabs to invade and survive in intertidal areas having a coarser substrate, where food material is not readily available. A slight advancement in this direction is seen with the modification of a number of hair tips to a deeply spooned shape in *U. pugnax,* with an accompanying ability of the crab to feed in sandy portions of the marsh. The modified hairs, which may approach thirty in number, extend from the upper portion of the second maxilliped meropodites, where they are effective in removing coarse material from the upper edges of the first maxilliped endites and withdrawing material from the central part of the buccal cavity. The spoon-tipped hairs present on the second maxilliped palps are similarly arranged in both *U. pugnax* and *U. minax,* as is also the luxuriant fringe of plumose hairs on the outer edges of the second maxillipeds and on the maxillae, which basically adapts *U. pugnax* to feed on a muddy substrate.

The greatest modification of the hairs of the mouth parts appears in *U. pugilator*. Here the terminal lobing of the spoon-tipped hairs is more pronounced, making the tips more rounded and deeply cupped, which, coupled with the greater number of spoon-tipped hairs present, greatly increases the efficiency of the mouth parts in handling coarse material. The most striking surfaces of closely set spoon-tips are those formed by the hairs extending medianly from the meropodite of the second maxillipeds. The number and size of the spoon-tipped hairs of the second maxilliped palps are also increased in *U. pugilator,* making those two opposing surfaces of spoon-tipped hairs on the dactylopodite and propodite more effective. Thus the palps further supplement the action of the meropodite in manipulating material to be discarded from the buccal cavity, as well as carrying accumulations of food to the maxilla for passage to the mouth. This efficiency in handling coarse material within the buccal cavity enables the crab to feed in such habitats as the protected sand beach.

In addition to the necessity of selecting against coarse mineral particles, another problem which appears to confront fiddler crabs living in a sandy habitat is the paucity of available food material, for there the organic matter is closely associated with the coarse sand. In such a form, the food material is not as readily available for ingestion as is the organic matter in the marsh, which is associated with fine silt. Thus, a beach-inhabiting fiddler crab must also be capable of efficiently separating from the sand a large percentage of the food material which is taken into the buccal cavity. In *U. pugilator,* the efficiency of the sorting process is increased by a cleaning action which involves the drawing of sand particles over the bristled surface of the first maxilliped basal endites by the second maxillipeds. The bristles of the basal endites are modified in *U. pugilator* in a manner similar to that of the tips of many of the hairs on the second maxillipeds. Such enlarged bristles projecting perpendicularly from the endites offer a brush-like surface against which sand grains may be scraped and food material removed as the second maxillipeds sweep the grains across the endites. The bulk of the freed food is then retained on the shallowly-cupped endite hairs, which are placed in such positions as to catch the maximum amount of loose food material which may be washed toward the base of the buccal cavity by respiratory water. The closely set spoon-tipped hairs of the second maxilliped meropodites facilitate this cleaning process by providing a sufficiently rigid surface to achieve efficient scouring action on the sand grains as they are carried across the first maxillipeds.

Distribution of *Uca* is generally believed to be governed by a complex of physical and biotic factors, operative during the pelagic developmental stages of the crab, as well as after it has invaded the intertidal areas. However, in considering the influence of the observed modifications of the mouth part hairs on the distribution of *Uca,* only the adult crabs and their intertidal habitat need be examined, for it is only in the adult stage that the mouth-part hairs are well developed, and thus fully functional. In *U. minax,* the condition of the mouth-part hairs indicates that they are not able to sort food material from coarse inorganic matter with any efficiency. This leads to the inference that the crab would be able to survive only in areas where it has access to an abundance of food material on the surface of a silty substratum, such as in the salt marsh. However, *U. minax* does not inhabit all portions of the tidal marsh, but it is generally restricted to the high regions, where a dense mat of *Spartina alterniflora* roots and rhizomes impart great stability to the substrate. In view of this restricted distribution within the marsh, though nutritive conditions appear fairly consistent throughout, there are doubtless limiting factors other than an inability of the crab to obtain food. It is probable that the stability of the substrate, which differs from that of the lower marsh, is critical for the crabs' burrowing activities.

The more advanced modification of some of the hairs on the mouth appendages in *U. pugnax* does appear to be an adaptation permitting the crab to be more widely distributed. This is demonstrated by the crabs' presence in the sandy areas of young marshes. There feeding is facilitated by the band of modified hairs extending from the upper portion of the second maxilliped meropodites, which can manipulate coarse material in the central region of the buccal cavity. The inability of *U. pugnax* to survive on protected sandy beaches, however, where surface food material is less available, reflects on the unmodified condition of the first maxilliped basal endites and the wide spacing of the spoon-tipped hairs present on the second maxilliped, which limits the efficiency with which food can be cleaned from the coarse grains. Thus the lack of modified hairs is one factor limiting *U. pugnax* to marshy areas, where food material can be more readily obtained.

The ability of *U. pugilator* to obtain sufficient food from its sandy habitat is seen to lie principally in the highly modified condition of the first and second maxillipeds. However, it is difficult to believe that this structural adaptation renders the crab incapable of surviving in marshy areas, particularly when its presence in the sand-fringed young marsh is considered. There *U. pugilator* inhabits primarily the high sand rim when it is flooded by a spring tide. During the neap tides, when the high sandy areas remain dry, the crab moves into the siltier areas of the marsh where the surface substratum may contain only 40% sand. With the next spring tide, a large portion of the population returns to the sand fringe, demonstrating an apparent preference for the sandier areas. Population pressure may play a role in this latter movement, for when *U. pugilator* is in the silty marsh areas, it shares the space with *U. pugnax.* But more generally, the ability of *U. pugilator* to feed with efficiency in a sandy area is looked to as a basis for this preference. When the crab feeds from a sandy substrate, the inorganic fraction of the material passed into the buccal cavity is of sufficient size that it may be readily discarded during the sorting process, and little inorganic material is ingested. This, coupled with the effective cleaning process in *U. pugilator,* indicates that material of a relatively high total nutritive value is ingested during feeding. In the marsh, however, the efficiency of the feeding process is diminished, since the silt with which the organic matter is associated is of too fine a texture to permit separation of the food material. This is further borne out by examination of the fecal material of *Uca,* which indicates that these fiddler crabs do not remove fine silt from the organic matter. Therefore, in the marsh the nutritive value of the material ingested by the crab is reduced in comparison to that of sand-inhabiting species. While observations show *U. pugilator* to be capable of surviving in areas with little sand in the substratum, the mouth part modifications which have aided it in surviving in sandy areas have also exposed it to such advantageous nutritive conditions that the sandy habitat has become the more favorable. However, since this factor appears to be more of a preference than an absolute requirement, there are probably other factors, such as the consistency of the substratum for burrowing, which prevent *U. pugilator* from living in the marsh.

SUMMARY

1. A study has been made of the feeding mechanism of three species of fiddler crabs: *Uca pugilator, U. pugnax* and *U. minax.*
2. Fiddler crabs feed by scraping organic matter from the surface of the substratum, with subsequent separation of food material from the coarser fraction within the buccal cavity.
3. The two basic aspects of the feeding mechanism are the coordinated actions of the mouth parts and a flotation process which utilizes water from the gill cavities.
4. Species-specific modification of the hairs which cover the mouth parts are described and correlated with the characteristic substratum on which each species feeds.
5. The distribution of the fiddler crabs is discussed, with consideration of ecological adaptations of the feeding mechanism within each species.

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EXPLANATION OF THE PLATE

Plate I

Fig. 1. Median portion of the right second maxilliped meropodite, *U. pugilator,* dorsal view.

Fig. 2. Median portion of the left second maxilliped meropodite, *U. pugnax,* dorsal view.

Fig. 3. Median portion of the right second maxilliped meropodite, *U. minax,* dorsal view.

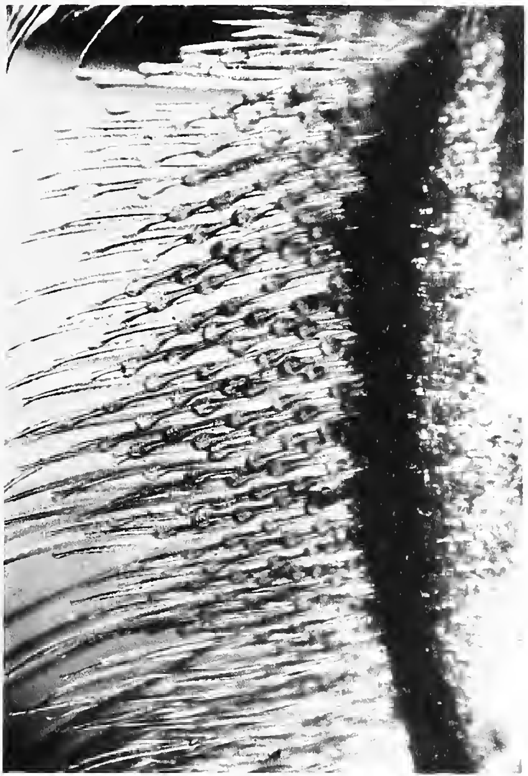

FIG. 1

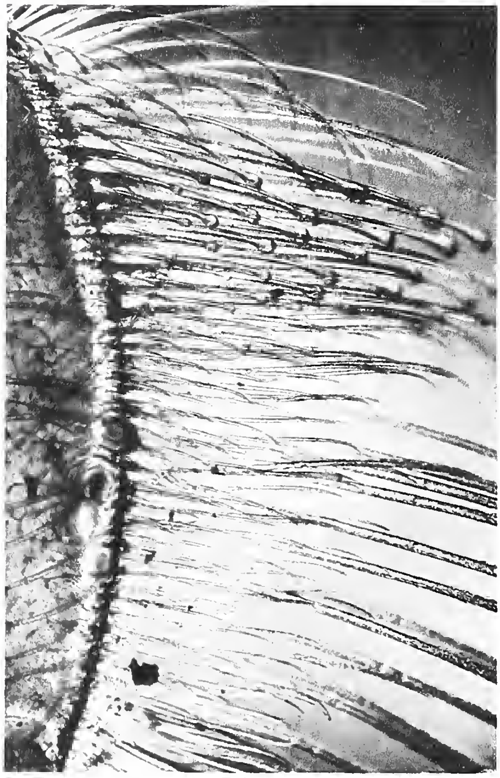

FIG. 2

FIG. 3

THE FEEDING MECHANISM OF FIDDLER CRABS, WITH ECOLOGICAL CONSIDERATIONS OF FEEDING ADAPTATIONS

9

Hybridization Experiments in Rhodeine Fishes (Cyprinidae, Teleostei). Intergeneric Hybrids Obtained from *Acheilognathus lanceolata* × *Rhodeus amarus* and *Rhodeus amarus* × *Acheilognathus tabira*

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(Plate I)

In a previous publication[1] the successful intergeneric hybridization of female *Rhodeus ocellatus* (Kner) and male *Acanthorhodeus atremius* (Jordan & Thompson), both of Japanese origin, was reported. All 15 hybrid specimens obtained were males. A number of these were crossed back to the maternal species, *R. ocellatus*, and the offspring obtained from this combination consisted of interfertile males and females. This paper deals with results obtained by crossing (1) female *Acheilognathus lanceolata* (Temminck & Schlegel) of Japanese origin with male *Rhodeus amarus* (Bloch) of western Europe, and (2) female *Rhodeus amarus* with male *Acheilognathus tabira* (Jordan & Thompson) of Japanese origin. Berg (1948-1949) considered *R. amarus* a subspecies of *R. sericeus*. Recently, however, Holčík (1959) has revised the taxonomic status of the different forms of European bitterling which had been considered to represent only minor variations within the taxon *R. sericeus amarus*. As a result of extensive comparative investigations he proposed to subdivide the group into three lower taxonomic units, "*Rhodeus sericeus amarus amarus* (Bloch)" which inhabits the system of the river Elbe in Germany, "*Rhodeus sericeus amarus danubicus*" which populates the Danube river in Austria, and "*Rhodeus sericeus amarus svetovidovi*" occurring in the Dnepr and Bug rivers in the U.S.S.R. The form that has been used in the present investigation is identical with the form called "*R. sericeus amarus amarus*" by Holčík.

Hybrids between *A. lanceolata* and *R. amarus*

Because of the incompatibility of their spawning behavior, *A. lanceolata* and *R. amarus* are incapable of interbreeding under laboratory conditions with the aid of freshwater mussels. Therefore, artificial insemination was applied in the usual way.

Thirty-four eggs of one female *A. lanceolata* were inseminated with sperm of one male *R. amarus*. Fertilization was complete. All the larvae hatched and 33 of them reached the free-swimming stage within 24 days. Twenty arbitrarily selected fry were raised to the adult stage. A representative adult hybrid specimen is illustrated in Plate I, Fig. 3, together with specimens of the parental species.

All hybrids showed a male phenotype and displayed full nuptial colors in the breeding season. Tubercles were abundant on top of the snout. Spawning behavior, however, was poorly developed. The quantity of milt produced by stripping was very small, and from this it is concluded that spermatogenesis was impaired. All hybrids were fairly uniform in size. With respect to body size they were, however, not intermediate between the parental species. They attained the body lengths of the maternal species, *A. lanceolata*, which is larger than *R. amarus*. The hybrids were more resistant to infectious diseases than either parental species. In their general appearance and liveliness they clearly demonstrated the presence of hybrid vigor.

Hybrids between *R. amarus* and *A. tabira*

In *R. amarus* and *A. tabira*, the patterns of spawning behavior are very similar. Therefore the interbreeding of both species with the aid of freshwater mussels was attempted under laboratory conditions.

One male *A. tabira* and two female *R. amarus* were placed together in an aquarium provided with three South African najads, *Aspatharia wahlbergi* (Krauss). After five weeks, five fry were seen swimming near the surface of the water. Only one specimen reached the adult stage. It is illustrated in Plate I, Fig. 6, together with specimens of the parental species.

The hybrid was more or less intermediate between the parental species and showed a male phenotype. In the breeding season it displayed full nuptial colors and spawning behavior. The red pigment, which is present in male *R. amarus* but lacking in *A. tabira,* was also absent in the hybrid. Tubercles were abundant on top of the snout, but the quantity of milt produced by means of stripping was extremely small.

A taxonomic description of the two hybrid forms recorded here will be published separately.

SUMMARY

With the aid of artificial insemination, intergeneric hybrids between female *Acheilognatus lanceolata* of Japanese origin and male *Rhodeus amarus* from western Europe were obtained. All hybrids were males. They displayed full nuptial colors in the breeding season, but spawning behavior was poorly developed and milt production was impaired.

The interbreeding of *Rhodeus amarus* and *Acheilognathus tabira* from Japan with the aid of mussels was successfully carried out under laboratory conditions. Only one hybrid reached the adult stage, however. It appeared to be a male. In the breeding season it displayed full nuptial colors and spawning behavior, but its production of milt was very limited.

ACKNOWLEDGEMENTS

I am greatly indebted to Prof. Tokiharu Abe and Dr. Yoshitsugu Hirosaki for supplying the species of Japanese bitterling, and to Mr. F. G. du Jardin for making photographs of the illustrated specimens. This investigation was generously supported by the Council for Scientific and Industrial Research of the Union of South Africa.

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EXPLANATION OF THE PLATE

PLATE I

FIG. 1. Female *Acheilognathus lanceolata.*

FIG. 2. Male *Rhodeus amarus.*

FIG. 3. Adult intergeneric hybrid between female *A. lanceolata* and male *R. amarus.* Standard lengths: 74, 56 and 105 mm.

FIG. 4. Female *Rhodeus amarus.*

FIG. 5. Male *Acheilognathus tabira.*

FIG. 6. Adult intergeneric hybrid between female *R. sericeus amarus* and male *A. tabira* Standard lengths: 55, 62 and 75 mm.

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[1] Zoologica, Vol. 46 (2): 25-26, 1961.

FIG. 1

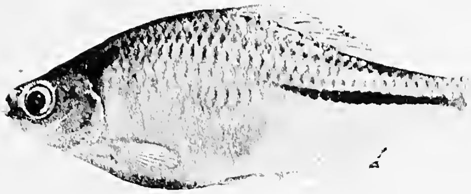

FIG. 2

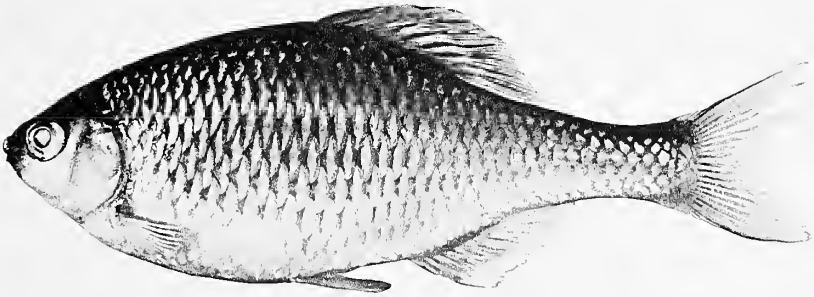

FIG. 3

FIG. 4

FIG. 5

FIG. 6

INTERGENERIC HYBRIDS OBTAINED FROM ACHEILOGNATHUS LANCEOLATA X RHODEUS AMARUS AND RHODEUS AMARUS X ACHEILOGNATHUS TABIRA

10

Some Observations on the Metamorphosis of the Frog *Rana curtipes* Jerdon

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(Plate I; Text-figure 1)

RANA CURTIPES Jerdon is relatively unknown scientifically, but is found in abundance in the thick forests of Dandeli, Londa, Castlerock, Supa, Nagargali, Anmode and other neighboring forest areas along the Kali River on the west coast of India. Tadpoles in various stages of development are often seen swarming together in the Kali River (Kali = black), where their jet black color blends with the black waters of the river.

The frog is often found in small puddles within the forest and seems to prefer the cool water of shady areas. It apparently estivates during the hot season extending from March to May, when all the pools and puddles dry up. In the rainy season (*i.e.*, from June to September) it is seen in large numbers.

Tadpoles collected from various forest areas along the Kali were brought to the Karnatak College laboratory at Dharwar, India, where they were allowed to metamorphose in an aquarium well supplied with algae and other food material.

It was not possible to undertake a detailed study of the development from fertilization of the egg to the emergence of the tadpoles, since the spawn could not be found; neither could early tadpole stages with external gills be collected. Only young tadpoles (Plate I, Fig. 1), jet black in color, are seen swimming in the Kali River in the months of September and October. A young tadpole measures about 2 inches in length. It has a long and coiled gut about 8 inches long. These tadpoles are voracious and are purely vegetarian in diet.

Growth takes place by an increase in size of the tadpole and by a relative elongation of the gut. No other morphological changes take place. The large tadpole (Plate I, Fig. 2) varies in length from 3 to 4 inches. It is very stout and its sluggish nature may be attributed to its feeding habits.

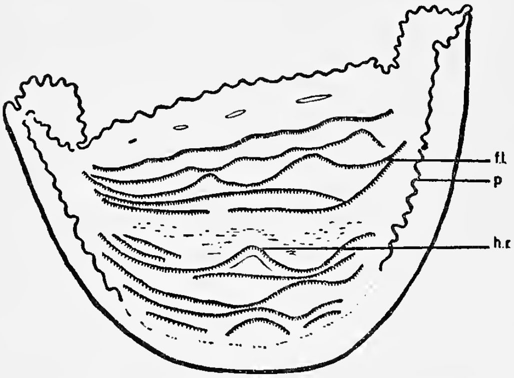
Large tadpoles are blackish-brown with a large head, and a distinct parotoid-like gland is found behind each eye. Posteriorly the long tail is flattened like a fin. The mouth is wide and bears two horny lips and 4 to 6 rows of labial teeth over the dorsal lip (Text-fig. 1). A row of papillae surrounds the mouth parts. Scattered irregularly along the body and tail are black dots, which are less prominent on the head region.

The following table shows the approximate growth of a tadpole from October to January.

Month	Length of Body	Breadth of Body	Length of Intestine	Remarks
October	2″	1″	8″	Small tadpoles
November	3″	1″	14½″	Large tadpoles
December	4″	1½″	15″	” ”
January	4½″	1½″	14″	” ”

No structural modifications occur in the tadpole during these four months, but only an increase in size. The tadpole attains its maximum growth about the middle of January, after which it becomes even less active. About the end of January the hind limbs make their appearance. At this stage the tadpole has a very long tail (2-3″) and the head is about 1 inch long (Plate I, Fig. 3). The gut now shows a reduction in length and measures 14 inches.

About the first week of February the fore limbs begin to bud and early in March the hind and fore limbs are fully formed. The tadpole is still jet black and has a long tail, as seen in Plate I, Fig. 4. The tail is gradually absorbed and a reduction in size of the gut ensues. By the end of March the last traces of the tail have disappeared and metamorphosis is complete.



TEXT-FIG. 1. Mouth parts of tadpole of *R. curtipes.*

The young frog (Plate I, Figs. 5 & 6) has now changed its diet and feeds on insects. The young frog is jet black but it gradually loses the black pigment on the dorsal and ventral surfaces, which take on a brown color as age advances.

The prolonged growth and metamorphosis in the forest-dwelling *Rana curtipes* is peculiar to its kind, and extends over a total period of 9 months, usually from July to March.

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I wish to express by appreciation to Dr. J. C. Uttangi, Dept. of Biology, Karnatak Science College, Dharwar, India, for help and facilities rendered to me in making my observations. Thanks are also due to Dr. R. S. Miller, Dept. of Biology, University of Saskatchewan, Saskatoon, Sask., Canada, for the necessary corrections made in the manuscript.

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EXPLANATION OF THE PLATE

PLATE I

FIG. 1. Small tadpole of *R. curtipes.*
FIG. 2. Large tadpole of *R. curtipes.*
FIG. 3. Tadpole with hind limbs.
FIG. 4. Tadpole with fore and hind limbs.
FIG. 5. Young frog with tail.
FIG. 6. Young frog with reduced tail.

FIG. 1

FIG. 2

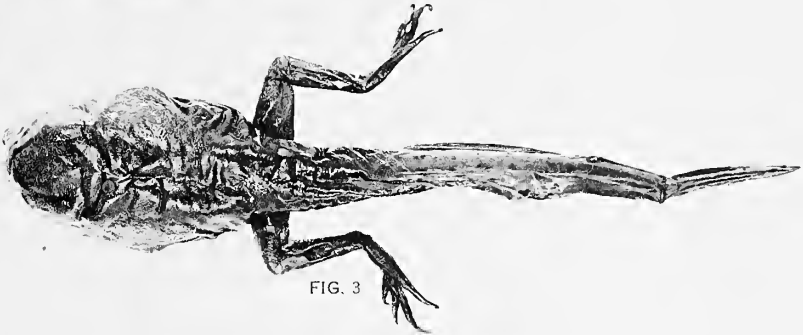

FIG. 3

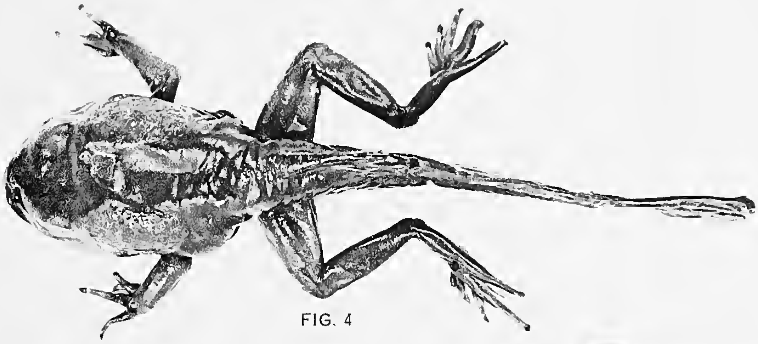

FIG. 4

FIG. 5

FIG. 6

SOME OBSERVATIONS ON THE METAMORPHOSIS OF THE
FROG RANA CURTIPES JERDON

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Contents

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A Study of the Biology and Behavior of the Caterpillars, Pupae and Emerging Butterflies of the Subfamily Heliconiinae in Trinidad, West Indies. Part II. Molting, and the Behavior of Pupae and Emerging Adults[1,2]

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(Plate I; Text-figures 1-3)

[This paper is one of a series emanating from the Tropical Field Station of the New York Zoological Society, at Simla, Arima Valley, Trinidad, West Indies. This station was founded in 1950 by the Zoological Society's Department of Tropical Research, under the direction of Dr. William Beebe. It comprises 200 acres in the middle of the Northern Range, which includes large stretches of undisturbed government forest preserves. The laboratory of the Station is intended for research in tropical ecology and in animal behavior. The altitude of the research area is 500 to 1,800 feet, and the annual rainfall is more than 100 inches.

[For further ecological details of meteorology and biotic zones, see "Introduction to the Ecology of the Arima Valley, Trinidad, B.W.I.," William Beebe, Zoologica, 1952, 37 (13): 157-184.

[The success of the present study is a large measure of the cooperation of the staff at Simla, especially of Jocelyn Crane and Constance Carter, the former contributing much of her knowledge of the animals, the latter helping with recording of observations].

CONTENTS

I. INTRODUCTION

A PRELIMINARY STUDY has been made of the behavior of as many species of larval heliconiines as were available over a period of 4½ months spent in Trinidad, W. I. A comparative account of the general activities, such as feeding, resting, locomotion and social and defensive behavior, has already been given (Alexander, 1961). Observations on the late larvae, however, made it clear that some of the significance of their behavior should be sought in the pupa and emergent adult. Hence descriptions of some of these aspects of heliconiine behavior are presented here as Part II of the report, together with observations on molting behavior throughout larval life.

The information relates to eleven of the fourteen species of heliconiines found in Trinidad, namely *Heliconius doris doris* (Linnaeus), *Heliconius sara thamar* Hubner, *Heliconius erato hydara* Hewitson, *Heliconius melpomene euryades* Riffarth, *Heliconius ricini insulana* Stichel, *Heliconius aliphera aliphera* (Godart), *Heliconius isabella isabella* (Cramer), *Dryadula phaetusa phaetusa* (Linnaeus), *Dryas iulia iulia* (Fabricius), *Agraulis vanillae vanillae* (Linnaeus) and *Dione juno juno* (Cramer).

Descriptions and illustrations of the external characteristics of the larvae referred to here are given by Beebe, Crane & Fleming (1960).

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[1] Contribution No. 1010, Department of Tropical Research, New York Zoological Society.

[2] This study has been aided by a grant from the National Science Foundation (G6376). Financial support from the Royal Commission for the Exhibition of 1851 and the South African Council for Scientific and Industrial Research is also gratefully acknowledged.

TABLE I. MOLTING BEHAVIOR IN SPECIES OF HELICONIINE CATERPILLARS
Records of times abstracted from approximately three cases for each species, but confirm general impressions of molts whose details were not recorded.

Species	Position on vine prior to molt	Pose prior to shedding skin	Time of day when skin normally shed
Dione juno	Stem	Arranged in circle, heads to center, bodies straight	Mid-a.m.
Agraulis vanillae	Midrib/stem	—	Mid-a.m. to mid-p.m.
Dryas iulia	Midrib/stem/ tendril	"J"/straight	Mid-a.m. to early p.m.
Dryadula phaetusa	Midrib	"J"/straight	Noon to early p.m.
Heliconius aliphera	Midrib	Curved	Mid-a.m.
Heliconius isabella	Leaf blade	Straight	p.m.
Heliconius melpomene	Midrib	Straight/head slightly to one side	Mid-a.m. to early p.m.
Heliconius erato	Stem, seldom midrib	Straight	Mid-a.m.
Heliconius ricini	Midrib/stem	?	Mid-a.m.
Heliconius sara	Stem, few on leaves	Straight	Early a.m. to noon

II. MOLTING BEHAVIOR

As in most butterflies, the Heliconiinae typically have four molts separating five larval stages. Six instars may occur occasionally in some species–*H. sara, H. isabella, H. aliphera* and to a lesser extent *H. melpomene* and *D. juno* (Beebe, Crane & Fleming, 1960).

Normal molting is very similar in all species observed and a generalized description will be given first.

During the afternoon before a caterpillar actually sheds its skin, it selects a site and takes up a position ready for molting, both of these actions varying slightly from species to species (Table I). The caterpillar rests with thorax sharply arched into the air and head bowed down. During this period the dorsal skin between the prothorax and head capsule becomes enormously stretched and a pale band appears beneath it–the new head capsule showing through the old skin. It is this which Wachter (1930), in her classical paper on the molting of the silkworm, calls the "triangle stage."

Next day at some time between 7:30 a.m. and 3 p.m. the skin is actually shed, the actual time tending to differ from species to species (Table I). From the initial appearance of the pale triangle until shortly before the shedding of the skin, the animal remains almost motionless. (Text-fig. 1a). It is, however, still able to walk and gives a perfectly recognizable defensive response if disturbed.

For about half an hour before the skin splits, the larva makes numerous small contortions within the old skin. The skin appears shrivelled and the scoli along thorax and abdomen are moved independantly of each other, due presumably to local contractions inside the larva as the muscle attachments are being freed from the old cuticle. This activity may continue for about ten minutes. The head is then bent gradually back so that the old capsule is at right angles to the long axis of the body (Text-fig. 1b). The cuticle between the old head capsule and the ventral part of the first thoracic segment spilts and the legs begin to emerge[3]. The whole skin is shifted backward as the larva surges forward and this puts a strain across the dorsal cuticle behind the old capsule. Thus about forty seconds after the third pair of legs has been freed, this connection between the old head capsule and dorsal prothorax breaks and the head is sud-

---

[3] Wachter (1930) says that in *Bombyx mori* the bending back of the head occurs because contact between old head capsule and skin is maintained at the mid-dorsal point after being broken ventrally and laterally. This may be true for the Heliconiinae as well.

TEXT-FIG. 1. Diagrammatic representation of four stages during the shedding of the skin of a heliconiid caterpillar. **a**, The pose adopted during the "triangle stage"; **b**, head bent sharply back as the skin begins to tear; **c**, head more extremely bent as the legs emerge; **d**, head jerked down and the caterpillar emerging from the skin which is gathered into folds as it passes back. Newly exposed cuticle is stippled, old head capsule is black and the "triangle" is marked with an arrow.

denly jerked down to its normal position, the old capsule now being stuck loosely onto the mouthparts of the new one. Meanwhile the rest of the old skin is being gradually shifted back by muscular movements which run along the body. In some species, *H. sara, H. ricini* and *D. juno,* which tend to be gregarious, the newly emerged caterpillar puts its feet onto the surface and begins to crawl forward out of its skin, so that waves of movement are assisted by the walking of the larva. There is no strict distinction between these two methods since the behavior is graded from *D. iulia,* in which there is almost no sign of walking forward, to *D. juno* where it is very marked.

Partially correlated with the extent of this walking forward is the degree to which the cast skin is gathered together as a heap or left spread. *D. iulia* and *D. phaetusa* are alone marked in their habit of producing a closely folded mass of exuvium.

The complete shedding of the skin, from the time the head is bent back until the anal prolegs are freed, takes about four minutes. There are, however, slight indications of specific differences in duration (Table II) although these are not consistent enough to evaluate satisfactorily without a larger sample from each species.

Once the skin is freed from the last segment, the hind end is lifted into the air. The anal prolegs contract and expand several times and then the caterpillar begins to rid itself of the old head capsule. This it does by wiping off the capsule against a leaf or stem. As soon as its new capsule is bared, the caterpillar puts its spinneret to the substratum and weaves a small patch of silk just ahead of it. It walks forward a few millimetres and weaves again.

There is a fairly wide variation in the pose adopted during the process of expanding the scoli and spinules of the head and body, but information is too limited to say whether any specific constancy exists among these variations. All species are alike, however, in having head and thorax held away from the substratum. Expansion seems to be effected by periodically raising the internal pressure of the body and blowing out the scoli and spinules. The contraction which produces this pressure increase is a shortening of the body, the legs being kept free of the ground and the head and thorax pressed back into the abdominal region. During this contraction the arch already present in the thorax may be flattened somewhat towards the substratum, as often happens in *A. vanillae.* The thorax and head may be lifted further and the extent of arching actually increased, an event which is more noticeable in *H. melpomene* and *D. iulia.* The principle effect, a longitudinal shortening of the body, is the same in all species and is often the only one visible. During any one contraction a scolus will expand from the base up and the spinules arising from this expanded portion will "click" into position. Normally it takes about 6-11 contractions to expand a scolus and its spinules fully. There is a very slight tendency for scoli and spinules to collapse again once the pressure which erected them has subsided. A series of 7 contractions lasts approximately 5 minutes, by the end of which time all scoli and spinules are usually fully expanded.

It may be noted that although spine expansion normally begins within a few minutes of the animal's being freed of its skin, disturbance by another caterpillar or other interference may delay its onset for at least 20 minutes without obvious effect upon the form of the spines. Once the expansion is complete, darkening and hardening begin.

The hardening and darkening process occurs gradually and there is no marked activity for 20 to 40 minutes. During this time all species rest with thoracic segments arched, legs somewhat spread and head slightly bowed. *D. iulia* and *H. aliphera* frequently adopt a "J" position as well, the head and thorax between O° and 90° to the rest of the body in both dorso-ventral and lateral planes. The caterpillar then relaxes so that its legs touch the leaf or stem again, but it may remain motionless for another one to four hours. It then turns slowly round, may rest again but finally walks back to its empty skin and begins to eat it.

Table II. Examples of Records of Behavior during Shedding of Larval Skin by Species of Heliconiine Caterpillars

Species	Duration of the shedding of the skin (mins., secs.)	Duration of spine expansion (mins., secs.)	Number of contractions in spine expansion
Dione juno	5,15	?	?
	5,00	?	?
	4,00	5, 20	7
	23,40	5, 00	5
	5,00	4, 10	5
	6,00	?	?
Agraulis vanillae	4,40	?	?
	4,45	6, 10	6
	3,10	5, 40	6
	5,35	6, 20	10
Dryas iulia	3,20	?	?
	2,20	6, 20	9
	2,30	4, 35	8
	?	5, 20	9
	2,45	8, 20	11
Dryadula	3,20	5, 30	5
phaetusa	?	4, 00	+3
Heliconius	?	10, 15	11
aliphera	2,30	?	?
	2,30	7, 45	7
Heliconius	3,40	10, 55	8
melpomene	3,30	5, 10-6, 50	11
	3,05	6, 50	5
	2,50	4, 55	7
Heliconius erato	5,00	3, 25-7, 25	?
Heliconius ricini	3,05	6, 15	2
Heliconius	3,20	6, 00	?
sara	3,30	7, 00	2, indistinct
	2,10+	3, 00+	?

In *D. iulia* and *D. phaetusa,* but not in other species, there is a great deal of arching of both head and hind end during the period between the end of hardening and eating the skin. The legs and anal and last abdominal prolegs are freed and arched up so that the hind end and head almost meet above the caterpillar's body.

If there happens to be the cast skin of another caterpillar immediately in front of a larva which has just finished its hardening and darkening, it will not turn round and go back to eat its own skin but will walk forward and accept the foreign one. This indicates that the behavioral sequence leading to the eating of exuvia is not strictly stereotyped. After the skin has been eaten there is a further period of rest before the caterpillar begins to feed on a leaf. If, however, a second skin is offered to a newly molted caterpillar it will eat this as well, a phenomenon often seen to occur naturally among gregarious species such as *H. sara.* Thus eating a single empty skin does not, in itself, provide the whole consummatory act.

D. juno and *H. sara,* two of the gregarious species, differ from *A. vanillae, D. iulia, D. phaetusa, H. aliphera, H. isabella, H. melpomene, H. erato* and to a lesser extent *H. ricini* in the appearance of the spine expansion process. Instead of being a definite series of clear-cut contractions, there is an indistinct period during which the scoli gradually expand. While this could be a genuine effect, it seems more likely that it is due to the observational set-up. The gregarious species have shorter scoli and less developed spination (Beebe, Crane & Fleming, 1960). Thus they provide less distinct indicators of the process which is being observed than would caterpillars of solitary species. In addition,

TABLE III. PREPUPATIONAL BEHAVIOR IN SPECIES OF HELICONIINE CATERPILLARS

Data on *Dione juno* relate to a single group of 13 larvae observed in this study but are confirmed by the record of another group kept by Constance Carter. *Dryadula phaetusa* record refers to a single animal. Information relating to *Heliconius sara* supplied by Constance Carter.

Species	Approx. duration of fast before site select. (hours)	Locomotion during site select., mins./meter	Normal time of day for:		
			Site select.	Hanging up	Shedding last larval skin
Dione juno	8	1,44–3,04	8:30 p.m.	11:00 p.m.	1:00-4:00 p.m.
Agraulis vanillae	6-14	2,22–7,59	Indistinct	5:00 p.m.	7:00-11:30 a.m.
Dryadula phaetusa	4	10,12	3:00 p.m.	5:00 p.m.	Before noon
Dryas iulia	5	1,24–1,58	2:30 p.m.	5:00-8:00 p.m.	8:00 a.m.-noon
Heliconius isabella	0	2,05–3,43	2:30 p.m.	3:00-7:00 p.m.	?
Heliconius aliphera	0	–5,30	2:00 p.m.	3:00-7:00 p.m.	9:30-noon
Heliconius melpomene	1-8	2,55–3,16	11:00 a.m.-1:30 p.m.	1:30-7:00 p.m.	7:00-11:30 a.m.
Heliconius erato	1	3,18–4,25	9:30 a.m.-12:30 p.m.	12:30-3:00 p.m.	8:00-10:30 a.m.
Heliconius ricini	¼-1	3,20–4,50	10:30 a.m.-1:30 p.m.	1:00-5:00 p.m.	7:30-9:30 a.m.
Heliconius sara	?	3,14–	10:30 a.m.	5:00 p.m.	?

the caterpillars are together in a group and more difficult to distinguish individually. Moreover they are frequently moving and disturbing each other.

III. PUPATION

a. *Selection of Pupational Site*

As can be seen from Table III, most species stop feeding some hours before they start to find a site suitable for pupation. There are no noticeable behavioral changes during this period but changes take place in the coloration (Beebe, Crane & Fleming, 1960). The caterpillar rests either on the leaf it had been eating or on the stem.

Once they have begun to search for a pupation site, the caterpillars no longer feed. *H. isabella* is exceptional for it may sometimes stop and eat again even after it has abandoned a feeding position and begun the ambulatory phase. None of the species which pupate on leaves has ever been seen to do so on a leaf which has already been chewed by a caterpillar or one on which another larva is resting.

Some indication of the speed with which caterpillars of different species walk is given in Table III. (This has not been corrected for the slightly different temperatures at which the observations were made.) Only *D. iulia* is exceptional in walking invariably fast. *D. phaetusa* is odd in being very slow but as only one individual was tested, no valid conclusions can be drawn from this. The rate of walking varies during the ambulatory phase, starting slowly, increasing, then apparently falling again—an effect which partially accounts for the wide variation in results presented here. Except in the cases of *A. vanillae, D. phaetusa* and *H. erato,* the walk is interrupted by few rests. A caterpillar may drop from one part of the vine to another or to the ground, covering a small area of its leaf or stem with silk and then swinging down from this on a safety thread. Sometimes the drop may be as much as a meter.

Once a caterpillar has started walking, it may pass numbers of what appear to be suitable pupation sites, a behavioral feature noted in the cecropia silkworm (v.d. Kloot & Williams, 1953). It should be noted, however, that at least some *D. iulia* and *H. melpomene* are able to settle at a pupation site after walking only a few inches. The location of such a site differs from species to species, as can be seen in Table IV, yet it is always such that the pupa can hang

TABLE IV. SITES SELECTED FOR PUPATION BY PREPUPATIONAL HELICONIINE CATERPILLARS HAVING FAIRLY UNRESTRICTED CHOICE

L=Living, D=Dead in reference to stem and tendril.

Species	Leaf tip	Leaf blade	Petiole	Midrib	Leaf margin	Stem, L., D.	Tendril
Dione juno	—	—	—	—	—	3L	10L
Agraulis vanillae	—	—	—	2	—	6L	1
Dryadula phaetusa	—	—	—	—	—	1L	—
Dryas iulia	—	1	3	2	—	2L & 12D	5
Heliconius isabella	—	12	—	—	—	—	—
Heliconius aliphera	—	4	—	12	—	—	—
Heliconius melpomene	—	—	—	10	3	—	2
Heliconius erato	2	—	—	1	1	2L	2
Heliconius ricini	—	—	—	10	3	1L	5
Heliconius sara	—	—	—	2	—	1	—

down below it, whether it be beneath a leaf, stem, tendril, flower stalk or some foreign horizontal surface. Occasionally a caterpillar may attach against a vertical surface.

One feature which normally determines if a site is suitable, is whether it provides enough free space beneath for the pupa. This is estimated by means of a behavior pattern which will be termed "trying for height." Taking *H. melpomene* as an example, the caterpillar walks along the ventral midrib of a fresh, unchewed leaf as it goes weaving on either side of the midrib. Towards the center of the leaf it stops and weaves more actively. Then it arches its head back and releases its foothold so that its head and thorax hang loose beneath the leaf. If it touches a second leaf or some other object below, it will usually abandon the position as a possible pupation site. If it does not touch anything, it releases the hold of its first and then second prolegs so that its body hangs further down beneath the leaf. It may swing gently, reaching out around it. If it now touches some object, the caterpillar does not grasp it and crawl onto it as it would if it were trying to move away, but will usually contract up onto the midrib again and walk off to try another leaf. A caterpillar usually tries for height three or four times, facing first one way along the midrib and then another. Finally, if the leaf is acceptable, it will rest before beginning to spin its pupal silk pad.

H. aliphera and *H. isabella* differ from the other species in that, when trying for height, the prolegs are never freed from the leaf above. In these two species the pupae do not hang directly but are bent almost at right angles to their support (Plate I). They therefore need less space beneath their pupation site.

Other immediate features which render a site desirable for pupation are less clear. Field observations show that all species except *H. isabella,* and to a more limited extent *H. aliphera,* choose to hang up on a ridge, a knob or other protuberance or irregularity, never on a smooth surface. In the laboratory *H. isabella,* given the choice of an irregular surface, such as aluminum gauze, or a smooth one such as glass, will hang up on the latter. The same preference is also shown by *H. aliphera* (Plate I), which in the field pupated on the ventral midribs of leaves in 12 out of 16 cases. Those four which were not on midribs were, nevertheless, in positions which would have been acceptable to *H. isabella.*

In broader terms a pupation site must, so far as possible, protect the pupa and permit a healthy butterfly to emerge. Protection against climatic factors is unlikely to be important, in that the pupae are in an environment which already supports the caterpillars. Nothing at all is known of pupal predators, although ants will attack pupae which are injured or unhealthy; they have not been seen to molest them otherwise. Various insect parasites, both dipteran and hymenopteran, can be bred out of pupae and it has been established that the eggs of one of these are laid by the female in the pupa itself. What does seem to be a significant hazard, however, is that another caterpillar, of the same or a different species, will begin to eat away the leaf from around a pupa so that the pupa falls to the ground. This has happened at least four times on the observational vines when there have been plenty of other leaves available for the larvae. Some of the consequences of a pupa falling to the ground will be considered later.

Meaningful discussion of the significance of a preferred pupation site, and consequently of the prepupational behavior of the caterpillar, must necessarily be unsatisfactory until more exact information is available about potential pupal predators and parasites. Those species which hatitually move off their leaves to pupate (*e. g., D. iulia*). risk attack by predators such as ants or mantids (and both have been observed to kill caterpillars searching for pupational sites) during this extended locomotory stage. There may be compensation for such dangers in their subsequent security from being chewed off a leaf.

b. *Pupational Behavior*

Once the site has been chosen, the caterpillar weaves generally around it and may tie the selected structure firmly onto the main vine. Then it spins silk more particularly in the region where the pupal pad is to be placed. There are several rest periods during which, if oriented under a leaf or along a stem, the caterpillar faces first one way, then the other. Eventually, after 30 minutes to two hours, spinning of the pad itself begins. The caterpillar first spins on one side of the midrib, stem or tendril, then, bringing its spinneret to the place where the pad is to be, touches the spot, lifts its head and pulls it backwards, touches the spinneret again to the position of the pad and then repeats the procedure, but turning this time to the other side. The process is repeated many times before the spinning becomes concentrated on the pad itself. The drawing back of the head over the area where the pad is being spun is concerned with forming loops of silk which make up much of the pad and has been discussed in Part I of this paper as "yawning." The pad is cone-shaped with an elliptical base whose long axis is about 1 mm. long. It is about 0.5 mm. tall and its apex is asymmetrically placed, being nearer to the caterpillar as it spins than the mid-point of the base. The color of the silk varies from species to species (Table V).

Once the pad is completed, the caterpillar moves forward until the pad is either just before or behind its fourth pair of prolegs. In this position the animal waits until it has emptied its gut of the last fecal pellet. Then it walks further forward so that its anal prolegs touch the pad. These structures work themselves into the silk,

TABLE V. COLORS OF SILK WHICH HELICONIINE PREPUPATIONAL LARVAE PRODUCED IN SPINNING THEIR PUPAL PADS

Species	White	Pale pink	Pink	Red	Indeterminate
Dione juno	13	—	—	—	—
Agraulis vanillae	9	—	—	—	—
Dryadula phaetusa	2	—	—	—	—
Dryas iulia	21	—	—	—	—
Heliconius isabella	9	—	—	—	—
Heliconius aliphera	11	—	—	—	—
Heliconius melpomene	—	1	—	12	2
Heliconius erato	—	2	2	—	2
Heliconius ricini	1	3	8	—	3
Heliconius sara	5	—	—	—	—

presumably much of the attachment being by way of the crochets. The caterpillar jerks its hind end forward and back, either to make the attachment more secure or to test it. Some species immediately loose their hold on the leaf or stem and hang freely from the pad, *e. g. D. iulia* and *H. melpomene*. Others such as *A. vanillae* may wait for as long as half an hour. When the prolegs release their hold on the substratum, it is the hindermost abdominal pair which let go first, then the third prolegs and finally the second and first. The caterpillar rests in this position until the following morning, when it sheds its skin.

If the observer pokes a larva which has just released its foothold and is hanging by its anal prolegs, it can be induced to climb back onto the leaf or stem. If the disturbance is rough enough, the caterpillar will break its attachment to the silk pad. It may then rest a while before reattaching itself once more to the original pad or may spin a second one either beside the first or a short distance off. There is no need for the caterpillar to revert to the previous locomotory stage before it can spin a second pad, in the way that cecropia larvae seem constrained to do if removed from a partly constructed cocoon (v. d. Kloot & Williams, 1953).

D. iulia and *H. melpomene* can be induced by continued teasing to spin a third silk pad, but this is invariably very thin and small. There appear to be differences in the ease with which different species can be teased into abandoning their pads or spinning a second. Thus *A. vanillae* is markedly reluctant to climb back onto the substratum and will twist and try to bite at the teasing forceps for much longer than would *H. melpomene*. No standards were established for the teasing, however, and a great deal more experimentation is necessary before evaluating any such specific differences.

A peculiar point emerging from these latter observations is that the pattern of hanging free after attachment to the pad changes with repetition. On the first occasion it is invariably the hinder of the prolegs which loose their hold first. On the second attempt the prolegs hang free in order from the front to the back. The possible interest attaching to the point is that it suggests that the second hanging-up is not in all ways a simple repetition of the first.

IV. PUPAL BEHAVIOR AND ADAPTATIONS

Of the ten species of heliconiine pupae which have been observed, all show a certain amount of movement. This is distinct from any flexibility which they may have at the point of attachment. This latter characteristic differs from species to

TABLE VI. BEHAVIORAL AND PIGMENTARY CHARACTERISTICS OF HELICONIINE PUPAE

Information relating to *Heliconius sara* supplied by Constance Carter; details of methods of measuring flexibility not strictly comparable with those of other species. Position of *H. sara* in the series is therefore open to suspicion.

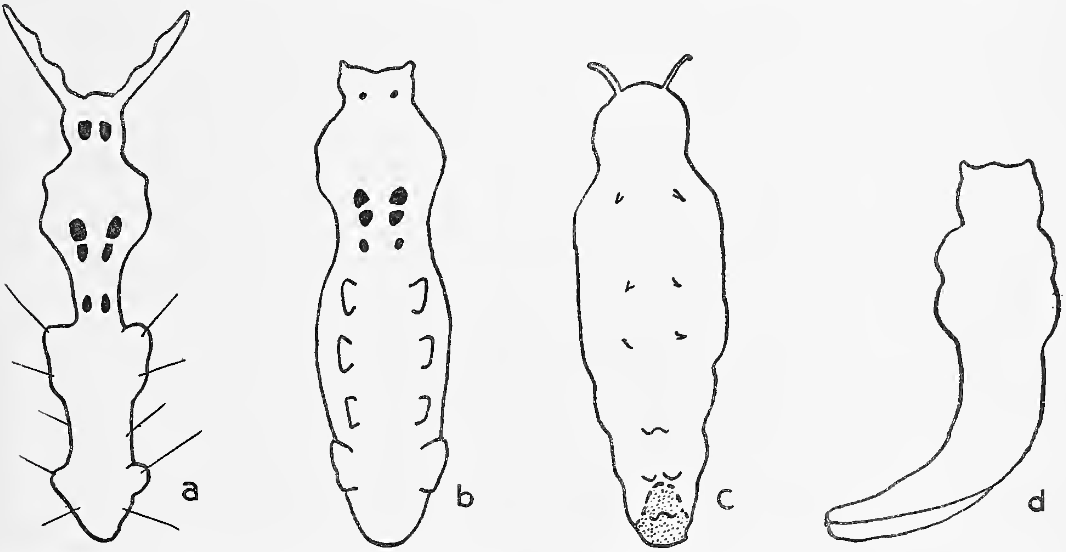
Species	Degree of flexibility	Bend in pupa	Odor production	Stridulation	Gold spots
Heliconius isabella	160	Permanent	–	+	–
Heliconius aliphera	160	Permanent	–	+	–
Agraulis vanillae	147	Changing	–	+	–
(Heliconius sara)	(144)	–	?	?	+
Dione juno	136	Changing	–	+	–
Dryas iulia	126	–	++	++	+
Dryadula phaetusa	125	–	–	–	++
Heliconius ricini	116	–	+	+	++
Heliconius melpomene	79	–	++	++	++
Heliconius erato	65	–	+	+	++

species, presumably depending on how tightly the pupa has attached its cremaster to the silk pad. Flexibility may be expressed by how far the pupa rotates while its support is rotated through 180° in the vertical plane. An absolutely rigid attachment gives the full 180° turn while a fully flexible pupa will not rotate at all. In this way, the ten species available for testing can be arranged in a series (Table VI).

Hinton (1955) discusses the selective advantage derived by such pensile pupae from their flexibility of attachment, contending that swinging hinders predators such as birds from getting an effective peck or that is it useful in shaking off ants. Whether such factors are involved among the heliconiine pupae or not, flexibility certainly adds to the effect of the movement of the pupa itself.

When the species of Heliconiinae studied are arranged according to other aspects of pupal behavior, the series established for flexibility of attachment tends to be repeated. Thus in the two species which are rigidly attached, *H. isabella* and *H. aliphera,* the posterior segments of the abdomen are bent so that the ventral surface lies near or touches the surface of the leaf and the angle between body and leaf is invariably less then 35°. The pupae of these species are the least active of all those studied. When touched by caterpillars or the experimenter, they swing from side to side but produce only faintly audible stridulation. Both are light colored, creamy white or translucent (with a suggestion of green in *H. aliphera*). Their markings are faint brownish or black without gold spots or other "attention calling" devices. Neither produces any perceptible odor. Considered as a whole, the behavior and appearance of the pupae of these two species suggest that the evolutionary trend in the pupal stages of *H. aliphera* and *H. isabella* has been towards camouflage and concealment rather than advertisement.

The next two pupae on the flexibility scale, *A. vanillae* and *D. juno,* share with *H. aliphera* and *H. isabella* the characteristic of bending their bodies so that they do not hang vertically down as do the other five species. They do not, however, bend up close to the support nor do they remain in this position until emergence. The major bend in the body of the pupa is more characteristically lateral as opposed to the dorso-ventral one in *H. aliphera* and *H. isabella;* further, *A. vanillae* and *D. juno* frequently have two distinct bends, one almost at right angles to the other (Text-fig. 2). During the nine or ten days of pupal life the orientation of these bends constantly changes; without any apparent environmental stimulus a pupa will twist its body into a new position. A few hours before emergence of the adult, they straighten out so that the pupa hangs vertically down. This again is distinct from the behavior of *H. aliphera* and *H. isabella,* whose adults emerge from a pupa which is still curved upwards towards the leaf. It may be noticed that, for the butterfly to expand its



TEXT-FIG 2 . Diagrammatic representation of the main morphological and some behavioral characteristics of the pupae discussed in the text. Dorsal view of: a, *H. erato;* b, *D. iulia;* c, *H. aliphera;* d, *A. vanillae.* Gold markings are shown in solid black and the ventral bend in the posterior end of *H. aliphera* is indicated by stippling.

wings, a free space is needed beneath the empty pupal case to which it clings. Since the pupae of *H. aliphera* and *H. isabella* remain bent during emergence, less space is required than when the pupae straighten out as do those of *D. juno* and *A. vanillae*. It will be remembered in this connection that *H. aliphera* and *H. isabella* do not release their prolegs when "trying for height" (see page 110) while *D. juno* and *A. vanillae* usually do.

The pupae of *D. juno* and *A. vanillae* make no audible sound when stimulated, nor do they produce any noticeable odor. Their movement is slight, rather slow and limited. They have no bright spots or grotesque frills or spines and their usual colors are neutral straw to brown-gray (Beebe, Crane & Fleming, 1960). *A. vanillae* pupates on fresh vine, either on the stem, tendrils, flowers or leaves, and almost invariably on its own food-plant. The brownish color combines with the bendings of the body to give the pupa a resemblance to a shrivelled leaf or withered flower of *P. foetida*. Like *A. aliphera* and *H. isabella*, the pupae of this species seem to be adapted rather toward concealment than warning or frightening away possible predators.

Of a group of 13 healthy *D. juno* caterpillars given a choice of pupational sites, all hung up on the stem, tendrils or leaves of a fresh vine. Although they resembled *A. vanillae* in their choice of a pupation site, they remained together as a group and it is hard to see any attempt at concealment in their behavior.

The fact that both *D. juno* and *A. vanillae* continually change from one twisted position to another during the pupal stage is interesting when compared with the constant bend of the abdomen of *H. aliphera* and *H. isabella*. The latter two are flexed directly back, *i. e.*, their bend is a bilaterally symmetrical one. It seems likely that if a pupa is asymmetrically twisted throughout its entire pupal life, the development of a bilaterally symmetrical butterfly would be more difficult. Thus a pupa of *D. iulia* which had been put onto a flat surface immediately after it had molted, and was still soft, hardened with a permanent twist to one side. When the butterfly emerged, the wing on the concave side was shorter than the other and buckled; the animal was barely able to flutter. Thus it may be advantageous for a pupa to remain bilaterally symmetrical. Since shrivelled leaves are seldom so, *A. vanillae* (perhaps *D. juno* as well) has to lose its symmetry to mimic such objects. Possibly the continual changing of the asymmetrical pose is a compensatory measure which ensures that the developing adult is not continuously exposed to a single pattern of asymmetric forces.

The pupae of the five remaining species hang vertically and are fairly flexible. With the exception of *D. phaetusa* (of which only one specimen was tested), they all wiggle suddenly and furiously when stimulated. Again with the exception of *D. phaetusa*, they produce a clearly audible squeaking. All except *D. phaetusa* produce a sudden and noticeable smell when disturbed. *H. melpomene* smells most strongly, an odor very like that produced by the adult butterfly when disturbed. This is unpleasant to some people, not to others – it passes very quickly. The pupae of *D. iulia* smell of musty honey, those of *H. ricini* and *H. erato* have a weaker odor somewhat like a mixture of *D. iulia* and *H. melpomene*. There are in addition bizarre anatomical effects (Beebe, Crane & Fleming, 1960). All five species, *D. phaetusa* included, have showy golden or silver spots on their backs (Text-fig. 2). *H. melpomene* is chestnut colored with black spines and is fairly obvious against the green of the leaves under which it normally pupates. *H. ricini* and *H. erato* have elaborate antler-like frontal horns, protuberances and spines on their bodies.

All these features, behavioral and structural, seem to advertise the pupae, and are thus the converse of the tendency shown in *H. aliphera, H. isabella, D. juno* and *A. vanillae*. However the site in which *D. iulia* chooses to pupate, up against the bark of a tree or on a shrivelled tendril or flower, combines with the mottled appearance of the animal to make it almost invisible in its natural position. The gold spots, so obvious in *H. melopene* and especially *D. phaetusa*, are in *D. iulia* very much smaller and the most anterior pair are almost invisible. In *D. iulia* and probably also *A. vanillae*, there is a tendency for the shade of the pupa to be influenced by the lighting conditions under which the larva hung up, a tendency well known among lepidopterous larvae and first investigated by Poulton as early as 1887. If *D. iulia* is kept in the dark during the day on which is it due to hang up, the resultant pupa is much darker than one kept in the light[4]. In these features therefore the pupa of *D. iulia* appears to be cryptic. In this case selection may be working in these two directions at once, and they do not necessarily cancel each other out nor are they mutually exclusive. A pupa which is concealed may be discovered accidentally by a predator and it would then be advantageous for it to have an intimidation display. If an "advertisement policy" like that of *H. mel-*

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[4] Although this experiment was carried out in a single room with only a partition separating "light" from "dark" larvae, there is no guarantee that some temperature difference did not exist.

pomene were modified so as to retain only those elements such as noise, movement or odor, which the pupa can control, then a condition like that in *D. iulia* would result. Perhaps the turning point in such an evolutionary pathway would be that of changing the pupational site from beneath a leaf to a stem, tendril or foreign surface. This change in its turn could have resulted from selection pressure of caterpillars eating the sheltering leaves or from the fact that leaves on the particular species of vine are scarce or very flexible—both of the latter tend to be true in the case of *P. tuberosa,* the vine on which *D. iulia* feeds.

H. ricini and *H. erato* are usually cryptically placed, but less so than *D. iulia* (Table IV). About half of the cases observed were not on living leaves. The explanation suggested for *D. iulia* can be put forward for these two species as well, although with perhaps less justification.

The case of *D. phaetusa* is not explicable in such terms. The only feature which could be regarded as intimidating is the spotting. It has no marked movement, sound or smell. On the other hand, the pupa does not appear to be well concealed, being light beige without striking peculiarities of any sort. Perhaps some simple adaptive explanation will be revealed by observations in the field.

V. Emergence from the Pupa

The duration of the pupal stage in the Heliconiinae is generally 9 or 10 days. There is a suggestion of some specific differences in what time of day emergence takes place; thus *H. erato* is usually out of its pupal case before 9 a.m., while *H. melpomene* tends to come out throughout the morning, with a concentration around 10:30 a.m.

The pupal case cracks across behind the head, down the center of the thoracic tergites, then across and down either side of the wing covers. The lateral edges of the split behind the head run down onto the ventral surface of the pupal case and along the ventral edges of the wing covers. Thus the thoracic part of the pupal case is divided longitudinally into three sections which can be easily separated from each other and from which the wings and legs of the butterfly can emerge without excess straining. The separation of the three sectors seems to be effected mainly by movements of the bases of the wings, which, expanding and contracting against the loose sections, gradually work them apart. Presumably in its normal hanging position this would be helped by gravity as the body tends to fall out of the pupal case. The legs do, however, grasp feebly at the edges of the case and within a few seconds either by their effort, by waves of movement passing back or by gravity, the swollen abdomen is freed of the case. The butterfly immediately turns around so that the small wing buds, antennae and abdomen hang down behind it.

The wings at this time are about one-third of their final length and during the next two to five minutes they expand until they reach full length. When fully expanded, the wings are almost completely flattened. Although they are soft and flaccid to the touch, they do not fall into folds as an equally soft cloth would do. The antennae hang straight back, lying between the wings. The labial palps have moved from their original position, folded down into the thorax, to their final one, bent up beside the compound eyes.

At intervals for a period of 15 to 30 minutes the wings flap slightly open and then close again, the intervals between the opening and closing depending to some extent on the species, examples of which are shown in Text-fig. 3. During these movements the two wings of each side are held together with the hind wing a little further forward relative to the front than is normally the case. As the wings open, the head moves slightly forward and the abdomen swings between them. When the wings close the head and abdominal movements are reversed. Extension of the proboscis occurs fairly frequently (Text-fig. 3), but without definite relation to the wing movements. The palps begin to tremble a short time after the wing fanning starts and there is a marked tendency for this to be associated with wing closing rather than opening.

At the end of wing fanning, the wings are closed and then the hind ones open again so that they are well separated from the forewings. The latter open only very slightly. The wings are then held motionless in this position for an interval which varies from less than four to more than 15 minutes; the interval will be referred to as the "relaxation period." Depending on the species, the palp trembling will or will not continue throughout the relaxation period (Table VII). The end of relaxation is marked by the hind wings closing up against the forewings. These are, however, still held slightly apart from each other for a further two to four minutes so that the ending is gradual.

A variable time after the start of wing flapping, the antennae move forward to the leading edges of the wings and, when the wings next close, fold back so as to lie lateral to the wings instead of between them. Subsequently the antennae, together with the head, can be seen to move forward and back as the wings flap. By the beginning of the relaxation period they come to lie together at the leading edge of the wings. During or after relaxation they move anterior to the wings and are held together for a variable time, sometimes opening and closing slightly. In some species, such as *A. vanillae,* this forward movement of the antennae is very characteristic and coincides with the end of palp trembling, though not usually at the end of relaxation.

The first drops of meconium are voided at a time which varies to some extent from species to species (Table VII). The meconium is a brownish, reddish or yellowish fluid, the shade depending on the species of butterfly (Table VIII)[5]. Three to six small drops fall together at

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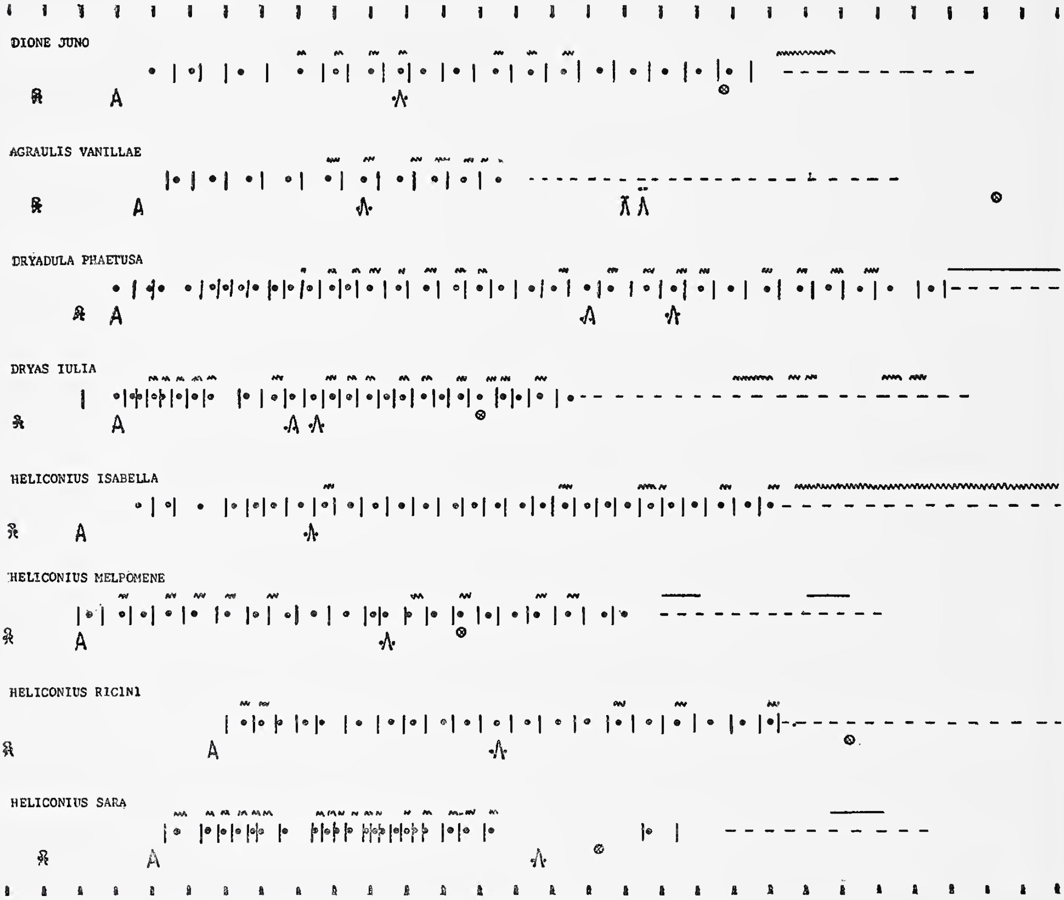
[5] No attempt has been made to establish whether the color of the meconium is influenced by the vine on which the larva had been feeding. It is surely not the direct cause, in that *H. isabella,* feeding on the same species as *H. melpomene* and *H. ricini,* produces yellow while that of the other two is primarily gray or chestnut. Conversely, *H. melpomene* and *H. erato,* feeding on different vines, produce the same colors. Nor can it be correlated simply with the general pigmentation of the scales.



TEXT-FIG. 3. Examples of records of emergence behavior of *D. juno, A. vanillae, D. phaetusa, D. iulia, H. isabella, H. melpomene, H. ricini* and *H. sara.* The time scale at the top and bottom of the figure is in minutes and zero time for each record is on the left hand side and is the moment when the pupal case split open. The upper saw-toothed line of each record represents activity of the palps, while a continuous line marks periods when inactivity was observed. A vertical stroke indicates wing opening during the period of wing fanning, wing closing being shown by a solid black circle, while a dotted line shows the relaxation period. The open circle with a cross in it marks the liberation of the first drop of meconium. Below this in each record is a representation of the state of the wings, starting with a pair of wavy lines at the left of each record and representing the crumpled wings at the last point of time when they were mentioned in the laboratory notes as being crumpled prior to the straightening, which is represented by straight lines. The position of the antennal tips is shown relative to the wings as pairs of black dots.

Table VII. Behavior of Emergent Heliconiine Butterflies

Number in [ ] after each species denotes number of cases watched in detail; number followed by an "e" indicates additional cases of experimental animals. (See Table IX).

Species	Number of flaps and number of minutes of phase	Number of flaps per minute	Palp movement obvious during flapping	Palp movement obvious during relaxation period	Minutes before 1st meconium shed. Co-efficient of variation (bracketed)	Final palp movement and head twisting
Dione juno [8]	14/16–18/17	1.0	+	+	20.7 (.07)	+++
Agraulis vanillae [5] [2e]	10/9.0–17/10	1.3	Absent or late	+	29.6 (.06)	+++
Dryadula phaetusa [1]	32/23	1.4	++		32.0 (?)	++
Dryas iulia [6] [9e]	18/13–24/13	1.6	++		17.8 (.21)	+++
Heliconius isabella [3] [1e]	26/17–28/16	1.6	++ late	+	32.0 (.03)	–
Heliconius aliphera [2]	21/16–29/19	1.55	–	–	21.5	–
Heliconius melpomene [8] [2e]	19/15–27/17	1.4	+++ (on shut)	–	10.8 (.45)	–
Heliconius erato [1] [3e]	22/15	1.4	+ (on shut)	–	23.1 (.58)	–
Heliconius ricini [4] [2e]	21/14–25/15	1.5	+ (on shut)	–	21.3 (.16)	–
Heliconius sara [2]	22/10	2.1	+++ (on shut)	–	13.7 (.10)	–

intervals of a few minutes until the animal is ready to fly. The first two or three sets of drops contain a fine precipitate but later drops become more transparent and paler in color until after a period varying from 6 to 40 minutes the fluid is altogether clear.

Once the relaxation period is over and the wings are once more apposed, only slight movements of the antennae take place before the animal flies off. They do, however, gradually move further and further apart until their normal position is achieved. Just before flight a peculiar pattern has been noticed in *D. juno, A. vanillae, D. phaetusa* and *D. iulia.* The butterfly twists its head from one side to the other, at the same time extending and waggling its palps, one after the other.

Flight takes place after the antennae are well apart, although a disturbance before this time may cause the butterfly to flap its wings. The stimulus for flight often comes from the movement of a nearby butterfly or a puff of wind but frequently there is no apparent external stimulus at all. The insect walks up its empty pupal case, flapping its wings, then suddenly takes off, sometimes still dropping meconium as it flies.

The specific differences among the 10 species observed are listed in Table VII and some can be seen in Text-fig. 3.

During a natural emergence there is very little rearrangement of the legs and body necessary before the butterfly is in the position appropriate for wing fanning. The preferred position is one in which the longitudinal axis of the thorax is not quite horizontal, sloping slightly upward at the anterior end. In the majority of species examined, the pupae have projections on the dorsal part of abdominal segments and these are placed so that the newly emerged butterfly can obtain its preferred pose by clinging onto them with the first pair of functional legs and onto the margin of the head case with the others. However, the stance does not invariably occur and it may not be correct to regard such projections as having been evolved primarily for this function.

Emergence from the pupal case is almost invariably prolonged when the animal is not hanging in the normal way. If, however, an object is placed near the butterfly's legs as soon as these are free, it is grasped and the body and wings pulled out quite quickly.

If a recently emerged butterfly is not provided with an appropriate resting place, it walks until it finds one and, in *H. melpomene* at least, is capable of searching for at least 27 minutes. Its walking is initially directed toward the lightest part of the horizon but this orientation gradually becomes confused and the butterfly tends to begin circling movements, possibly due to mechanical difficulties with its wings.

During this walking stage the inflation of the wings is not delayed and within five minutes of its exit from the pupal case the animal's wings are fully expanded and flattened. If the butterfly is allowed to hang up at this stage, it shows no apparent deviation from the normal pattern of wing flapping, palp trembling, relaxation and finally flight. Yet even when it is forced to walk on a flat surface there may be some damage to the wings, either from scraping on the pupal case or injury during walking. There may also be a fair amount of bleeding from both wings, although the effects are not as serious as Brocher (1919) reported for punctures made at an earlier stage in the wings of Agrionidae where full expansion was prevented. In natural conditions, after a pupa has fallen to the ground as a result of the chewing activity of some other caterpillar, its newly emerged butterfly may have to walk over twigs, leaves or other debris and will probably damage its wings more severely.

If the butterfly is forced to continue walking after the wing expansion is completed, the wings trail limply behind it and during this time there are no signs of any flapping behavior, nor are the palps trembled at all. Meconium will nevertheless be dropped at the appropriate time. When eventually the butterfly is allowed to hang up, the wing flapping may be prolonged, or much curtailed; it may be much faster, or just irregular (Table IX). If the butterfly is forced to walk for as long as 10 minutes, its wings, when eventually it does hang up, fall into folds instead of remaining flattened. Although they appear still to be soft, the animal seems unable to hold them in such a way that they harden as flat surfaces. Thus, although they are fully expanded, the ability to flatten the wings seems to disappear about eight minutes after emergence from the pupal case. Normal wing expansion may sometimes take as long as five minutes, so the flattening process cannot be postponed for more than about three minutes after its normal time. Hardening of the wings must occur very shortly after the loss of flattening ability, for when hanging up is delayed for longer than eight minutes, the wings begin to take on the shape they had when trailing along behind the walking butterfly. If they were twisted over the right side, the hardened wings later show the same twist. Indeed, it is likely that straightening and hardening normally take place concurrently.

Even if its wings have hardened in a distorted and useless condition, the butterfly, after a period of some wing flapping and violent palp trembling, relaxes for a period, rests and then finally tries to fly. Although the wings may be so twisted that their pattern is unrecognizable, the butterfly still rests for the normal relaxation period and then attempts to flap the wings prior to take off.

Like the wings, the antennae may remain unstraightened unless they are allowed to hang straight back between the wings. The critical period for this event appears to be the same as that for wing flattening and hardening.

Wing flapping and palp trembling are definitely not concerned with wing expansion. They normally occur during the process of flattening and hardening the wings and are curtailed when enforced walking is continued so long that the wings are never correctly flattened and hardened. It thus seems likely that these behavioral activi-

TABLE VIII. COLORS OF FIRST MECONIUM PRODUCED BY EMERGENT HELICONIINE BUTTERFLIES

Species	Brown	Chestnut		Yellow	Gray
		Dark	Light		
Dione juno	4	4	1	—	—
Agraulis vanillae	—	5	—	—	—
Dryadula phaetusa	—	—	1	—	—
Dryas iulia	2	5	6	—	—
Heliconius isabella	—	—	—	4	—
Heliconius aliphera	—	—	—	3	—
Heliconius melpomene	2 (+ red)	1 (+ red)	—	—	15
Heliconius erato	—	—	—	—	5
Heliconius ricini	—	4 (+ red)	3	—	—
Heliconius sara	—	—	—	—	3

ties are concerned in some manner with processes associated with hardening.

In relation to survival the important point is that if within eight minutes of emerging from its pupal case an adult can reach a position suitable for flattening and hardening of its wings, no harm should result if it had fallen or been chewed from its natural support. It must however be recognized that a pupa lying on the ground may well be more susceptible to attack by ants or small mammals or to destruction by molds. Thus the critical significance of selection of pupation sites, in relation to being chewed free by other larvae, cannot be dismissed yet.

VI. Phylogenetic Discussion

Similarities and differences between the larval behavior of some of the species of the Heliconiinae became apparent in Part I of this paper and phylogenetic considerations dependent on them have already been briefly dealt with. Part II provides more material and, in addition, features of the pupae and behavior patterns of the emerging imago. As in Part I, attempts have been made throughout the description to explain the presence of these in terms of the factors which control their appearance and of their possible selective advantage to the species. It is necessary now to see how far these latter behavioral characteristics may be of phylogenetic importance.

Considering the phylogenetic age of the molting patterns and the improbability that they are strongly influenced by the food plant of the caterpillar, it is in fact surprising that there are any specific differences here at all. Yet the position taken up on the vine prior to molting is specifically determined to a large extent and can be partially correlated with feeding and resting positions (Part I). Those species, *H. erato, D. iulia* and *A. vanillae,* which tend to rest on the stem, frequently molt there as well—in contrast to *H. aliphera, H. melpomene, H. isabella* and to a lesser extent *H. ricini.* It is noticeable that *H. aliphera* is more like the other species of *Heliconius* in molting under the midrib of a leaf than is *H. isabella,* which molts on the blade of the leaf. This intermediate position of *H. aliphera* was evident in the consideration of feeding of the larvae and will be remarked later in relation to behavior of pupae and emergent butterflies.

Table IX. Examples of Records of Fanning Behavior in Emergent Butterflies under Normal Conditions and when Forced to Walk for Various Lengths of Time after Emerging from Pupal Case

Species	Number of wing beats during wing fanning, given in minutes and seconds:	
	Normal conditions	Butterfly forced to walk for number mins. shown in parentheses
Agraulis vanillae	17 in 10	? in 9, 30 (15)
	13 " 10	
	11 " 10	
	11 " 9	
	10 " 9	
Dryas iulia	24 in 13	18 in 10, 30 (6)
	21 " 13	17 " 10 (5½)
	18 " 13	15 " 7 (12)
Heliconius isabella	26 in 17	23 in 15 (7)
	25 " 17	
	28 " 16	
	+22 " +13	
Heliconius melpomene	27 in 17	31 in 22 (6)
	22 " 17	20 " 10, 50 (2¼)
	20 " 15	
	19 " 15	
	? " 15	
	22 " 14	
Heliconius erato	22 in 15	24 in 13, 10 (12)
		12 " 11 (7½)
Heliconius ricini	22 in 17	
	25 " 15	21 in 11, 15 (8)
	21 " 14	16 " 10, 35 (6½)

Gregarious species, *D. juno, H. sara* and, less markedly, *H. ricini,* molt together on a stem although in normal resting they are often found on the leaf they had been eating.

Likewise the pose adopted prior to molting is related to that usually taken by a resting caterpillar and is thus species-specific to some extent.

There is less variation between the species when it comes to the actual shedding of the skin and subsequent expansion of the scoli and spines. There is but a slight suggestion that *A. vanillae* waits longer during the triangle stage than do the others and that *H. sara, H. ricini, H. erato, H. melpomene, H. aliphera* and *D. juno* are towards the other extreme in a short wait. Once the caterpillar does begin shedding its skin, the process is almost invariably slower in *A. vanillae* than other species, a feature which might well appear among caterpillars which are relatively unspecialized or unlikely to be interrupted by others during the molting process. Conversely the habit of shedding empty head capsules before the skin is free is one which might be retained in an unspecialized species or acquired as part of the plasticity necessary when gregarious caterpillars are molting together. It occurs in *A. vanillae, D. juno, H. ricini* and *H. sara.*

D. iulia and *D. phaetusa* were seen in Part I to share many behavioral characteristics of feeding and resting. Both show two peculiarities in molting behavior—leaving the cast skin in a small heap instead of spreading it out as they emerge from it, and the marked head-to-tail arching after spine expansion.

There are quite marked differences in the choice of pupation site and these can be correlated to some extent with specific differences in feeding, resting and weaving behavior. In feeding both *H. aliphera* and more noticeably *H. isabella* orient on the blade of a leaf. Resting always takes place on the blade and molting has not been seen to occur anywhere other than on a leaf blade in these two species. The emphasis on weaving has already been mentioned in *H. aliphera* and *H. isabella* and its possible correlation with larval life spent primarily on a smooth blade. Selection of a pupation site can be seen as part of the same syndrome: fifth instar *H. isabella* caterpillars invariably hang up on the ventral surface of a leaf blade if possible while those of *H. aliphera* show definite indications of the same tendency. *H. isabella* is in many other characteristics more specialized than *H. aliphera* and these two species are alone among the Trinidad heliconiines in their choice of pupation site. Thus it seems justifiable to assume that the choice of the smooth surface of a leaf blade as a pupation site is a specialization among them.

Among the species studied, *D. iulia* is unique in its rapid and almost invariably long journey to find a pupation site on a foreign plant or surface. On the other hand, it is the most catholic in its final choice—a characteristic which is seldom associated with specialization. *D. phaetusa,* which in respect of feeding, resting and molting is very like *D. iulia,* cannot really be judged from observations on a single individual, but it seems probable that its behavior in selecting a site for pupation is more like that of *H. erato* than *D. iulia.*

H. melpomene, H. ricini and to a lesser extent *H. erato* show clear similarities in their choice of pupation site. This is not surprising since they have shared characteristics of feeding and resting. There is little information on *H. sara* and because I did not see the behavior myself, I feel unable to comment on it. There is no evidence to suggest that it should be considered different from *H. melpomene* in its choice of pupation site.

If it were assumed, then, that leaf surfaces were the pupation site among primitive heliconiines and in particular the midrib of the leaf, the situation has changed in *D. iulia, D. phaetusa, H. erato, A. vanillae* and *D. juno.* The argument relating to scarcity of leaves and their flimsiness and flexibility on *P. tuberosa* might explain the abandoning of the presumed primitive site in the cases of *D. iulia, D. phaetusa* and *H. erato.* Much of the manner of eating, position and posture in feeding and resting as well as position during molting has already been related to characteristics of this vine. It might be possible to extend the argument to cover *A. vanillae* as well, in that its food plant *P. foetida,* has fairly thin leaves. *D. juno,* which pupates as a group, could perhaps have been forced into abandoning leaves as pupational sites because of lack of available space. It seems more reasonable, however, to suggest that midrib selection is a specialization adopted within the genus *Heliconius* and that the more basic choice was wider, including stem, petiole, flower stalk and midrib. The tendency to orient with respect to the midrib is particularly strong when the latter is as defined as that in a leaf of *P. laurifolia.* It has already been pointed out that *H. melpomene* and the solitary *H. ricini* and *H. sara* orient to the midrib in feeding, rest along it and molt on it.

If *H. aliphera* were "in the course of" abandoning a preference for protuberances in favor of flat surfaces as pupation sites, a shift to the midrib or a fine vein would be the last stage before reaching a leaf blade position like that of *H. isabella.*

In other prepupational behavior *H. aliphera* and *H. isabella* are distinct from *H. melpomene, H. erato, H. ricini* and *H. sara*. They eat until the search for a pupation site begins and *H. isabella* may even stop to eat during the walk. The search begins distinctly later in the day than it does among other *Heliconius* species. Differences in "trying for height" behavior are almost certainly part of a syndrome of pupal adaptation and adult emergence and in all this *H. aliphera* and *H. isabella* are alike and differ from other *Heliconius*. The significance of the color differences in the silk of the pupal pads is obscure. It is worth noticing, however, that *H. aliphera* and *H. isabella* again resemble each other in producing white silk and in differing from *H. melpomene, H. erato* and *H. ricini*. The red or pink silk characteristic of these latter species is certainly a specialization in that *D. iulia, D. phaetusa, D. juno, A. vanillae* and even *H. sara* produce white silk like that of *H. aliphera* and *H. isabella*.

D. iulia, D. phaetusa and especially *D. juno* select their sites late in the day. *A. vanillae*, on the other hand, has usually chosen its site and often hung up by mid- or late afternoon, only slightly later than is usual for *H. aliphera* and *H. isabella*. It spends an even longer period between its last larval feed and the time when it hangs up, sometimes resting almost motionless from 6:30 a.m. until the afternoon. A long fast also occurs in *D. iulia* but since fifth instar caterpillars of this species do not normally feed during the morning, its relation to the fast in *A. vanillae* is not clear. Among lepidoptera in general it seems that pupational behavior only starts some time after the last larval meal—thus Crowell (1943) describes a protracted resting period in *Prodenia* larvae prior to pupation and the larvae of the cecropia silk moth do not begin their wandering phase until they have emptied their guts of the last larval meal (v.d. Kloot & Williams, 1953). This would support the idea that *H. aliphera* and *H. isabella* are specialized in having no rest period at all prior to site selection and that *A. vanillae* is in this respect the most primitive of the species considered here.

The time of day at which a pupating caterpillar sheds its last larval skin is linked at least partially with the time at which it spun its pad and hung up on the day before. Thus *H. erato, H. melpomene* and *H. ricini* tend to be earliest. *H. aliphera, H. isabella, A. vanillae* and *D. iulia* are somewhat later. *D. juno* is the latest and here the last larval skins are still being shed during mid-afternoon of the day after hanging up.

Among the behavioral characteristics of the pupa itself, those of *H. isabella* and *H. aliphera* again are in an association separating them sharply from *H. melpomene, H. ricini, H. erato, D. iulia* and *D. phaetusa*, though less strongly from *A. vanillae* and *D. juno*. The two species mentioned last are perhaps more closely allied to each other in their pupal than in any other behavior.

The behavior described for *H. melpomene* and *D. iulia* so far has been fairly distinct. In pupal behavior, however, they are very alike, movements, sounds and odor production being very similar. The odors are perhaps different enough to suggest that this might be due to convergence. Yet it has already been pointed out that *D. iulia* might be losing the gold spots which are suggested as part of the equipment of advertisement. The direction of evolution in such a case can only be estimated in the light of other closely related species. It is thus very unfortunate that information on pupal behavior of *D. phaetusa* is so limited as to be valueless in such a connection. Since this species so often shows characteristics intermediate between those of *D. iulia* and the *Heliconius* species, it might well be of importance here.

The close relationship of *H. ricini* and *H. erato* with each other and with *H. melpomene* has been continually evident throughout the study and these two species are so alike in pupal behavior and form that it is possible to confuse them.

Information on the emergence behavior of the species is admittedly scanty, yet it is sufficient to divide the butterflies into four groups.

H. melpomene, H. erato, H. ricini and *H. sara* compose the first of these. All of the four show palp movements clearly during the wing fanning phase of emergence but lack them altogether during the subsequent relaxation. None of them extend the palps or twist their heads from side to side just prior to flight. The actual fanning phase is variable, both in duration and number of wing beats. With the exception of *H. sara*, however, the range of variation of this group would have to be extended to accommodate any of the other species studied in Trinidad. The rate of wing beating is very close in *H. melpomene, H. erato* and *H. ricini*, though *H. sara* beats very much faster.

H. aliphera and *H. isabella* are closer to the other *Heliconius* in the emergence behavior of their imagos than any other aspects so far. Movement of the palps during fanning is however less marked and tends to occur later. There is also a slight tendency to include palp movements in the relaxation period. The wing beats of fanning are somewhat quicker in the other *Heliconius* but the duration of the behavior is the same.

The six species considered so far show no sign of palp twitching and twisting of the head before flight but all of the remaining species do. Of these *D. iulia* and *D. phaetusa* form one group, *A. vanillae* and *D. juno* the other. The former are like the four *Heliconius* species in that palp movement is obvious during fanning and does not extend into the relaxation period. The rate of fanning in *D. phaetusa* is approximately the same as that in the *H. melpomene* group, but in *D. iulia* it is much higher.

Both *D. juno* and *A. vanillae* show practically no palp movement during fanning but have a very distinct burst of it during relaxation. The wing beats during fanning are much slower than in any other species.

Information relating to the period between emergence from the pupal case and shedding of the meconium is surprising. Firstly because the co-efficient of variation varies so much between species, being very high in *H. melpomene* and *H. erato* and only an eighth the size in *D. juno* and *A. vanillae.* It seems possible that the point at which meconium is shed is changing in some species or that an unsuspected feature, such as the sex of the individual, is coming into the matter.

The second point for comment is that the time of shedding meconium differs so between species which would be regarded as closely related on other grounds. Thus *D. iulia* and *D. phaetusa* contrast strongly, as do *H. aliphera* and *H. isabella.* Since the liberation of meconium is not effected by whether or not fanning occurs, it is logical that the point of time at which it occurs bears no relation to duration or rate of fanning. Nor is there any direct correlation between the color of meconium and time of shedding.

Consideration of the color of the meconium supports all previous evidence linking *H. aliphera* and *H. isabella* and separating them from the other species of *Heliconius.* This character also links *D. juno* and *A. vanillae* as do other features of their larval and pupal behavior. Meconium of *D. iulia* and *D. phaetusa* cannot be distinguished by color. All of the remaining species of *Heliconius* clearly belong together as a group for, although *H. ricini* produces none of the gray meconium typical of the others, it is nevertheless closer to *H. melpomene* than to any other species in respect of color.

In conclusion, much of the information given in this paper supports the hypotheses raised tentatively in the discussion of Part I. Relationships gauged on general behavior of the caterpillars corresponds fairly closely with those suggested by considering larval molting, and the behavior of pupae and emerging adults. It is interesting, however, that the apparent degree of relationship between species differs at different stages. Thus the early larval instars of *H. aliphera, H. isabella* and *D. juno* are very alike in their feeding patterns. As they grow, similarities decrease. Pupal behavior is distinctly different and the question arises as to whether the points of agreement could not be due to convergence. The behavior of emergent *H. aliphera* and *H. isabella* is no closer to that of *D. juno* than any other species. Conversely, the relationship between these two species of *Heliconius* and the *H. melpomene, H. erato, H. ricini, H. sara* group is extremely obscure during larval and pupal life and it is only at the emergence of the adult that similarities become apparent.

The last mentioned group of *Heliconius* may indeed warrent Michener's description of "large and relatively homogeneous" (1942) in adult behavior but among the larvae there are at least some cases of non-conformity. Thus *H. erato,* on the basis of late larval, pupal and emergent behavior, is obviously closely related to *H. melpomene.* In its general larval behavior, however, even including molting, this is masked by modifications which probably arose in relation to its particular food plant. There are indications that it may originally have lived on a vine other than its present *P. tuberosa* and it has been suggested that this was somewhat like the thick-veined *P. laurifolia* on which *H. melpomene* and *H. ricini* feed.

Thus the behavior of larval lepidoptera can clearly be of taxonomic significance. The point which is perhaps of more importance is that such behavioral features, together with those of pupal and adult stages, are essential to form a picture of evolutionary relationships which is anywhere near complete. Taxonomists once favored the use of characteristics which are as far as possible independent of the environment, preferentially those with no apparent functional significance: in the past features which could be regarded as adaptive have been taxonomically suspect. There is now a wider recognition of the fact that to understand the pathway along which an animal has evolved, its mode of life, in as many aspects as possible, must be considered. While nobody is ever likely to suggest that animals be classified on their behavioral characteristics alone, or on their physiology, biochemistry or ecology, such disciplines are essential in interpreting anatomical features.

VII. Summary

1. Behavioral observations were made on cater-

pillars of butterflies of the subfamily Heliconiinae. Some of these observations concern 11 of the 14 species present in Trinidad, some concern 10 and some 9.

2. A brief description is given of the general molting behavior. This comprises a quiescent period, the movements during the actual shedding of the skin, expansion of the scoli, hardening and darkening of the cuticle and a further period of rest. The larva then turns to eat its cast skin before resuming normal feeding. Slight systematic differences occurring within the behavior are described.

3. It is established that caterpillars will eat several cast skins, one after the other, and that they do not distinguish between those of their own and other species. The age of the exuvium is of no significance but that of a fifth instar animal, *i.e.*, last larval molt, is usually rejected.

4. Behavior leading to the selection of a pupation site is described. This involves a locomotory phase and some estimation of the amount of free space beneath a potential site. Differences in the preferred pupation sites of the species are described and the significance briefly considered.

5. Behavior leading to the attachment of a late fifth instar larva to its silk pad is described and the results of experimental interference related. The degree of flexibility of attachment, the extent of movement, stridulation and production of odor are compared in the ten species whose pupae were studied. These features are correlated with pupation site, form and color of the pupae.

6. Emergence of a butterfly from its pupa is described and specific differences in this behavior noted. Observations made when recently emerged butterflies were forced to walk for varying periods before wing expansion are described and the implications of the results are discussed in relation to larval behavior and the pupation site.

7. The phylogenetic implications of the systematic differences in molting, late larval, pupal and emergent adult behavior are discussed. Practically all the information suggests the same picture of the relationship of the species concerned. This coincides to a great extent with that drawn from considering general larval behavior.

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EXPLANATION OF THE PLATE

Plate I

Pupa of *H. aliphera*, showing the dorso-ventral bend in the body which causes it to lie almost parallel to the surface under which it is attached, in this case a sheet of glass.

FIG. 1

A STUDY OF THE BIOLOGY AND BEHAVIOR OF THE CATERPILLARS, PUPAE AND EMERGING BUTTERFLIES OF THE SUBFAMILY HELICONIINAE IN TRINIDAD, W.I.

12

Melanoma, Renal Thyroid Tumor and Reticulo-endothelial Hyperplasia in a Non-hybrid Platyfish[1]

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(Plates I & II; Text-figure 1)

MELANOMAS consistently occur in certain platyfish-swordtail and other interspecific hybrid combinations among fishes of the genus *Xiphophorus*. Tumors of the thyroid gland develop in certain species and strains of *Xiphophorus* when they are maintained in iodine-poor water. Genetic factors are important in the etiology of these atypical growths of pigment and thyroid cells (Gordon, 1958; Atz, 1959; Baker, 1958a; Mac Intyre, 1960).

The discovery, in our laboratory, of a *nonhybrid* platyfish with a melanoma evoked considerable interest, and when this specimen was also found to exhibit tumorous thyroidal growths in both the pharyngeal region and kidney, an abnormal pituitary gland and hyperplasia of reticulo-endothelial cells, the value of studying it in detail became even more apparent. During the study large pigmented masses, interpreted as melanin-laden macrophages, were found in the kidney—a phenomenon not known to have been previously described.

In these fishes, melanosis necessarily precedes melanoma. One melanotic specimen of the same species was found and is described.

DESCRIPTION OF MELANOMATOUS FISH WITH THYROID TUMOR

History and Gross Anatomy.—The specimen was an adult male spike-tailed platyfish, *Xiphophorus variatus xiphidium,* from the sixth laboratory-reared generation of fish collected in 1939 from the Rio Purificacion, Tamaulipas, Mexico (strain 53). The identification was verified by Dr. Donn E. Rosen of the American Museum of Natural History. The fish measured 28 mm. in standard length and 12 mm. in depth. It carried the dominant sex-linked gene, *Sp,* for macromelanophore spotting on the body. In this specimen much of the body was intensely black. Externally a swelling, which was caused by the melanoma, was visible on the right side underneath the dorsal fin (Text-fig. 1). The extent of the swelling was approximately 8 × 6½ × 2 mm. Its surface was smooth. The thyroid tumor was not visible externally.

The specimen was fixed in Bouin's fluid, decalcified in formic acid, embedded in paraffin and sectioned at 3, 7 and 10 microns; staining was with Harris's hematoxylin and eosin, Masson's trichrome stain or Heidenhain's iron hematoxylin. Before staining, the melanin in some sections was bleached with hydrogen peroxide or with potassium permanganate and oxalic acid.

Microscopic Appearance of the Melanoma.—The melanoma was essentially similar in microscopic appearance to those previously described in hybrids between *Xiphophorus maculatus* and *X. hellerii* by Reed & Gordon (1931), Gordon & Smith (1938), and others. It has caused extensive destruction of the body musculature (Plate I, Fig. 2). The dermis was infiltrated with tumor cells, and it contained melanin-laden macrophages. The epidermis was considerably thickened in some regions and also contained melanin-laden macrophages (Fig. 3). The scales were in abnormal positions.

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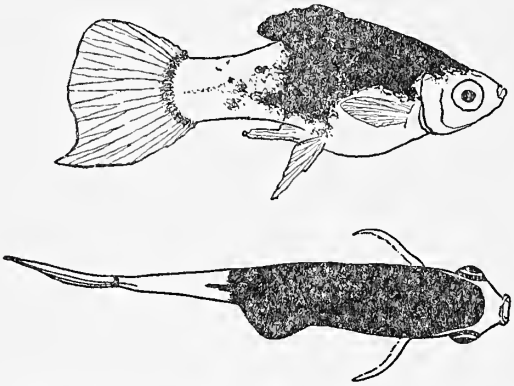
The predominant type of cell in the melanoma was the lightly pigmented melanocyte. There were scattered melanin-laden cells — macrophages and melanophores.[3] In areas adjacent to muscle cells, the melanocytes were arranged in whorls, and their nuclei were spindle-shaped and small, generally 2½ × 5 microns (Fig. 4). Elsewhere the nuclei of the melanocytes were round, oval or irregular and generally somewhat larger; the size varied but the majority were under 5 microns in diameter (Fig. 5). The cell outlines of the melanocytes were indistinct. The nuclei contained one or more prominent nucleoli. Mitotic figures were rare. A number of nuclei showed median or budlike constrictions, possibly indicating amitosis. Some giant nuclei were observed (Fig. 6).



Text-fig. 1. Lateral and dorsal views of a male *Xiphophorus variatus xiphidium* with a melanoma.

Microscopic Appearance of the Thyroid Tumor.—The pharyngeal thyroid was markedly hyperplastic and extended into the bases of the gills (Plate II, Figs. 10, 11). This hyperplasia was chiefly made up of small to large follicles, all filled with dense, acidophilic colloid. The colloid was not vacuolated and varied from a very dense homogeneous condition in the small follicles to a course granular state in the large ones. Some afollicular cells occupied interstices between the follicles. Thyroid tissue was present in small amounts in the chorioid gland of the eye and was either follicular with granular colloid or afollicular. In the kidneys, thyroid tissue was represented by many large, colloid-filled follicles, similar to those in the pharynx, and by swollen cystic follicles almost or completely devoid of colloid (Figs. 12, 13). No thyroid tissue was seen elsewhere in the body. The renal thyroid was different from the thyroid tumors in the kidneys of *Xiphophorus maculatus* described by Baker *et al.* (1955) in that the normal kidney tissues were less disturbed by the proliferation of thyroid tissue than they were in *X. maculatus* during the late stages of tumor growth and that large amounts of colloid were present throughout both the pharyngeal and renal thyroid masses.

Hyperplasia of the Pituitary Gland.—Although the pituitary gland of this fish was not sectioned or stained in a manner suitable for the demonstration of cell types, it was at once apparent that the gland was abnormal (Plate II, Fig. 14). In the anterior part of the gland, the vertical cross-section was much elongated; the elongation was made up of a homogeneous mass of light-staining basophils. Eosinophils were not delineated by the staining methods employed. Normally, at that cross-sectional level, the pituitary is round and chiefly composed of darkly staining basophils; the paler cells appear in small numbers along the ventral border and increase somewhat in the posterior direction, as the neural portion of the hypophysis appears. In the tumorous fish, the anterior basophilic overgrowth also continued along the entire length of the pituitary, making the entire organ misshapen.

Hypertrophy of the paler basophilic elements of the pituitary gland has been found in *X. maculatus* with goitrous thyroids or with thyroid tissue regeneration following radioiodine treatment (Baker-Cohen, unpublished data). In such fish, however, the proliferation of pale basophils did not assume the proportions seen in the tumorous *X. v. xiphidium.*

Hyperplasia of the Reticulo-endothelial System.—In the kidney there were numerous areas of cells heavily laden with melanin (Plate II, Figs. 12, 13). These cells were spherical and from 5 to 10 microns in diameter. Their nuclei, which were visible only in bleached sections, were compressed at the periphery of the cells because of the large amount of melanin. The nuclei were oval or irregular in shape, generally 1 to 3 microns long and ½ to 1½ microns wide (Figs. 8, 9). In the anterior part of the melanoma there was a distinct area that consisted of a large mass of melanin-laden cells similar to the ones described above. This appeared to be a distinct growth and not part of the melanoma proper. This mass was ventral to the vertebral column, surrounding the urinary ducts and dorsal to the air bladder, which it compressed (Plate I, Fig. 7). Posteriorly this mass was reduced to a few cells ventral to the vertebral column (Fig. 1). A few such cells were found in

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[3] In lower vertebrates, the melanocyte, which is the melanin-synthesizing cell common to all classes of vertebrates, differentiates into a pigment-effector cell called the melanophore; the melanophore is not a pigment-carrying macrophage (Gordon, 1953).

the liver, surrounding the brain, and in blood vessels within the brain. These cells appear to be macrophages, present in hyperplastic proportions (see Discussion).

Closely associated with the melanin-laden macrophages in the kidney were many nodular areas containing pale cells with indistinct outlines and round nuclei (2½ to 5 microns in diameter). Scattered macrophages were found in many of these nodules. We interpret these pale cells as primitive reticular cells, present in hyperplastic proportions. Some of these cells were also found associated with the area of melanin-laden cells in the anterior part of the melanoma. Extensive nodules of these cells were found among the pharyngeal thyroid follicles (Fig. 11). In the pharyngeal area, macrophages were rarely found in these nodules.

DESCRIPTION OF MELANOTIC FISH

History and Gross Anatomy.—This specimen also was a laboratory-reared, adult male *X. v. xiphidium.* It was identified by Dr. Donn E. Rosen. The fish measured 27 mm. in standard length and 11 mm. in depth. It carried the *Sp* gene; in this specimen the body was almost completely black (blacker than the body of the melanomatous fish). The specimen was fixed in formalin, decalcified in formic acid, embedded in paraffin and sectioned at 7 and 10 microns; staining was with Harris's hematoxylin and eosin.

Microscopic Appearance of the Melanosis.—In the posterior part of the body, melanophores were present not only in the dermis but had spread along the septae of the body musculature. Some lightly pigmented melanocytes had also spread among the muscles, and in the left dorsal side melanocytes had multiplied sufficiently to compress some of the muscles. There was slight destruction of the musculature. The epidermis in this area was thickened. It is not possible to say whether, if the fish had remained alive, this melanosis would have developed into a melanoma.

Microscopic Appearance of the Thyroid Tissue and Pituitary Gland.—The pharyngeal thyroid appeared normal. It consisted of a number of follicles, 20 to 175 microns in diameter, none containing colloid (probably an artifact, resulting from formalin fixation). The follicular cells were squamous to cuboidal. No thyroid tissue was observed in the kidney. The kidney contained many pearl-like basophilic concretions, similar to those described by Berg *et al.* (1954) in regressing thyroid tumors that had been treated with potassium iodide.

The hypophysis of this fish, although not well preserved because of the slow formalin fixation, appeared to be normal. Its structure seemed to be the same as seen in *X. maculatus.*

Macrophage Hyperplasia.—There was a large mass of spherical melanin-laden cells, similar to those described as macrophages in the melanomatous *X. v. xiphidium,* ventral to the vertebral column, surrounding the urinary ducts and urinary bladder. Numerous masses of these cells were present in the kidney, and some were found in the lining of the air bladder. Some primitive reticular cells were also present in the kidney.

DISCUSSION

Melanoma.—Thousands of wild and laboratory-reared poeciliid fish, including *Xiphophorus variatus xiphidium,* have been examined in this laboratory and elsewhere, but melanomas have been found only in interspecific hybrids, with the exception of the *Fu* (*fuliginosus*) domesticated strain of *X. maculatus* (Kosswig, 1938) and a few specimens of *X. montezumae cortezi* carrying the genetic factor for spotted caudal peduncle, *Sc* (unpublished data). The macromelanophore genes in platyfish require genetic buffering to control their growth, which is upset in hybrid combinations (Gordon, 1958; Atz, 1959). In the melanomatous *X. v. xiphidium,* which carried the *Sp* gene for macromelanophore spotting, the normal genetic constitution of the species was presumably altered, perhaps as a result of mutation or inbreeding.

Thyroid Tumor.—The discovery of extensive thyroidal proliferation in the kidneys of the melanomatous *X. v. xiphidium* adds another species to the growing roster of teleosts in which extra-pharyngeal thyroid tissue has been found. These have been summarized recently by Baker (1959) and Baker-Cohen (1959). In all cases to date, the fish exhibiting displaced thyroid tissue have had some history of a domesticated environment, sometimes with a known iodine deficiency, and all were freshwater species.

Both the pharyngeal and renal thyroidal growths in the *X. v. xiphidium,* like those described in a cherry barb, *Barbus titteya* (Baker, 1959), and unlike those reported in common platyfish, *X. maculatus* (Baker *et al.,* 1955), were extensively colloidal. They thereby more nearly resembled colloid goiter than adenoma, as described in man.

Thyroid tumors have rarely been found in this species. However, they may be more frequent than previously supposed if they are not visible externally, as was the present one. Thyroid tumors in poeciliid fish generally produce externally-visible swellings of the gill region or of the body.

Relation between the Melanoma and Thyroid Tumor.—Stolk (1959) recently reported that the incidence and development of melanomas was increased in thiouracil-fed (hypothyroid) platyfish-swordtail hybrids and reduced in thyroxine-fed (hyperthyroid) ones. Similar results were obtained by Meites (1958) for carcinogen-induced skin tumors in Swiss mice; his article gives references to the few and contradictory studies that have been made on the influences of the thyroid gland on tumor growth.

Endocrine secretions, particularly certain pituitary hormones, play an important role in pigment cell formation and maturation and in melanin synthesis in normal fish (Pickford, 1957; Chavin, 1959; Kosto *et al.,* 1959). Considering the extreme rarity of melanomas in *X. v. xiphidium,* it is conceivable that the development of the melanoma described here might have been influenced by the abnormal state of the fish's endocrine system, particularly in view of the unusual state of hyperplasia of the hypophyseal basophils.

Hyperplasia of the Reticulo-endothelial System.—Morphologically, the melanoma and melanosis described here are similar to those reported in platyfish-swordtail hybrids, with the exception of the large number of heavily melanin-laden cells in the kidney and ventral to the vertebral column. Metastases are very rare in fish melanomas. Breider (1938, 1953) described pigment deposits in the kidneys of melanomatous platyfish-swordtail hybrids. He considered them to be acellular pigment deposits but did not make any studies of bleached sections; the deposits shown in his figures appear very similar to the masses of melanin-laden cells found in our *X. v. xiphidium.* Mac Intyre (unpublished data) has found similar, though less extensive, masses in the kidneys of some melanomatous *X. maculatus* belonging to the *Fu* strain and in some melanomatous platyfish-swordtail hybrids. We do not believe that these represent metastases, but rather macrophages that have both multiplied to hyperplastic proportions and massed in these areas. Cell movement alone could not account for the large quantity of these cells.

What happens to melanin after it has been produced by the melanocyte is not fully known (Dalton & Felix, 1953). Much of it is transferred to macrophages. The recent findings of Speece *et al.* (1959) in human melanomas indicate that macrophages not only ingest dead cells but pick up melanin discharged into the intercellular fluid by active tumor cells. Observation of cut pigment cells in amphibians showed that some melanin-containing macrophages remained at the area of ingestion, while others migrated and entered the blood stream (Lehman, 1953). Melanin debris may not be immediately ingested by macrophages; it may be removed from tissue spaces by the lymphatics (Smith, 1931) and thus carried to lymph nodes or other areas of macrophage concentration. Ultimately, the melanin may be broken down intracellularly in macrophages, or, as Cowdry (1950) stated, "once formed, melanin is a very stable substance and may be excreted in the urine." In fish and amphibians, some melanin-containing macrophages are sloughed off through the epidermis (Goodrich & Hansen, 1931; Gordon & Lansing, 1943; Niu, 1959). Niu (1959) has suggested that in amphibians the pigment debris is generally carried by macrophages to the liver, where it is eliminated via the biliary system.

In teleost fishes, the kidney is the organ richest in lymphoid tissue (Bertin, 1958) and the principal hemopoietic organ (Grassé, 1958). In the kidney sinuses of normal poeciliid fish, large numbers of lymphocytes and erythrocytes and small numbers of primitive reticular cells and macrophages are found. Primitive reticular cells can differentiate into lymphocytes and macrophages (Walvig, 1958). Studies in the eel have shown that injected dye and bacteria are picked up chiefly by lymphoid cells in the kidney (Schmidt, 1959). Bertin (1958) stated that ink or carmine injected into a fish's body is eliminated in the urine, after several hours, across the renal lymphoid tissue.

In the two specimens of *X. v. xiphidium,* histological observations indicate that much of the enormous amount of melanin produced by the melanoma and melanosis was picked up by macrophages in the kidney, which were evidently not able to eliminate the melanin, by intracellular digestion or by transfer into the urine, at the rate that it was produced by the abnormally large number of melanocytes. In addition to this process, much of the melanin was phagocytized by macrophages in the immediate area, some of which remained there and others of which were sloughed off through the epidermis. That these are methods of melanin disposal in poeciliid fishes is confirmed by observations we have made in normally pigmented fish: *e.g.* small numbers of macrophages containing melanin are customarily found in the kidney and other lymphoid tissues.

The nodules of primitive reticular cells found in the kidney and in the pharyngeal thyroid tumor resemble cells that were found by Rasquin & Rosenbloom (1954) in *Astyanax* raised in the dark and described by them as reactive hyperplastic reticulo-endothelial cells. Baker (1958b) found similar masses, associated with degenerating thyroid follicles, in *X. maculatus* whose renal thyroid tumors had been treated with potassium iodide.

Relations between the Reticulo-endothelial Hyperplasia and the Melanoma and Thyroid Tumor.—Several studies have been made in mammals of the relation between the reticulo-endothelial system and neoplastic diseases (*e.g.* Pelner, 1957; Old *et al.*, 1960). The latter found a slight increase of RES activity during the growth of certain spontaneous mouse tumors and suggested that this activation might represent response to some unique property of the tumor, stimulation by products of tumor growth, or response to associated necrosis, hemorrhage, or infection. In the fish with the melanoma and thyroid tumor, we conclude that there was a marked reactive hyperplasia of the reticulo-endothelial system, particularly of macrophages and primitive reticular cells, as a result of the presence of the tumors, for one or more of the above reasons. Reaction to excessive melanin production may have been a causative factor in the hyperplasia, or the melanin phagocytosis may have been a secondary phenomenon. The cells of this reactive hyperplasia were present in such great quantity that they themselves probably caused considerable damage through pressure on the air bladder and kidney tissue.

SUMMARY

Melanomas consistently occur in certain platyfish-swordtail and other interspecific hybrid combinations of xiphophorin fishes. A melanoma in a non-hybrid xiphophorin, the spike-tailed platyfish, *Xiphophorus variatus xiphidium,* is described, in which the normal genetic mechanisms buffering pigment cell growth were presumably upset. The melanoma consisted mainly of lightly pigmented melanocytes and had destroyed much of the body musculature on the right side. This fish had an extensive thyroid tumor in the pharyngeal region and in the kidney, consisting of colloid-filled and swollen cystic follicles. In the pituitary gland, a hyperplasia of light-staining basophils was present.

In addition to these abnormal growths, there was a marked reactive hyperplasia of the reticulo-endothelial system, consisting of large masses of melanin-laden macrophages in the kidney and ventral to the vertebral column, and of nodules of primitive reticular cells in the kidney and in the pharyngeal thyroid tumor. The disposal of melanin and the reticulo-endothelial system in fish are discussed; it appears that much of the melanin produced by pigment cells is picked up by phagocytic cells in the lymphoid tissue of the kidney.

Another specimen of *X. v. xiphidium* is described, in which melanosis was present. Large masses of melanin-laden macrophages were also found in its kidney and ventral to the vertebral column.

ACKNOWLEDGMENTS

We wish to acknowledge our gratitude to the late Dr. Myron Gordon for encouraging this research and to Dr. James W. Atz for critical reading of the manuscript.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. Cross section of a melanomatous male *Xiphophorus variatus xiphidium* through the posterior part of the melanoma, which consists mainly of lightly-pigmented melanocytes. 13×.

FIG. 2. Infiltration of the musculature by melanocytes. 140×.

FIG. 3. Thickening of the epidermis. 260×.

FIG. 4. Bleached section in an area of muscle infiltration, showing whorls of melanocytes with small spindle-shaped nuclei. The large dark objects are muscles. 345×.

FIG. 5. Bleached section from a more central part of the tumor. The nuclei are larger and round or irregular in shape. 920×.

FIG. 6. A giant nucleus. 700×.

FIG. 7. Cross section through the anterior part of the melanoma. To the left are melanocytes which have invaded the musculature. A large hyperplastic mass of melanin-laden macrophages occupies part of the region where the air bladder is normally located. 70×.

PLATE II

FIG. 8. Bleached section showing the edge of the melanin mass of Fig. 7. At the right are melanin-laden macrophages which, after bleaching, appear as empty shells. At the left are melanocytes. 450×.

FIG. 9. Bleached melanin-laden macrophages. The small, irregularly-shaped nuclei are compressed at the periphery of the cells. 945×.

FIG. 10. Thyroid tumor in the same fish. Pharyngeal region. 70×.

FIG. 11. Detailed view of pharyngeal region. At the right are thyroid follicles. At the left is a mass of hyperplastic primitive reticular cells. 375×.

FIG. 12. Thyroid tumor in the kidney showing large colloid-filled follicles and a large cyst at the upper right. The solid black areas are groups of hyperplastic macrophages, laden with melanin. 70×.

FIG. 13. Detailed view of melanin-laden macrophages and thyroid tissue in kidney. 375×.

FIG. 14. Hyperplasia of light-staining basophils in the pituitary gland of the same fish. 100×.


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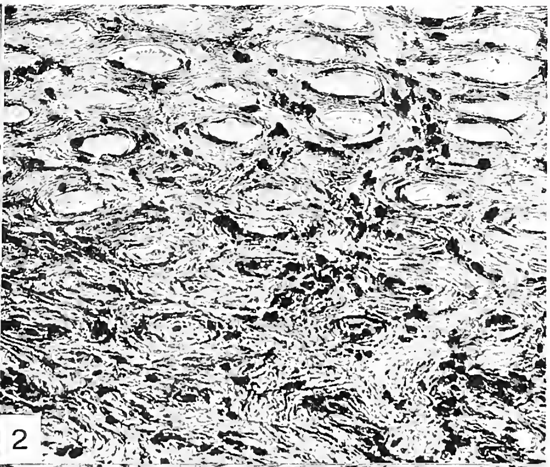
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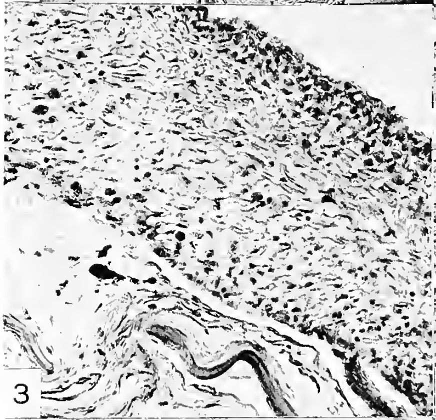
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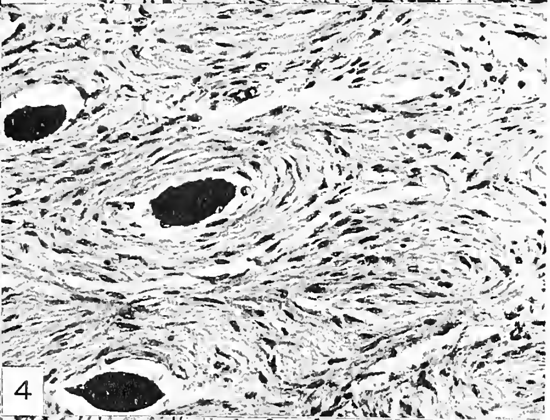
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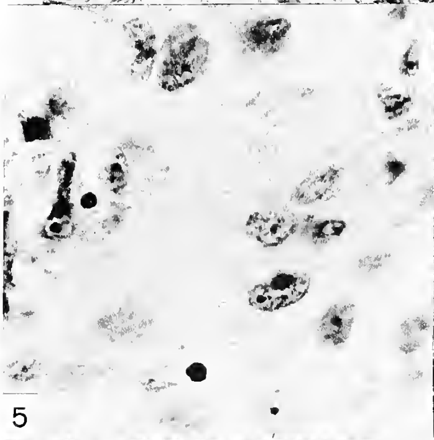
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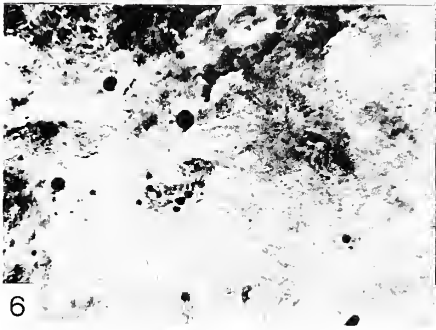
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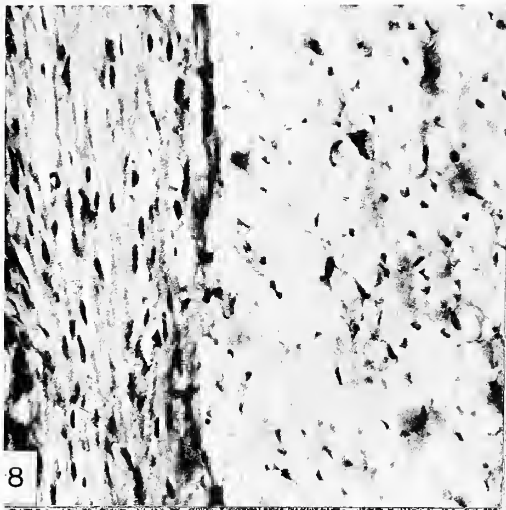
MELANOMA, RENAL THYROID TUMOR AND RETICULO-ENDOTHELIAL HYPERPLASIA IN A NON-HYBRID PLATYFISH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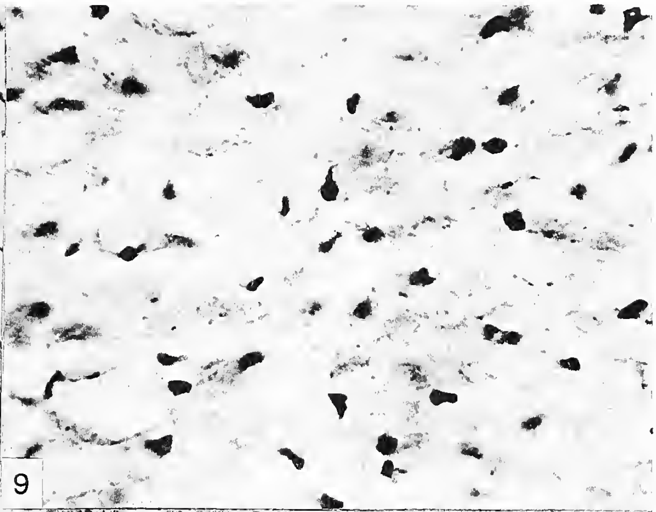
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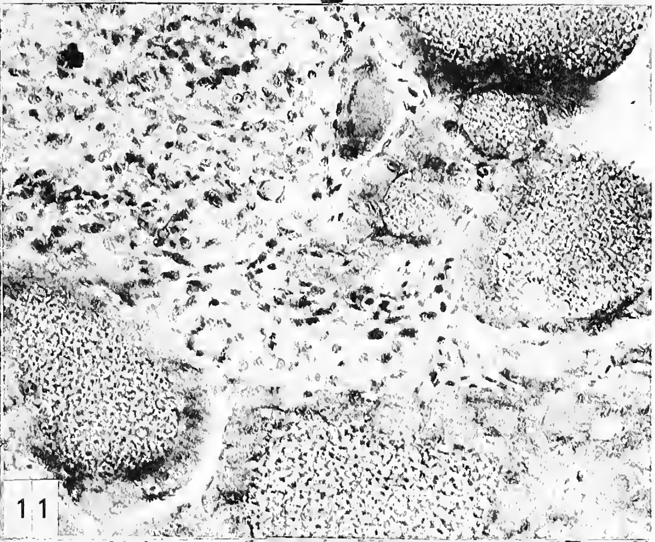
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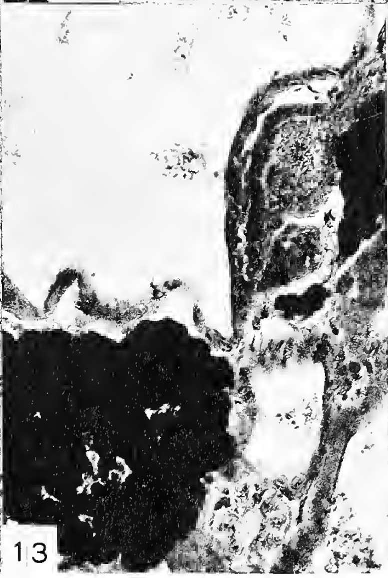
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MELANOMA, RENAL THYROID TUMOR AND RETICULO-ENDOTHELIAL HYPERPLASIA IN A NON-HYBRID PLATYFISH

13

Eastern Pacific Expeditions of the New York Zoological Society. XLV. Non-intertidal Brachygnathous Crabs from the West Coast of Tropical America. Part 2: Brachygnatha Brachyrhyncha[1,2]

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Allan Hancock Foundation,
University of Southern California

(Plate I; Text-figures 1 & 2)

[This is the forty-fifth of a series of papers dealing with the collections of the Eastern Pacific Expeditions of the New York Zoological Society made under the direction of William Beebe. The present paper is concerned with specimens taken on the Templeton Crocker Expedition (1936) and the Eastern Pacific "Zaca" Expedition (1937-1938). For data on localities, dates, dredges, etc., refer to *Zoologica,* Vol. XXII, No. 2, pp. 33-46, and Vol. XXIII, No. 14, pp. 287-298.]

Contents

Tribe Brachyura
Subtribe Brachygnatha
Superfamily Brachyrhyncha
Family Portunidae

Family Xanthidae

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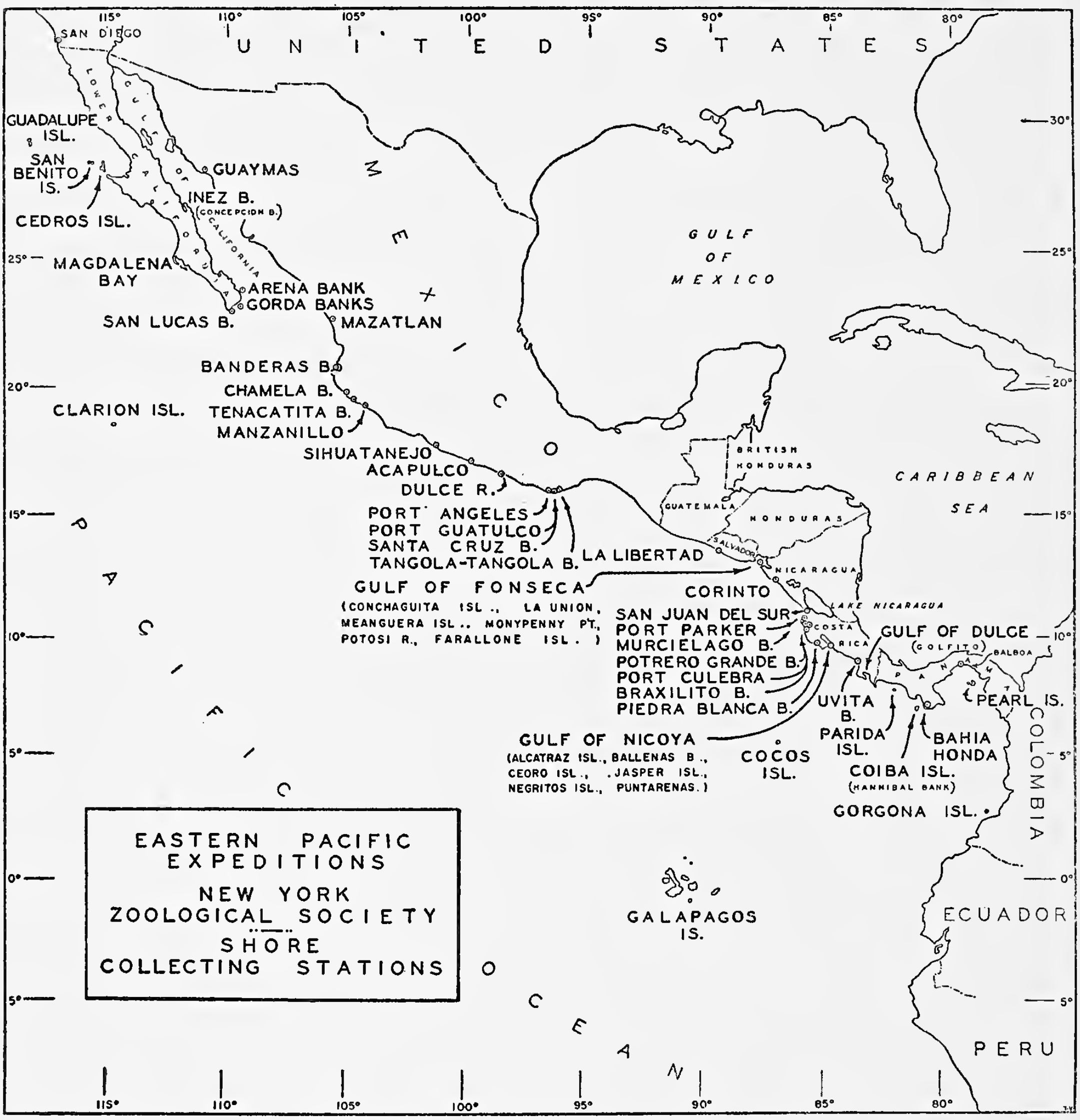
[1] Contribution No. 1011, Department of Tropical Research, New York Zoological Society.

[2] Contribution No. 243, Allan Hancock Foundation, University of Southern California.

INTRODUCTION

THE brachygnathous crabs of the families Portunidae, Xanthidae, Goneplacidae and Cymopoliidae constitute the subject matter of the second part of this paper, the Majidae and Parthenopidae having been treated in part one. The Pinnotheridae are not included, since they present problems in identification not encountered in the other families, which for the area covered are much better known.

The general statements made in the introduction to part one apply equally to part two. Insofar as these were restricted to the Oxyrhyncha, however, they need supplementation for the



TEXT-FIG. 1. Shore collecting stations of the Eastern Pacific Expeditions of the New York Zoological Society. For exact locations of associated dredge stations, refer to *Zoologica*, vol. XXII, no. 2, and vol. XXIII, no. 14.

Brachyrhyncha. Of this group the cancroid or cyclometopous crabs were the subject of monographic treatment as recently as Rathbun (1930), while the grapsoid or catometopous crabs have received no comprehensive survey since Rathbun (1918). It is to be expected, therefore, that the largest number of new records will be found among the latter group.

Ecological Considerations

As in the earlier part of this report, the field notes on color, behavior and food habits provided by Miss Jocelyn Crane have been utilized freely and fully to supplement the routinely systematic portions of this paper. Of the 15 species of Portunidae, color in life is recorded for all but a few; these perhaps were not recognized as different in the field. Color notes on populations of the same species from widely separated localities are included in order to establish a basis for a future consideration of the geographical variation of color and pattern. Notes on behavior are given for *Portunus (Achelous) tuberculatus, Callinectes arcuatus* and *Cronius ruber*. The food habits of *Arenaeus mexicanus* are discussed, and the use of *Euphylax dovii* and *E. robustus* as food for the fish, *Caranx caninus,* is mentioned. Among the Xanthidae, color in life is recorded for but 9 of the 21 species, and usually from but a single locality. Color notes for the Goneplacidae and Cymopoliidae are not available.

The species that are duplicated from Crane's (1947) report on the intertidal forms are few in number. All come from her vertical zones 3 to 7, inclusive. Common to zone 3 (stones near low tide level) are *Xanthodius stimpsoni* and *Pilumnus pygmaeus*. Common to zone 4 (tidepools) are the above two plus *Menippe obtusa*. Common to zone 5 (*Pocillopora* coral) are *Medaeus spinulifer, Xanthodius stimpsoni* and *Micropanope xantusii*. Common to zones 6 and 7 (mangroves and mudflats, respectively) are *Panopeus purpureus* and *Eurytium tristani*. The fact that the *Pocillopora* coral zone is more properly subtidal or adtidal than intertidal, and that mangrove and mudflat biotopes continue below low-tide level, was discussed in the previous section of this report (Garth, 1959). Furthermore, since the specimens of *Menippe obtusa, Panopeus purpureus* and *Eurytium tristani* here reported carry no indication of depth, while *Micropanope xantusii* and *Pilumnus pygmaeus* were taken once each in a dead pearl oyster, also without indication of depth, it is possible that all five species were collected intertidally, but that lacking also evidence assigning them to a particular intertidal zone or habitat, they were set aside for later treatment with the non-intertidal material. Certainly, they form a marginal group when considered in this category.

Geographical Considerations

The present collection adds appreciably to our knowledge of distribution within the Panamic faunal province, broadly defined as extending from Lower California and the Gulf of California to southern Ecuador or northern Peru. (See Text-fig. 1).

Of species heretofore known to occur in the Gulf of California, the following may be reported as having ranges extended southward along the mainland, those marked with an asterisk having been reported previously from the Galapagos Islands (Garth, 1946) as well: *Euryplax polita* to southern Mexico, **Micropanope (?) maculatus, Speocarcinus granulimanus* and *S. californiensis* to Costa Rica, *Portunus (Achelous) iridescens* and **Micropanope polita* to Panama. Of species known to occur in Mexico south of Cape Corrientes, *Hexapanopeus orcutti* and *Pilumnus stimpsonii* may be reported southward to Costa Rica, while of species known to occur in Costa Rica, *Hexapanopeus nicaraguensis* and *H. costaricensis* may be reported southward to El Salvador and Panama, respectively.

Of species known to occur in Peru, *Speocarcinus ostrearicola* may be reported northward to Nicaragua; known to occur in Colombia, *Pseudorhombila xanthiformis* may be reported northward to Costa Rica; known from Panama, *Pilumnus limosus* and *Chasmophora macrophthalma* may be reported northward to west Mexico; while *Menippe obtusa* and *Pilumnus pygmaeus,* known from Nicaragua and Costa Rica, respectively, may be reported northward to southern Mexico. (See also Table I).

Three species, **Medaeus spinulifer, *Micropanope polita* and *Cymopolia lucasii,* are reported for the first time from Clarion Island, Mexico. An important Panama record for *Heteractaea peterseni* links the previous records from Colombia and the Gulf of California for that species. The southern record of *Arenaeus mexicanus* of Callao, Peru, is confirmed.

Systematic Considerations

The 45 species of Brachyrhyncha compare with the 44 species of Oxyrhyncha previously reported in part one of this paper. The number of species by families is as follows: Portunidae, 15; Xanthidae, 21; Goneplacidae, 8; Cymopoliidae, 1. While the total number of species for the eastern Pacific is not as readily available for the Brachyrhyncha as for the recently monographed Oxyrhyncha (Garth, 1958), it may be stated that of the Portunidae, largely a tropical

TABLE I. EXTENSIONS OF RANGE

	From	To
XANTHIDAE		
Medaeus spinulifer	Mainland	Clarion I.
Hexapanopeus costaricensis	Costa Rica	Panama
Hexapanopeus nicaraguensis	Costa Rica	El Salvador
Hexapanopeus orcutti	NW Mexico	Costa Rica
Micropanope polita	Gulf of California, Galapagos	Clarion I., Panama
Micropanope (?) maculatus	Gulf of California, Galapagos	Costa Rica
Menippe obtusa	Nicaragua	Mexico
Pilumnus pygmaeus	Costa Rica	Mexico
Pilumnus limosus	Panama	Mexico
Pilumnus stimpsonii	Mexico	Costa Rica
GONEPLACIDAE		
Pseudorhombila xanthiformis	Colombia	Costa Rica
Euryplax polita	Gulf of California	S Mexico
Chasmophora macrophthalma	Panama	Mexico
Speocarcinus granulimanus	Gulf of California	Costa Rica
Speocarcinus californiensis	Gulf of California	Costa Rica
Speocarcinus ostrearicola	Peru	Nicaragua
CYMOPOLIIDAE		
Cymopolia lucasii	Gulf of California, Galapagos	Clarion I.

family and exclusively estuarine or pelagic, the "Zaca" obtained a complete representation for the territory covered, lacking only the few endemics from Chile-Peru, the Galapagos, and the Gulf of California (the latter obtained by the "Zaca" in 1936 and reported by Crane in 1937) to complete the list of species known from the entire eastern Pacific.

Among the Xanthidae, a new species of *Hexapanopeus* is described from Corinto, Nicaragua, and the megalops of *Quadrella nitida* is described and figured for the first time. The depth range is increased for several species, among them *Pilumnus stimpsonii* and *P. limosus. Panopeus convexus* Bott (not A. Milne Edwards) is considered a synonym of *Eurytium tristani* Rathbun, the subspecies *minor* Bott (1955) as belonging to that species also, hence a new combination.

It is among the Goneplacidae, however, that the greatest number of "firsts" has been established. The first specimens since the types may be reported for *Pseudorhombila xanthiformis* Garth, *Speocarcinus ostrearicola* Rathbun and *Hexapus williamsi* Glassell. Moreover, each was known from but a single specimen, and the opposite sex of each is now made known, *i.e.*, the male of *Pseudorhombila*, the female of the other two.

RESTRICTION OF SYNONYMIES

In keeping with the format established in the earlier section of this report, synonymies are restricted to the original description, the first use of the name in its current combination, and the citation placing it in the territory covered, if not included in the above two. Reference is also made to the appropriate monograph of Rathbun, either the cancroid (1930) or the grapsoid (1918) volume, and to all reported occurrences of the species in the eastern tropical Pacific since then.

MEASUREMENTS

To the total length of the largest and smallest specimen examined in each class, male, female and ovigerous female, as given for the Oxyrhyncha, a second measurement, that of total width including spines, if any, has been added. In the Portunidae, where length of lateral spine is frequently a diagnostic character, a third measurement, width excluding spines, is given. Thus the figures 14.5 × 29.5 (21.5) imply length and breadth with (and without) lateral spines. The figures 29.5 – 21.5 divided by 2 will give the length of the lateral spine, in this case, 4 mm.

ACKNOWLEDGMENT

In addition to those to whom gratitude was expressed in the earlier part of this study, the writer wishes to thank Dr. Jens W. Knudsen, Pacific Lutheran College, Tacoma, Washington, an authority on larval development of the Xanthidae, for the illustration of the magalops of *Quadrella nitida* that appears as Text-fig. 2.

SYSTEMATIC DISCUSSION

Tribe BRACHYURA

Subtribe BRACHYGNATHA

Superfamily BRACHYRHYNCHA

Family PORTUNIDAE

Portunus (Portunus) xantusii (Stimpson)

Achelous xantusii Stimpson, 1860, p. 222.

Portunus (*Portunus*) *xantusii*, Rathbun, 1923, p. 620 (part); 1930, p. 50, pl. 18. Glassell, 1935, p. 105.

Not *Portunus* (*Portunus*) *xantusi*, Boone, 1930, p. 163, pl. 56, figs. A, B.

Range. — From Santa Barbara, California (Glassell), to Cape San Lucas, Lower California; Gulf of California at Agua Verde and Concepción bays. (Rathbun, 1930).

Material Examined. — San Benito Islands, west coast of Lower California, Mexico, November 9, 1937, Station 178, L-1 (night light), 8 males, 2 females.

Measurements.—Male specimen, 15.6 × 31.2 (22.6) mm., female specimen, 14.5 × 29.5 (21.5) mm.

Habitat.—Pelagic.

Color in Life.—Exceedingly variable: dullest specimen grayish speckled with black and white, pepper-and-salt fashion. Tips of legs, including chelae, pinkish; ambulatories banded white and brown. Brightest specimen pinkish pepper-and-salt with entire gastric region raspberry red. Others pinkish pepper-and-salt with anterolateral margin and that of front ringed, well inside spines, with black. One with carapace and chelae mottled brown, on the carapace a Y of brown, the prongs extending from the inner margins of the eyes to gastric region, the base along midline through cardiac and intestinal regions to posterior edge of carapace. Gastric and frontal regions between forks of Y rosy pink. Chelae with pinkish chestnut band across base and another across tips. Ambulatories overcast with pink above and below. (J. Crane, field notes).

Remarks.—A megalops possibly of this species was taken at the same time as the adults; it is translucent with large black spots.

Portunus (Portunus) acuminatus (Stimpson)

Achelous acuminatus Stimpson, 1871, p. 112.

Portunus (*Portunus*) *acuminatus*, Garth, 1940, p. 73, pl. 19, figs. 1-3; 1948, p. 33. Not Rathbun, 1930, p. 56, pl. 19.

Range.—From Isabel Island, Mexico, to La Libertad, Ecuador. 2-50 fathoms. (Garth, 1948).

Material Examined.—88 specimens from 12 stations:

Mexico

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 29 males, 26 females (8 ovigerous).

17 mi. SE × E of Acapulco, November 29, 1937, Station 189, D-1, 20 fathoms, 1 male.

4 mi. SSW of Maldonado Point, November 30, 1937, Station 192, D-1, D-2, 26-33 fathoms, 2 males.

Port Guatulco, Station 195, December 4, 1937, D-2, 3 fathoms, 2 young; December 6, 1937, D-11, 5 fathoms, 1 young; D-12, 6 fathoms, 1 young; December 7, 1937, D-19, 17 fathoms, 2 young.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-6, D-7, 7-6 fathoms, 2 young; December 12, 1937, D-14, D-15, 5 fathoms, 3 young; December 13, 1937, D-16, 16 fathoms, 2 young.

Nicaragua

Corinto, January 7, 1938, Station 200, D-27 to D-30, 3 fathoms, 2 young males.

Costa Rica

Port Parker, Station 203, January 20, 1938, D-1 to D-3, 10-15 fathoms, 2 males, 2 females (1 ovigerous); January 22, 1938, D-11, 2-4 fathoms, 1 male.

Port Culebra, January 30, 1938, Station 206, D-2, 14 fathoms, 1 female, 1 young.

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 1 young.

14 mi. S × E of Judas Point, March 1, 1938, Station 214, D-2, D-3, 43-50 fathoms, 1 male.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-4, D-5, 6 fathoms, 1 young male.

Panama

Bahia Honda, March 18, 1938, Station 222, D-1 to D-3, D-5, 3-11 fathoms, 3 young.

Measurements. — Males from 9.3 × 18.5 (14.3) to 16.0 × 41.9 (27.2) mm., females from 8.0 × 16.4 (12.1) to 16.2 × 37.7 (26.8) mm., ovigerous females from 8.0 × 16.4 (12.1) to 15.3 × 36.2 (26.0) mm., young from 5.0 × 9.0 (7.3) mm.

Habitat.—Shelly mud, shelly sand; gravelly mud, gravelly sand; sandy mud; crushed shell; mangrove leaves; rock; dead coral.

Color in Life.—Of an Acapulco, Mexico, specimen: Chestnut mottled with darker. Of Manzanillo, Mexico, specimens: "Plain" and "orange branchialed"; eggs raspberry. Gastric spot present or absent as in *Portunus (Achelous) affinis.* (J. Crane, field notes.)

Remarks.—The identification of this and the following two species of *Portunus (Portunus)* occurring widely throughout the Panamic Province has been facilitated by a prior study (Garth, 1940, p. 73) based on Hancock Expedition material in which the true *P. (P.) acuminatus* (Stimpson) was recognized and a neotype established. The result was to accord equal and full specific rank to *acuminatus* (Stimpson), *panamensis* (Stimpson), and *asper* (A. Milne Edwards) [= *transversus* (Stimpson)], rather than to consider them members of the so-called "*acuminatus-asper-panamensis* group" (Rathbun, 1930, p. 53). The acuminate lateral spine and the slender, almost filiform chelae are diagnostic, now that ample material is available to show these distinctive features.

Portunus (Portunus) asper (A. Milne Edwards)

Neptunus asper A. Milne Edwards, 1861, p. 325, pl. 30, figs. 3-3c.

Portunus (*Portunus*) *asper*, Rathbun, 1930, p. 56, pl. 20, figs. 2, 3, pl. 21, pl. 22, figs. 1, 2. Garth, 1948, p. 33; 1957, p. 36, synonymy.

Range. — From Mazatlan, Mexico, to Chile. To 16 fathoms. (Garth, 1957).

Material Examined.—33 specimens from 10 stations:

Mexico

17 mi. SE × E of Acapulco, November 29, 1937, Station 189, D-1 to D-3, 13-20 fathoms, 2 males, 1 ovigerous female.

Mouth of Dulce River, November 30, 1937, Station 191, D-1, 8 fathoms, 1 male, 1 young.

Port Guatulco, December 6, 1937, Station 195, D-11, 5 fathoms, 1 young; D-12, 6 fathoms, 1 male.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-1, D-2, D-5, 5-9 fathoms, 4 young; December 13, 1937, D-16, 16 fathoms, 2 males, 1 female, 1 young.

El Salvador

Meanguera Island, Gulf of Fonseca, December 23, 1937, Station 199, D-1, 16 fathoms, 1 male.

Costa Rica

Murcielago Bay, January 23, 1938, Station 204, D-1, D-2, D-4, 2-4 fathoms, 2 young.

Port Parker, January 30, 1938, Station ?, depth ?, 1 young male.

Piedra Blanca Bay, Station 208, February 1, 1938, L-1, surface at light, 1 male, 1 ovigerous female, 7 young; February 5, 1938, D-1 to D-3, D-6, D-7, D-9, 3-6 fathoms, 1 male, 2 young.

Cedro Island, Gulf of Nicoya, February 21, 1938, Station 213, L-1, surface at light, 1 male.

Golfito, Gulf of Dulce, March 7, 1938, Station ?, depth ?, 1 female without chelipeds.

Measurements. — Males from 10.2 × 22.0 (15.9) to 41.9 × 96.5 (69.3) mm., females from 14.7 × 32.8 (23.7) to 39.6 × 85 (65.2) mm., ovigerous females from 38.1 × 85.5 (61.7) mm., young from 5.2 × 9.3 (7.6) mm.

Habitat. — Sand, mud, sandy mud, gravelly sand, crushed shell, rocks, algae.

Color in Life.—Of Piedra Blanca, Costa Rica, specimens: 39 mm. ovigerous female and 20 mm. male olive buff above except for white-tipped spines. Carpus, manus and dactyls of ambulatories (but not of swimming legs) lilac. Swimming legs with posterior half of paddle only lilac. Middle of fixed finger with a band of brick red; tips of both dactyls white. Underparts white. Eggs bright orange. 11 mm. young lack the violet and are grayer, not buffy, with suggestions of red bar across fixed finger. (J. Crane, field notes).

Of Gulf of Fonseca, El Salvador, male: Carapace and all legs pale olive brown; a white spot on posterior lateral margin. Movable dactyl dull violet basally, distal part and fixed finger white; dactyls of ambulatories rose red, tips white, distal half of swimmerets violet red. (J. Crane, field notes).

Of Acapulco, Mexico, specimens: Carapace and chelipeds pale olive brown, ridges darker. Ambulatories and cheliped wine colored. Manus and dactyls of swimmerets white with two longitudinal stripes of dark brown or pale buff. One patch faintly visible on posterolateral region of large male; below this, on margin, a white spot. (J. Crane, field notes). Underside white.

Remarks. — The broad anterolateral arc and teeth that show little reduction are characters useful in separating this species from both *Portunus (P.) acuminatus* and *P. (P.) panamensis*, while the heavier cheliped will serve to separate this species from *P. (P.) acuminatus* even in the young, where the relative lengths of the lateral spines might not suffice.

Portunus (Portunus) panamensis (Stimpson)

Achelous panamensis Stimpson, 1871, p. 112.

Portunus (*Portunus*) *panamensis*, Rathbun, 1910, pp. 577, 610; 1930, p. 58, pl. 20, fig. 1, pl. 22. fig. 3, pls. 23, 24. Finnegan, 1931, p. 626, text-fig. 5. Garth, 1948, p. 34.

Range.—From Panama Bay to Bay of Sechura, Peru (from Angeles and Mulege Bays, Gulf of California, Mexico, only if Rathbun's synonymy of *Amphitrite paucispinis* Lockington be accepted). To 33 fathoms. (Garth, 1948).

Material Examined.—115 specimens from 6 stations:

Nicaragua

Corinto, January 5, 1938, Station 200, D-12 to D-19, 1-13 fathoms, 2 young.

Costa Rica

Port Parker, Station 203, January 20, 1938, D-1 to D-3, 10-15 fathoms, 29 males, 24 females (10 ovigerous), 1 young; January 22, 1938, D-4, 7 fathoms, 3 males, 3 females; D-6, 1 fathom, 1 young; D-7, 9-5 fathoms, 1 female; D-8, 9 fathoms, 1 female; D-9, 1.5-4 fathoms, 1 male, 1 female; D-11, 2-4 fathoms, 1 male, 1 ovigerous female; D-12, 2 fathoms, 1 male; D-15, 9-2 fathoms, 2 males, 2 females, 1 young.

?Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-8, 6 fathoms, 1 male, 1 young.

Panama

Bahia Honda, March 18, 1938, Station 222, D-1 to D-3, D-5, 3 fathoms, 2 males, 3 females, 2 young.

Colombia

At sea near Gorgona Island, March 27, 1938, from mangrove seeds floating in tide rip, 5 young, questionably of this species.

Gorgona Island, March 31, 1938, Station 232, D-1, 2-8 fathoms, 1 male, 25 young.

Measurements.—Males from 4.4 × 6.7 (6.0) to 11.2 × 22.0 (17.6) mm., females from 4.5 × 7.3 (6.2) to 11.0 × 21.5 (16.8) mm., ovigerous females from 5.5 to 9.1 (7.8) to 8.6 × 15.6 (12.7) mm., young from 3.0 × 5.0 mm. All but the largest male came from the first Port Parker series.

Habitat.—Sandy and shelly mud, shelly sand, crushed shell, gravel, rocks, coral, algae, and mangrove leaves. (These all from Station 203).

Remarks.—A small species, as shown by the size of the ovigerous females, which would be in the size range of young in either the *Portunus (Portunus) asper* or *P. (P.) acuminatus* series. The young taken at sea off Gorgona Island, the largest of which is only 3.5 mm. in length by 5.6 mm. in width, may be of two species. The two larger specimens show the alternation of large and small anterolateral teeth expected in *P. (P.) panamensis;* the three smaller specimens have anterolateral teeth of equal size, as in *P. (P.) asper.* The species finds its optimum conditions in the shallow bays of Costa Rica and Panama, judging from the tremendous breeding population found by the "Zaca" at Port Parker, and occurs sparingly to the north and south.

Portunus (Achelous) brevimanus (Faxon)

Achelous spinimanus, Faxon, 1895, p. 23. Not *Portunus spinimanus* Latreille.

Achelous brevimanus Faxon, 1895, p. 23.

Portunus (Achelous) brevimanus, Rathbun, 1898, p. 593 (part: not the Galapagos specimens); 1930, p. 68, pls. 29, 30.

?*Portunus (Achelous) spinimanus,* Finnegan, 1931, p. 628. Not *Portunus spinimanus* Latreille.

Range.—Revilla Gigedo Islands, Mexico, and Cocos Island, Costa Rica. (Rathbun, 1930).

Material Examined. — 2 specimens from 2 Revilla Gigedo Islands stations: Sulphur Bay, Clarion Island, May 11, 1936, from night light, 1 male. 3 mi. off Pyramid Rock, Clarion Island, May 12, 1936, Station 136, D-2, 55 fathoms, 1 female.

Measurements. — Male 8.4 × 14.3 (11.3) mm., female 17.6 × 28.0 (23.8) mm.

Habitat.—Not given.

Remarks.—Aside from the suggestion by Glassell (1934, p. 454) that specimens from Perlas Islands, Panama, and Puntarenas, Costa Rica, attributed to *Portunus (Portunus) xantusii* (Stimpson) by Boone (1930, p. 163, pl. 56, figs. A, B), might instead represent Faxon's species, a suggestion questioned by this writer, *P. (Achelous) brevimanus* has not been reported from the Central or South American mainland, unless specimens from Gorgona Island attributed to the Atlantic *P. (Achelous) spinimanus* Latreille by Finnegan (1930) be of this species. Specimens from the Galapagos Islands earlier attributed to *P. (A.) brevimanus* by Rathbun (1898) were subsequently described by her (1902) as *P. (A.) stanfordi.*

Portunus (Achelous) pichilinquei Rathbun

Portunus (Achelous) pichilinquei Rathbun, 1930, p. 78, pl. 37. Crane, 1937, p. 67.

Range.—From Magdalena Bay, west coast of Lower California, and Cape Tepoca, Gulf of California, to Cape San Lucas. 0.5 to 33 fathoms. (Crane, 1937).

Material Examined.—San Lucas Bay, Lower California, Mexico, November 13, 1937, Station 135, D-27, 2-6 fathoms, 1 young male.

Measurements.—Young male, 6.0 × 9.8 (8.2) mm.

Habitat.—Sand bottom.

Color in Life.—Mottled olive and grayish and black. Legs grayish banded with black. Underside pure white. (J. Crane, field notes).

Remarks.—The single specimen was taken in the same dredge haul with *Arenaeus mexicanus.*

Portunus (Achelous) affinis (Faxon)

Achelous affinis Faxon, 1893, p. 155 (part: not the Guaymas, Mexico, specimens); 1895, p. 23.

Portunus (*Achelous*) *affinis,* Rathbun, 1898, p. 595; 1930, p. 80, pls. 38, 39.

Portunus affinis, Coventry, 1944, p. 538.

Range.—From Cape San Lucas, Lower California, Mexico, to Ecuador. (Rathbun, 1930).

Material Examined. — 59 specimens from 5 stations:

Mexico

Tenacatita Bay, November 21, 1937, Station 183, D-2, D-3, 30-40 fathoms, 4 males, 6 females.

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 7 males, 7 females (5 ovigerous).

Port Guatulco, Station 195, December 3-5, 1937, light, 1 male; December 6, 1937, D-13, 8 fathoms, 2 young; D-16, 10 fathoms, 1 male, 7 young; December 7, 1937, D-17, 6 fathoms, 1 young; D-19, 17 fathoms, 1 young male; D-20, 23 fathoms, 2 males; D-21, 18 fathoms, 1 young male.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-1, D-2, D-5, 5 fathoms, 3 young; D-6, D-7, 7-6 fathoms, 1 young; December 13, 1937, D-16, 16 fathoms, 1 young; D-17, 23 fathoms, 13 young.

Colombia

Gorgona Island, March 31, 1938, Station 232, D-1, 2-8 fathoms, 1 young.

Measurements. — Males from 8.0 × 12.5 (11.2) to 25.4 × 44.6 (38.4) mm., females from 14.8 × 25.1 (22.0) to 21.1 × 36.8 (30.9) mm., ovigerous females from 16.0 × 27.3 (24.0) to 21.1 × 36.8 (30.9) mm., young from 4.0 × 5.9 mm.

Habitat. — Sand, mud, sandy mud; gravelly sand, gravelly mud; crushed shell.

Color in Life.—Of Tenacatita Bay specimens: Apricot buff, striations brown, chelipeds and ambulatories, especially inner sides of manus and dactylus, streaked with violet. A constant white spot in middle of posterolateral margin.

Of Manzanillo specimens: White spot on gastric region and spot above base of swimming legs on abdomen may be present, absent, or faint. Posterolateral spot, however, constant. Eggs raspberry. (J. Crane, field notes).

Remarks.—This species is at once separated from the *Portunus (Portunus)* species of the Panamic Province with which it ranges coextensively by its short lateral spine and spinulous merus of the fourth ambulatory (or natatory) leg. In the latter respect it resembles *P. (P.) xantusii* of southern California — west coast of Lower California.

Portunus (Achelous) tuberculatus (Stimpson)

Achelous tuberculatus Stimpson, 1860, p. 223.

Portunus (*Achelous*) *tuberculatus,* Rathbun, 1898, p. 596; 1930, p. 90, pl. 44. Finnegan, 1931, p. 629. Crane, 1937, p. 68. Garth, 1946, p. 421, pl. 71, fig. 2; 1948, p. 34.

Range.—From Cape San Lucas, Lower California, Mexico, to off Ecuador. Galapagos Islands. 3-70 fathoms. (Garth, 1948).

Material Examined.—200 specimens from 6 stations:

Mexico

Chamela Bay, November 17, 1937, Station 182, D-4, 16 fathoms, 1 female.

Port Guatulco, Station 195, December 4, 1937, D-3, 3.5 fathoms, 1 female; December 5, 1937, D-8, D-9, 6-7 fathoms, 4 males, 6 females; December 7, 1937, D-16, 10 fathoms, 2 males, 11 young; D-17, 6 fathoms, 1 male, 2 females, 4 young; D-18, 6 fathoms, 8 males, 6 females.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-1, D-2, D-5, 5-9 fathoms, 1 female, 3 young; D-6, D-7, 6-7 fathoms, 2 males, 2 females (1 ovigerous), 26 young; D-8, 9 fathoms, 4 males, 2 females, 43 young; December 12, 1937, D-9 to D-12, 4.5 to 7.5 fathoms, 3 young; D-13, 10 fathoms, 1 male, 4 young; D-14, D-15, 5 fathoms, 3 males, 3 females (2 ovigerous), 27 young; December 13, 1937, D-16, 16 fathoms, 1 young.

Costa Rica

Port Parker, Arriba rocks, January 16-17, 1938, 2 males; Station 203, January 22, 1938, D-11, 2-4 fathoms, 1 female.

Piedra Blanca Bay, February 5, 1938, D-1 to D-3, D-6, D-7, D-9, 3-6 fathoms, 1 male, 1 young.

Colombia

Gorgona Island, March 31, 1938, Station 232, D-1, 2-8 fathoms, 1 male, 1 female, 22 young.

Measurements. — Males from 6.0 × 11.6 (8.6) to 11.3 × 26.4 (18.3) mm., females from 6.9 × 14.4 (10.5) to 10.2 × 22.9 (16.0) mm., ovigerous females from 7.8 × 15.1 (11.2) to 9.0 × 18.5 (13.7) mm., young from 4.6 × 7.3 (6.3) mm.

Habitat. — Predominantly sand, often with crushed shell or algae; occasionally rock; rarely mud.

Color in Life. — Of Chamela Bay, Mexico, female: General color light brown marbled with black; base of lateral spines tinged with crimson. Legs barred cream and brown. Underparts white (J. Crane, field notes).

Behavior. — Kept alive in aquarium in 2½ inches of water on native sand (coarse sandy bottom with tiny shells). Much more responsive to light than *Cycloes* and more nervous and active; continually changing position, burrowing quickly in sand, hind end first. Digs with ambulatories, kicking sand out forward to chelipeds. Sinks all of self except front and eyes. (J. Crane, field notes).

Remarks. — Of small size but distinctively ornamented with tubercles, and bearing a spine at the posterolateral angles of the carapace, as well as a long, straight lateral spine, *Portunus* (*Achelous*) *tuberculatus* is easily segregated from the several other species of *Portunus* with which it customarily occurs, often in the same dredge hauls.

Portunus (Achelous) iridescens (Rathbun)

Neptunus (*Hellenus*) *iridescens* Rathbun, 1893, p. 240.

Portunus (*Achelous*) *iridescens,* Rathbun, 1930, p. 93, pl. 46. Crane, 1937, p. 66.

Portunus (*Achelous*) *spinicarpus,* Finnegan, 1931, p. 628. Not *Achelous spinicarpus* Stimpson.

Range.—From off Santa Margarita Island, west coast of Lower California, and from off Diggs Point to off La Paz Bay, Gulf of California, Mexico. 18-112 fathoms. (Rathbun, 1930). Gorgona Island. (Finnegan).

Material Examined.—31 specimens from 2 stations:

Costa Rica

14 mi. S × E of Judas Point, March 1, 1938, Station 214, D-2, D-3, 50 fathoms, 13 males, 13 females (6 ovigerous).

Panama

Gulf of Chiriqui, March 13, 1938, Station 221, D-3 to D-5, 35-40 fathoms, 2 males, 3 females.

Measurements.—Males from 14.4 × 29.3 (21.5) to 24.7 × 48.0 (36.5) mm., females from 16.2 × 31.0 (24.9) to 25.3 × 48.5 (37.4), ovigerous females from 17.0 × 36.3 (25.6) to 21.1 × 42.7 (31.3) mm.

Habitat.—Mud; sandy mud.

Color in Life—Not recorded.

Remarks.—Distinguished from all other Pacific Portunidae by the long inner carpal spine, a character shared with the Atlantic *Portunus* (*Achelous*) *spinicarpus* (Stimpson). The Costa Rican and Panamanian localities above would represent the first records for the species from the Bay of Panama, were it not for the previous report of the "St. George" from Gorgona Island, Finnegan (1931) attributing it to the Atlantic species.

Callinectes arcuatus Ordway

Callinectes arcuatus Ordway, 1863, p. 578. Rathbun, 1930, p. 121, pl. 52. Garth, 1948, p. 35; 1957, p. 36, synonymy. Holthuis, 1954, p. 27. Bott, 1955, p. 56.

Range.—From Anaheim Slough, California, to ? south Chile. (Garth, 1957).

Material Examined.—100 specimens from 13 stations:

Mexico

Chamela Bay, North Lagoon, November 17, 1937, 1 male, 3 females (2 ovigerous).

Acapulco Beach, November 26-28, 1937, 1 male.

Honduras

Cutuco and Potosi Lights, Gulf of Fonseca, December 20, 1937, 4 males, 6 females, 3 young.

El Salvador

La Union, Gulf of Fonseca, December 27, 1937, Station 199, D-8 to D-16, 5-6 fathoms, 1 female.

Nicaragua

Corinto, December 29, 1937, Station 200, D-7, 2 fathoms, 1 young; January 7, 1938, D-20 to D-26, 1.5-6.5 fathoms, 2 young. Castenones Lagoon and mid-harbor, January 6, 1938, 4 males, 4 females.

Costa Rica

Port Parker, January 13, 1938, shore, 1 male, 3 females (1 ovigerous), 4 young; January 22, 1938, Station 203, D-9, 1.5-4 fathoms, coral, 1 young.

Port Culebra, January 26, 1938, 8 males, 3 females, 2 young; January 30, 1938, 1 young male.

Piedra Blanca, February 6, 1938, 2 males, 2 females.

Cedro Island, Gulf of Nicoya, February 12, 1938, 1 male.

Golfito, Gulf of Dulce, March 5, 1938, 1 male, 1 female, 4 young; March 9, 1938, Station 218, D-4, D-5, 6 fathoms, 3 young; D-8, 6 fathoms, 2 young; same date, mudflats, 1 young.

Panama

Bahia Honda, March 16, 1938, 3 males, poor condition; March 19, 1938, tidepool, 1 male, lacks chelipeds.

Bella Vista, Panama City, 1944, 5 males.

Canal Zone

Balboa, April, 1938, night light, 3 males, 2 females, 8 young; 1940, 3 males, 5 young.

Ecuador

Puerto Bolivar, April, 1944, 1 male.

Measurements.—Males from 17.0 × 39.6 (29.2) to 42.8 × 98 (75.2) mm., females from 16.0 × 35.2 (26.4) to 51.8 × 102.8 (85.7), ovigerous females from 24.7 × 52 (42) to 51.8 × 102.8 (85.7) mm., young from 5.7 × 12.9 (10.5) mm.

Habitat.—Mud, shells, mangrove leaves; frequently taken in mudflats or shallow lagoons, at Gulf of Dulce "mostly salt, slightly brackish." Came often to shipside light at night. One coral station.

Color in Life.—Of Port Parker male: Carapace dull olive gray-green. Chelipeds olive green dorsally, whitish ventrally, washed with bluish-violet and chelae tipped with pale yellow-brown. Legs turquoise washed with olive; hairs straw gold; swimming legs olive green with suggestion of turquoise, paddles washed with black; hairs straw; tubercles at leg joints golden orange; eyes straw with brownish streaks; underparts pure white. (J. Crane, field notes).

Of Chamela Bay females: Carapace in general blue with olive pile: central portion (without pile) blue-violet; anterolateral margins deep purplish-vinaceous. Base of merus of cheliped olive, inner margin of manus blue-violet, rest of cheliped purplish. Spines of cheliped and tips of anterolateral spines white. Chelae varied, fixed finger usually tipped with white; both fingers barred with purple. Ambulatories Italian blue, hairs olive; swimming legs same with tubercles at joints and all margins narrowly violet; swimming feet sometimes turquoise green. Abdomen violet, joints white; plastron white; under sides of legs, however, colored like upper sides. (J. Crane, field notes).

Behavior.—Of Chamela Bay females: 8 large females [were seen] near the mouth of the lagoon, all with buffy eggs. [Each was] swimming singly, at least 25 feet away from the nearest other one. [There were] no males in the vicinity, nor were there [any] small females. (J. Crane, field notes).

Remarks.—In view of the overlapping ranges of this and the following species, it was considered more than likely that some of the young listed above would prove to be *Callinectes toxotes,* to which only two large males are referred. Specimens from Acapulco Beach, Mexico, Golfito, Costa Rica (Sta. 218, D-8), and Puerto Bolivar, Ecuador, all of which showed blunted or rounded frontal teeth, were compared with specimens of like size in the Hancock collections determined by M. J. Rathbun as *C. toxotes.* Not only did the "Zaca" young fail to show the narrow intramedial area, but they proved dissimilar from *C. toxotes* in other characters as well. It was concluded, therefore, that all young *Callinectes* obtained by the "Zaca" were *C. arcuatus.* (See also *Remarks* under the following *C. toxotes*).

Callinectes toxotes Ordway

Callinectes toxotes Ordway, 1863, p. 576. Rathbun, 1930, p. 127, pl. 54. Garth, 1948, p. 35; 1957, p. 37, synonymy. Holthuis, 1954, p. 27. Bott, 1955, p. 56.

Range.—From Cape San Lucas, Lower California, Mexico, to mouth of River Tumbes, Peru. Juan Fernandez Island, Chile. (Garth, 1957).

Material Examined.—2 specimens from as many stations:

Costa Rica

Piedra Blanca, February 6, 1938, 1 large male.

Golfito, Gulf of Dulce, March 6, 1938, 1 large male.

Measurements.—The two males measured 46.5 × 89.2 (76.4) and 52.8 × 101.3 (87.5) mm., respectively.

Habitat.—Not stipulated, but presumably as in the preceding species.

Color in Life.—Not noted.

Remarks.—A more granulate species than *Callinectes arcuatus, C. toxotes* is further characterized by having the frontal teeth rounded, the middle pair equally advanced with the outer pair in the young. The intramedial area, that portion of the gastric region behind the posterior of the gastric carinae, is as long as its posterior width. These characters are all apparent in young from Costa Rica from among Hancock collections determined for the writer prior to 1935 by the late Mary J. Rathbun. The absence of young, and of mature females, from the "Zaca" series would indicate that *C. toxotes* is much less abundant, and that it may have narrower ecological tolerances than *C. arcuatus.*

Arenaeus mexicanus (Gerstaecker)

Euctenota mexicana Gerstaecker, 1857, p. 131, pl. 5, figs. 3, 4.

Arenaeus mexicanus, Faxon, 1895, p. 22. Rathbun, 1930, p. 137, pl. 58, fig. 1, pl. 61. Garth, 1948, p. 35. Holthuis, 1954, p. 28.

Range.—From Ballenas Bay, Lower California, and Carmen Island, Gulf of California, Mexico, to Ancon, Peru. (Garth, 1948).

Material Examined.—101 specimens from 18 localities:

Mexico

San Lucas Bay, Lower California, November

13, 1937, Station 135, D-27, 2-6 fathoms, 1 male, 3 young.

Chamela Bay, lagoon shore, November 17, 1937, 2 males. Passavera Island, November 19, 1937, 1 male, 3 females (1 ovigerous).

Acapulco beach, November 26-28, 1937, 1 male.

Port Guatulco, December 3-5, Station 195, L-1 to L-3 (light), 2 females.

Tangola-Tangola Bay, Station 196, December 9, 1937, D-1, D-2, D-5, 5-9 fathoms, 9 young; December 12, 1937, D-9 to D-12, 7.5-4 fathoms, 8 young.

Nicaragua

Corinto, Station 200, January 5, 1938, D-12 to D-19, 3-13 fathoms, 1 male, 6 young; January 7, 1938, D-20 to D-26, 1.5-6.5 fathoms, 12 young.

San Juan del Sur, January 9, 1938, 1 carapace.

Costa Rica

Potrero Grande Bay, January 20, 1938, 1 male, 2 ovigerous females.

Murcielago Bay, January 23, 1938, Station 204, D-1, D-2, D-4, 2-4 fathoms, 9 young.

Piedra Blanca Bay, February 5, 1938, Station 208, D-1 to D-3, D-6, D-7, D-9, 3-6 fathoms, 1 young; February 6, 1938, 1 male.

Cedro Island, Gulf of Nicoya, February 12, 1938, 1 male, 3 females, 3 young.

Ballenas Bay, Gulf of Nicoya, [February 25, 1938], 1 male, found dead in mangroves.

Uvita Bay, March 3, 1938, seine, 6 males, 2 females.

Panama

Isla Parida, Gulf of Chiriqui, March 12, 1938, 1 male.

Bahia Honda, March 14, 1938, 1 male.

Pacheca Island, Pearl Islands, July 4, 1933, tidepools, "Antares," 1 male.

Colombia

Gorgona Island, March 28, 1938, 2 males; March 31, 1938, Station 232, D-1, 2-8 fathoms, 7 young.

Peru

Immediately S of Callao, 1941, 9 males, caught by natives, gift of Mrs. Sherman P. Haight.

Measurements. — Males from 8.1 × 19.1 (13.8) to 35.0 × 80.3 (60.1) mm., females from 12.4 × 31.0 (21.0) to 29.0 × 65.5 (47.4) mm., ovigerous females from 17.6 × 39.0 (30.0) to 29.0 × 65.5 (47.4) mm., young from 4.0 × 7.5 (6.0) mm.

Habitat.—Sand, rarely with mangrove leaves, rocks, or algae.

Food.—Four stomachs: amphipods (2), sand, algae, and iridescent, fine nacre shell (very thin, inner layers only apparently) (2). (J. Crane, of 15 to 33 mm. specimens seined at Piedra Blanca).

Color in Life.—Of San Lucas Bay specimens: All mottled, gray and black, spotted with white. Underside of legs and carapace, except abdomen, speckled with black. Chelipeds and legs grayish spotted with black. Two conspicuous black spots on carapace, one on each side of mid-gastric region. (J. Crane, field notes).

Of Chamela Bay specimens: Olive-tinged pepper-and-salt with bright white spot in middle of posterior gastric region and another on intestinal region. (J. Crane, field notes).

Of Passavera, Chamela Bay, specimens: Olive spotted finely with white. Eggs bright orange. Distal segments of legs pale gray. (J. Crane, field notes).

Remarks.—The Haight specimens, included here for convenience although not of "Zaca" collecting, confirm the southern limit of range for the species, Callao, Peru, being just a few miles south of Ancon, where a single specimen was obtained by R. E. Coker (Rathbun, 1930).

Cronius ruber (Lamarck)

Portunus ruber Lamarck, 1818, p. 260.

Cronius ruber, Stimpson, 1860, p. 225. Rathbun, 1930, p. 139, pls. 62, 63. Finnegan, 1931, p. 630. Garth, 1946, p. 422, pl. 72, figs. 3, 4; 1948, p. 36. Holthuis, 1954, p. 28, text-fig. 10.

Range.—From Point San Bartolome, Lower California, Mexico, to Paita, Peru. Galapagos Islands, 4-20 fathoms. Occurs also in the Atlantic. (Garth, 1948).

Material Examined.—48 specimens from 12 stations or localities:

Mexico

Chamela Bay, November 17, 1937, Station 182, D-1, 8 fathoms, 1 young.

Manzanillo, November 22, 1937, Station 184, D-1, 25 fathoms, 1 young.

Port Guatulco, Station 195, December 5, 1937, D-5, D-7, 2-4.5 fathoms, 2 males, 1 female, 3 young; D-8, D-9, 6-7 fathoms, 1 female, 7 young; D-8, D-9, 6-7 fathoms, 1 female; December 6, 1937, D-14, 4 fathoms, 1 female; December 7, 1937, D-18, 6 fathoms, 1 young.

Tangola-Tangola Bay, December 9, 1937, Station 196, D-6, D-7, 7-6 fathoms, 1 male, 1 young; December 12, 1937, D-14, D-15, 5 fathoms, 2 young.

Honduras

Cutuco and Potosi Lights, Gulf of Fonseca, December 20, 1937, 1 male.

Gulf of Fonseca, date?, fumarole, 1 young, 1 ovigerous female.

Costa Rica

Port Parker, Arriba rocks, January 15-18, 1938, 1 male, 1 female.

Port Culebra, January 30, 1938, 1 male, broken.

Piedra Blanca, February 2, 1938, 1 female; same locality, February 5, 1938, Station 208, D-1, D-2, D-3, D-6, D-7, D-9, 5 fathoms, 6 young.

Panama

Bahia Honda, Station 222, March 18, 1938, D-1, D-2, D-3, D-5, 3-11 fathoms, 2 young.

Colombia

Gorgona Island, March 30, 1938, 1 male; from coral, 1 ovigerous female; same date?, 2 males.

Gorgonilla Island, April 2, 1938, 1 male, 1 female, 3 young.

Measurements. — Males from 10.9 × 16.8 (14.9) mm. to 23.9 × 39.3 (35.0) mm., females from 9.0 × 13.0 (11.9) mm., ovigerous females from 15.3 × 24.3 (21.9) mm. to 44.2 × 68.0 (59.7) mm. The largest specimen, a male from Port Culebra, is in damaged condition. It measures aproximately 47 × 72 mm. in length and breadth.

Habitat. — Off Mexico and Costa Rica, from sand bottom with algae, rocks, or crushed shell; coral. Off Panama, from mud bottom with rocks, dead coral, shell, and leaves. "Under stone completely out of water and in upper tidal zone; alive and all right."

Color in Life. — Of Chamela Bay young female: Dark brownish-black streaked with gray. Paddle legs chestnut brown. (J. Crane, field notes).

Of Piedra Blanca female: Carapace olive brown speckled finely with cream. Transverse ridges blue-black. A prominent oval cream spot on middle of posterolateral margin. Anterolateral spines violet tipped with reddish-brown. Chelipeds like carapace, both as to background, ridges, and spines, above, but ridges definitely dark blue or green. Lower (outer) half of chelipeds creamy white. Chelae purple, tips buffy white, a greenish spot in middle of movable dactyls above. Ambulatories mottled dark green and white. Dactyls reddish-brown. Swimming feet pumpkin orange. Underside of carapace and maxillipeds and anterior edge of sternum orange streaked with white. Rest of sternum, abdomen, and under side of merus of ambulatories white. A purple line down middle of abdomen. Carpus to dactylus of ambulatories like upper side. (J. Crane, field notes).

Of Gulf of Fonseca ovigerous female: Dark purplish-black; pile dark buff; ridges and carapace and legs purplish-red and purplish-blue. Same color on abdominal crests. Swimmerets rusty orange. Chelae dark purple. Under side white with buffy pile, except carpus, manus, and dactylus of legs, which are like upper parts of same. Eggs buffy orange. (J. Crane, field notes).

Behavior.—Threatens. (J. Crane, field note).

Remarks. — The young of this species were frequently included in mixed lots of *Portunus* species, which they resemble greatly. The alternation of large and small anterolateral teeth and narrow carapace even suggests the subgenus *Achelous.* The presence of four spines on the manus, however, serves at once to distinguish them from all other eastern Pacific Portunidae.

Euphylax dovii Stimpson

Euphylax dovii Stimpson, 1860, p. 226, pl. 5, figs. 5, 5a. Rathbun, 1930, p. 147, pl. 65. Boone, 1930, p. 190, pl. 65. Garth, 1946, p. 423, pl. 72, figs. 1, 2. Coventry, 1944, p. 539.

Euphylax dowi, Garth, 1957, p. 38.

Range.—West coast of Mexico? Panama to Talcahuano, Chile. Galapagos Islands.

Material Examined. — Identifiable material from 3 stations, as follows:

Panama

Bahia Honda, March 15, 1938, fragments, food of *Caranx caninus* Günther.*

Hannibal Bank, March 20, 1938, Station 224, D-2, D-3, 35 fathoms, 3 chelae.

22 mi. ESE of Jicaron Island, March 20, 1938, Station 226, L-1 (night light), 1 female.

Measurements. — Female specimen, length 23.4 mm., width 37.8 mm.

Habitat.—Pelagic. Frequently comes to light at night.

Color in Life.—Carapace and merus of all legs deep purple; other segments of legs wine red. Underside of carapace, meri of legs, and maxillipeds blue; sternum white; abdomen brownish; undersides of rest of ambulatories wine red. (J. Crane, field notes).

Remarks. — The three chelae from Hannibal Bank were taken in a dredge from a bottom of either rocks, mud, and dead coral or sand, shells,

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*The fish *Caranx caninus* Günther is considered by some authors to be a synonym of the Atlantic *C. hippos* (Linnaeus).

and algae. A specimen was seen by John Tee-Van swimming at the surface in daylight, on the same day, above Hannibal Bank. (J. Crane, field notes). This corresponds with the experience of the "Velero III," which encountered the crabs in numbers at Cocos Island, Costa Rica, (Garth, 1946), and of the "Askoy," which encountered them at Malpelo Island, Colombia. The observations of Dr. R. C. Murphy are recorded in Garth (1948, p. 9).

Euphylax robustus A. Milne Edwards

Euphylax robustus A. Milne Edwards, 1874, p. 249; 1879, p. 205, pl. 37. Rathbun, 1930, p. 148, pls. 66, 67. Coventry, 1944, p. 540. Garth, 1948, p. 37.

Range.—From Isabel Island, Mexico (Coventry), to Octavia Bay, Colombia (Garth).

Material Examined.—9 specimens from 5 stations:

Mexico

17 mi. SE × E of Acapulco, November 29, 1937, Station 189, D-1, 20 fathoms, 1 female.

Tangola-Tangola Bay, Station 196, December 12, 1937, D-9 to D-12, 7.5-4 fathoms, 1 young; December 13, 1937, D-17, 23 fathoms, 2 young.

Costa Rica

Port Culebra, January 30, 1938, Station 206, D-3, 14 fathoms, 2 young females.

Panama

Parida Island, Gulf of Chiriqui, March 12, 1938, 1 damaged specimen, food of *Caranx caninus.*

Bahia Honda, March 18, 1938, Station 222, D-5, 11 fathoms, 2 young.

Colombia

At sea near Gorgona Island, March 27, 1938, from mangrove seeds floating in tide rip, 1 young.

Measurements.—Large female, length 60 mm., width 96 mm., exorbital width 84.2 mm., frontal width 15 mm., cheliped 130 mm., chela 74 mm., dactyl 44.2 mm., height of palm 30 mm., first walking leg 116.5 mm. Young from 3.5 mm. length.

Habitat.—Sand, mud, sandy mud; with shell, leaves, or algae.

Color in Life.—Carapace and legs above gray blue-green. Chelipeds gray blue-green except olive brown manus and dactyls. Ridges and dactyls tinged with pink. Tubercles on chelipeds white. Eyestalks bright violet. Swimmerets pale horn. Abdomen barred with violet and white. Legs barred with violet and white below. Eggs pale salmon. (J. Crane, field notes).

Remarks.—While credit for the rediscovery of A. Milne Edwards's lost species rightfully belongs to the Fifth George Vanderbilt Expedition (Coventry, 1944), "Zaca" scientists may consider as one of their more significant contributions the finding of a specimen of the opposite sex and of a size comparable to the 56 × 90 mm. holotype of *Euphylax robustus.* This 60 × 96 mm. female, dredged on sandy mud bottom near Acapulco, Mexico, by its detailed resemblance to A. Milne Edwards's unique male, upholds the writer's conviction, based upon the examination of immature specimens only (Garth, 1948, p. 37), that "[*E.*] *robustus* is a valid species and not conspecific with [*E.*] *dovii,* as suggested by Rathbun (1930, p. 148)." That specimens of this large and distinctive species have not escaped the eyes of discriminating collectors, but have merely failed to be reported in the literature, is attested by a pair of comparable size from Peru sent the writer by Dr. Albert Panning of the Hamburg Museum. A redescription based on this new material, together with photographs, will appear in a subsequent monograph.

Family XANTHIDAE

Medaeus lobipes Rathbun

Medaeus lobipes Rathbun, 1898, p. 583, pl. 44, fig. 1; 1930, p. 275, text-fig. 44, pl. 114. Crane, 1937, p. 70. Garth, 1946, p. 442, pl. 77, fig. 2; 1948, p. 39.

Range.—From Santa Inez Bay, Gulf of California, Mexico, to Guayabo Chiquito, Panama. Galapagos Islands. 5.5-150 fathoms. (Garth, 1948).

Material Examined.—35 specimens from 4 stations:

Mexico

Manzanillo, November 22, 1937, Station 184, D-1, 25 fathoms, 1 young male; D-2, 30 fathoms, 15 males, 13 females (1 ovigerous).

Costa Rica

Port Parker, January 20, 1938, Station 203, D-1 to D-3, 10-15 fathoms, 2 males, 1 female.

Port Culebra, January 30, 1938, Station 206, D-1, D-3, 14 fathoms, 1 male.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-4 to D-7, 4-6 fathoms, 1 female, 1 young.

Measurements. — Males from 4.9 × 6.9 to 18.6 × 28.1 mm., females from 5.7 × 8.1 to 16.4 × 24.2 mm., ovigerous female 11.6 × 17.1 mm., young from 3.0 × 4.0 mm.

Habitat.—Sand, gravelly sand; sandy mud and crushed shell; mangrove leaves, mud, and shell.

Color in Life.—Of Manzanillo specimens: At least half had orange carapace of varying degrees of brightness with dark brown median longitudinal band and same brown on anterolateral angles. In the rest the orange was replaced by light brown or white. Chelipeds orange or light brown externally, white internally and on distal part of manus. Underparts white sprinkled posteriorly with brown. (J. Crane, field notes).

Breeding.—Mexico in late November.

Remarks.—The Port Culebra male, a young specimen, is granulate to the point of spinulosity.

Medaeus spinulifer (Rathbun)

Pilumnus spinulifer Rathbun, 1898, p. 585, pl. 42, figs. 6-8. Finnegan, 1931, p. 643.

Medaeus spinulifer, Rathbun, 1930, 276, text-fig. 45. Garth, 1946, p. 443, pl. 75, figs. 5, 6; 1948, p. 40. Crane, 1947, p. 75.

Range.—From Cape San Lucas, Lower California, Mexico, to Utria Bay, Colombia. Galapagos Islands. Shore to 73 fathoms. (Garth, 1948).

Material Examined. — 3 specimens from 2 stations:

Mexico

3 mi. off Pyramid Rock, Clarion Island, May 12, 1936, Station 163, D-3, D-4, 50 fathoms, 1 female.

Manzanillo, November 22, 1937, Station 184, D-2, 30 fathoms, 1 male, 1 young.

Measurements.—Male specimen 9.0 × 13.3 mm., female specimen 7.0 × 10.1 mm., young specimen 2.6 × 3.3 mm.

Habitat.—Gravelly sand.

Color in Life.—Not noted.

Remarks.—The Manzanillo specimens were sorted out from among a large number of *Medaeus lobipes* Rathbun. The species is now recorded from the Revilla Gigedo Islands.

Xanthodius stimpsoni (A. Milne Edwards)

Xantho stimpsoni A. Milne Edwards, 1879, p. 252, pl. 46, figs. 2-2b. Finnegan, 1931, p. 631. Buitendijk, 1950, p. 277.

Xanthodius stimpsoni, Rathbun, 1930, p. 315, pl. 143, figs. 5-7. Crane, 1947, p. 77. Garth, 1948, p. 41.

Daira ecuadorensis Rathbun, 1935, p. 49.

Range.—From Cape San Lucas, Lower California, Mexico, to Santa Elena Bay, Ecuador. 7-27 meters. (Garth).

Material Examined. — 15 specimens from 3 stations:

Mexico

Banderas Bay, November 16, 1937, from oyster-bearing rocks, 3 young.

Port Guatulco, Station 195, December 4, 1937, D-3, 3.5 fathoms, 1 male, 1 young; D-4, 4.5 fathoms, 1 male, 2 ovigerous females, 1 young; December 5, 1937, D-5, 2 fathoms, 2 males, 2 females (1 ovigerous), 1 young.

Nicaragua

Corinto, January 5, 1938, Station 200, D-15, 1 fathom, 1 young.

Measurements.—Males from 4.9 × 7.2 to 6.2 × 9.3 mm., females from 5.0 × 7.2 to 6.0 × 9.0 mm., ovigerous females from 5.0 × 7.3 to 6.0 × 9.0 mm., young from 2.8 × 3.7 mm.

Habitat.—Sand, with algae or crushed shell; mangrove leaves.

Color in Life.—Of Port Guatulco specimens: Carapace white speckled with violet; chelipeds bright orange; legs dark brown except last (white). (J. Crane, field notes). Marked with shades of white and dark red. (*Idem.*).

Breeding.—Mexico in early December.

Remarks. — Since depth is not mentioned in Rathbun (1930), the "Askoy" records of 6-10 feet and 7-27 meters and the "Zaca" records of 1-4.5 fathoms aid materially in defining the bathymetric range.

Hexapanopeus costaricensis Garth

Hexapanopeus costaricensis Garth, 1940, p. 79, pl. 21, figs. 1-4.

Range.—From Port Parker and Puerto Culebra, Costa Rica. 3-10 fathoms. (Garth).

Material Examined. — 14 specimens from 2 stations:

Costa Rica

Port Parker, January 20, 1938, Station 203, D-1 to D-3, 10-15 fathoms, 5 males, 3 females (1 ovigerous); January 22, 1938, D-7, 5-9 fathoms, 1 male, 1 female; D-8, 9 fathoms, 1 male.

Panama

Bahia Honda, March 18, 1938, Station 222, D-1 to D-3, D-5, 3-11 fathoms, 2 males; D-3, 8 fathoms, 1 male.

Measurements.—Males from 4.1 × 5.3 to 6.0 × 7.7 mm., non-ovigerous females from 4.0 × 5.5 to 4.6 × 6.0 mm., ovigerous female 3.5 × 4.9 mm.

Habitat. — Sandy mud, crushed shell; shelly sand, algae; shelly mud; dead coral.

Color in Life.—Not noted.

Breeding.—Costa Rica in late January.

Remarks.—The "Zaca" records confirm those of the "Velero III" from Port Parker, the type locality, and extend the range of the species south from Costa Rica to northern Panama.

Hexapanopeus nicaraguensis (Rathbun)

Lophopanopeus nicaraguensis Rathbun, 1904b, p. 162.

Hexapanopeus nicaraguensis, Rathbun, 1930, p. 395, text-fig. 61.

Range.—Known only from the type locality, Realejo [Corinto], Nicaragua.

Material Examined.—4 specimens from 2 stations:

El Salvador

La Libertad, December 16, 1937, Station 198, D-1, 13 fathoms, 1 male.

Nicaragua

Corinto, Station 200, December 29, 1937, D-1, D-3, D-8, 2-6.6 fathoms, 1 male, 1 ovigerous female; January 7, 1938, D-27 to D-30, 3 fathoms, 1 male.

Measurements.—Males from 4.7 × 6.9 to 7.3 × 10.6 mm., ovigerous female 5.4 × 7.7 mm.

Habitat.—Mud, mangrove leaves.

Color in Life.—Not noted.

Breeding.—Nicaragua in late December.

Remarks.—The above specimens are smaller than would be suggested by the unique male holotype, an 8.7 × 13 mm. specimen. Their presence at the original locality is confirmed, Realejo being the classic locality, Corinto its modern counterpart. A fine specimen from La Libertad, which is widest opposite the last marginal tooth, extends the known range a full degree of latitude north to El Salvador.

Hexapanopeus orcutti Rathbun

Hexapanopeus orcutti Rathbun, 1930, p. 397, pl. 170, figs. 3, 4.

Range.—Known only from the type locality, near Modesto, Sinaloa, Mexico.

Material Examined. — 32 specimens from 6 stations comprising 8 separate localities:

Mexico

Banderas Bay, November 16, 1937, from oyster-bearing rocks, 1 male, 5 young.

Chamela Bay, November 17, 1937, Station 182, D-3, 15 fathoms, 1 ovigerous female.

Port Guatulco, Station 195, December 4, 1937, D-1, 2.5 fathoms, 1 male; D-2, 3 fathoms, 1 male, 1 young; December 6, 1937, D-10, 4 fathoms, 1 ovigerous female.

Nicaragua

Monypenny Point, Gulf of Fonseca, December 24, 1937, Station 199, D-2, 5 fathoms, 1 male; D-5, D-6, 4-7 fathoms, 2 females (1 ovigerous).

El Salvador

La Union, Gulf of Fonseca, December 27, 1937, Station 199, D-8, 6 fathoms, 6 males, 4 females (2 ovigerous); D-17, D-21, 3-4 fathoms, 2 males, 1 female.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-4, 7 fathoms, 1 female, 1 young; D-10, 2.5-6 fathoms, 1 male, 4 females (2 ovigerous).

Golfito, Gulf of Dulce, March 9, 1938, D-4 to D-7, 4-6 fathoms, 1 male, 1 female.

Measurements.—Males from 3.0 × 4.0 to 6.0 × 8.3 mm., females from 3.0 × 3.9 to 3.7 × 5.1 mm., ovigerous female 3.7 × 5.0 mm., young from 2.2 × 2.8 mm., Gulf of Fonseca specimens: males from 4.1 × 5.2 to 9.5 × 12.6 mm., females from 4.0 × 5.3 to 7.0 × 9.2 mm., ovigerous females 4.6 × 6.0 to 5.5 × 7.2 mm., young not present.

Color in Life. — Of Chamela Bay, Mexico, specimens: Black all over except ambulatories, which are barred with black and cream. Chelae tipped with cream; abdomen pale buffy. (J. Crane, field notes).

Of Port Guatulco, Mexico, specimens: Gray marked with white. (J. Crane, field notes).

Habitat.—Sand, algae; gravelly sand, crushed shell, dead coral; mud, mangrove leaves; gravel, algae.

Breeding.—Mexico in mid-November and early December; Nicaragua in late December.

Remarks.—As will be noted under *Measurements* above, the Gulf of Fonseca specimens (Monypenny Point, La Union) represent a giant race as compared to Mexican and Costa Rican specimens. They appear also to have longer legs and somewhat different chelae, and should perhaps on this account be segregated from the above series. It is clear, however, that the range of the species should be extended from northwest Mexico all the way to the Gulf of Dulce, Costa Rica, the depth to 15 fathoms. The Port Guatulco male has two minor chelae, possibly the result of regeneration.

Hexapanopeus sinaloensis Rathbun

Hexapanopeus sinaloensis Rathbun, 1930, p. 398, pl. 170, figs. 1, 2. Garth, 1948, p. 41.

Hexapanopeus setipalpus Finnegan, 1931, p. 641.

Range.—From Boca Tecapan, Sinaloa, Mexico, to Málaga Bay, Colombia, 4-9 meters. (Garth, 1948).

Material Examined. — 31 specimens from 6 stations:

Nicaragua

Meanguera Island, Gulf of Fonseca, December 23, 1937, Station 199, D-1, 16 fathoms, 2 males.

Corinto, Station 200, December 29, 1937, D-1, D-3, D-8, 2-6.6 fathoms, 1 male, 1 female; January 7, 1938, D-26, 2.5 fathoms, 1 ovigerous female; D-27 to D-30, 3 fathoms, 1 male.

Costa Rica

Port Parker, January 22, 1938, Station 203, D-5, 3 fathoms, 1 male; D-13, 7-9 fathoms, 1 ovigerous female.

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 7 males, 6 females (2 ovigerous).

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-4 to D-7, 4-6 fathoms, 3 males, 3 females (2 ovigerous); D-8, 6 fathoms, 2 males, 2 young.

Panama

Bahia Honda, March 18, 1938, Station 222, D-3, 8 fathoms, 1 male.

Measurements.—Males from 3.2 × 4.4 to 5.7 × 8.7 mm., females from 3.0 × 4.0 to 4.2 × 5.9 mm., ovigerous females same; young from 2.9 × 3.8 mm.

Habitat. — Mangrove leaves; shells and dead coral or algae; mud, sand, and crushed shell.

Color in Life.—Not noted.

Breeding.—Nicaragua in early January; Costa Rica from late January to early March.

Remarks.—Previously recorded from Taboga Island, Panama, as *Hexapanopeus setipalpus* Finnegan. The records for Nicaragua and Costa Rica are new.

Hexapanopeus beebei, new species

(Plate I)

Type.—Male holotype, A.H.F. No. 377, and male and female, paratypes, N.Y.Z.S. No. 37,718, from Corinto, Nicaragua, December 29, 1937, "Zaca" Station 200, D-1, 3, 8, 2-6.6 fathoms. For additional paratypes see *Supplementary Material* below.

Measurements. — Male holotype, length 5.4 mm., width 7.2 mm., of fronto-orbit 5.4 mm., of front 2.6 mm., length of cheliped 8.3 mm., of chela (lower margin) 6.1 mm., of dactyl 3.5 mm., height of palm 3.5 mm. Female paratype, length 5.2 mm., width 7.1 mm.

Diagnosis.—Fifth lateral tooth minute. Supraorbital lobe advanced to level of outer orbital tooth. Fingers white, the whitened portion not continuing backward and upward on palm. Major dactyl strongly curved in male, a denticle but no large tooth at base. Male first pleopod with a reflected medial spine, an opposing lanceolate lobe, and a truncated or collared hood with three terminal setae.

Description.—Carapace nearly flat, almost devoid of pubescence, smooth anteriorly but granulate posteriorly, clearly divided by furrows into regions each surmounted by one or more rows of granules, disposed as follows: two protogastric, of which the posterior is the more oblique, one epibranchial, in line with the last tooth, one metagastric, interrupted at the middle, one cardiac, also medially divided, and one metabranchial; in addition to these an hepatic, in line with the anterior protogastric, and a postfrontal, in advance of the anterior protogastric. Front not exceptionally narrow, a little less than one-third carapace width, produced, thin-edged, lateral margins oblique, anterior margins somewhat oblique, straight or slightly sinuous, frontal lobes separated by a shallow but distinct median V, a suggestion of an outer lobe. Inner supraorbital border elevated, swollen, orbital emarginations V-shaped, the included lobe equally advanced inwardly with the outer orbital tooth. Second anterolateral tooth low, rounded, and separated from the first or exorbital by a shallow sinus, their combined width greater than that of the third tooth; third tooth with a short, straight, oblique anterior margin and a long, arcuate posterior margin, tip rectangular; fourth tooth spiniform, narrower at base than third and more projecting, directed obliquely forward, anterior margin transverse or slightly concave, posterior margin straight or nearly so; fifth tooth minute, separated from fourth by a closed fissure, and appearing as a notch on the posterior margin of the fourth.

Merus of outer maxilliped subrectangular, broader than long, anterolateral angle slightly produced and rounded, anterointernal angle shallowly notched at insertion of palpus.

Chelipeds markedly unequal in the male; merus with a superior distal tubercle; carpus with a marginal carina incompletely outlining a rhomb enclosing a rectangular sulcus in its outer portion, a scattering of tubercles above; manus of major chela smooth, inflated, two parallel ridges above. Fingers white, the white "color" not continued appreciably on palm, major dactylus strongly curved, almost forming a quarter-circle, a granulate ridge above, a small denticle basally in place of the customary larger tooth;

this with a small denticle distally defining a gape into which a somewhat larger tooth of the pollex fits incompletely; pollex not deflexed. Minor manus slenderer than major, fingers elongate, ridged and compressed, meeting without a gape but with crossed tips; pollex deflexed.

Male abdomen with sides of third segment rounded, sides of fused segments 3-5 concave, narrowest at base of segment 6, segment 7 triangular with a blunt tip. Male first pleopod with a long, backward-pointing medial spine, an equally long and oppositely directed lanceolate lobe, and a rimmed hood bearing three terminal setae.

Female noticeably more convex than male, ridges more prominent, interspaces more felted. Chelipeds less robust, carpus and minor manus more nodose, palms more granulate. Fingers compressed, those of major manus meeting without a gape but possessing larger teeth than those of minor manus.

Supplementary Material.—In addition to specimens mentioned under *Type* above, the following specimens, also from "Zaca" Station 200: D-7, 2 fathoms, 1 female, paratype; D-27 to 30, 3 fathoms, mangrove leaves, 1 male, 1 ovigerous female, paratypes. The male has two minor chelipeds, the result of regeneration of a major cheliped.

Remarks. — The proposed new species is most closely allied to *Hexapanopeus caribbaeus* (Stimpson), from which it differs in having the fingers white, the whitened portion not running backward and upward on the palm, the front not especially narrowed, and the fingers of the major chela of the male conspicuously gaping.

I take pleasure in naming this diminutive panopeid for Dr. William Beebe, director emeritus of the Department of Tropical Reseach, New York Zoological Society, whose "Book of Bays" (1942) so delightfully describes the expedition on which it was collected.

Panopeus purpureus Lockington

Panopeus purpureus Lockington, 1877b, p. 101. Rathbun, 1930, p. 344, pl. 158, fig. 1, pl. 159. Crane, 1947, p. 79.

Range.—From Magdalena Bay, Lower California, and Guaymas, Sonora, Mexico, to mouth of Rio Tumbes, Peru. (Rathbun).

Material Examined.—Puntarenas, Costa Rica, February 22, 1938, 1 male.

Measurements. — Male specimen length 11.5 mm., width 16.5 mm.

Habitat. — In stony mud on edges of mangrove swamps and open mudflats. (Crane).

Remarks.—It was undoubtedly due to an oversight that the above specimen was not reported by Crane (1947), who listed the species from Culebra, Ballenas, and Golfito, Costa Rica, and Puerto Bolivar, Ecuador.

Panopeus bermudensis Benedict & Rathbun

Panopeus bermudensis Benedict & Rathbun, 1891, p. 376, pl. 20, fig. 2; pl. 24, figs. 14, 15. Rathbun, 1930, p. 360, pl. 165, text-fig. 56.

Range.—Eastern Pacific from Magdalena Bay, Lower California, Mexico, to Matapalo (near Capon), Peru. Western Atlantic from Florida and the Bahamas to Brazil. Bermuda. (Rathbun).

Material Examined.—Chamela Bay, Mexico, November 17, 1937, Station 182, D-1, 8 fathoms, 17 males, 6 ovigerous females.

Measurements.—Males from 4.3 × 5.7 to 6.3 × 8.5 mm., ovigerous females from 3.9 × 5.3 to 4.9 × 6.6 mm.

Habitat.—Sand and algae. Fine bits of seaweed were entwined among specimens.

Remarks. — When considered independently, the Chamela Bay specimens could scarcely be reconciled with specimens from Bermuda to which the name *bermudensis* was originally applied. It is when considered in the context of a larger representation of this geographically variable complex from many west coast localities that their relationship to a similar array from numerous Caribbean localities is appreciated. While as a result of studies now in progress it may be decided to segregate the Pacific material from the Atlantic as a distinct species, to do so now on the basis of a single lot of specimens from an isolated locality would be premature. The Chamela Bay specimens serve to link the present representation from Lower California and the Gulf of California with that from Central and northern South America. The small size of the females, all of which are ovigerous, is noteworthy.

Eurytium tristani Rathbun

Eurytium tristani minor (Bott), n. comb.

Eurytium tristani Rathbun, 1906, p. 100; 1910, pp. 543, 585, pl. 47, fig. 1; 1930, p. 425, pl. 176, fig. 3; pl. 177, fig. 3. Crane, 1947, p. 80.

Panopeus convexus minor Bott, 1955, p. 57, pl. 6, figs. 9a, 9b. (Not *P. convexus* A. Milne Edwards, 1880).

Range. — From El Triunfo, El Salvador, to Salto (near Capon), Peru.

Material Examined.—Puntarenas, Costa Rica, February 22, 1938, 26 males, 29 females (1 ovigerous), 1 young.

Measurements.—Males from 3.5 × 4.5 to 12.0 × 17.8 mm., females from 3.7 × 5.1 to 9.9 ×

15.1 mm., ovigerous female 6.1 × 8.7 mm., young 3.2 × 4.3 mm.

Habitat.—Not stated, but most certainly from mud flats exposed at low tide.

Color in Life.—Not noted.

Remarks. — Of the two pairs of specimens from Puntarenas sent to Frankfurt, Germany, for comparison with the types of *Panopeus convexus minor* Bott, Dr. Richard Bott writes as follows: "They agree with the type in all characteristics mentioned by me; the first pleopods are completely identical." It was noted that, while in the published figure of the male holotype the left cheliped is larger than the right, among "Zaca" specimens the right cheliped is larger than the left in most instances. Dr. Bott affirms that this is also true of the remaining male from the type series of *P. convexus minor.*

The decision to transfer Bott's subspecies to Rathbun's species of another genus was the result of examining a male specimen from El Triunfo reported as *Panopeus convexus convexus* (Bott, 1955, p. 57) and loaned the writer by Dr. Bott for use in connection with studies on the family Xanthidae. This proved to be none other than *Eurytium tristani* Rathbun. It therefore follows that the somewhat smaller specimens reported by him as *Panopeus convexus minor* should be called *Eurytium tristani minor* (Bott) instead. The small size of the "Zaca" specimens, and in particular, of the ovigerous female, support their continued distinction.

Micropanope polita Rathbun

Micropanope polita Rathbun, 1893, p. 238; 1930, p. 440, text-fig. 40, pl. 180, figs. 3, 4, synonymy. Crane, 1937, p. 71. Garth, 1946, p. 459, pl. 77, fig. 4.

Range.—From Magdalena Bay, Lower California, and Santa Inez Bay, Gulf of California, Mexico, to Cocos Island, Costa Rica. Galapagos Islands. 3-150 fathoms. (Garth).

Material Examined. — 35 specimens from 3 stations:

Mexico

SE of Cedros Island, Lower California, November 10, 1937, Station 126, D-19, 25 fathoms, 1 male, 1 female. From holes in rocks.

3 mi. off Pyramid Rock, Clarion Island, May 12, 1936, Station 163, D-2, 55 fathoms, 30 specimens.

Panama

Hannibal Bank, March 20, 1938, Station 224, D-1 to D-3, 35-40 fathoms, 1 male, 2 females.

Measurements.—Males from 4.0 × 6.0 to 5.4 × 8.6 mm., females from 2.9 × 4.2 to 4.8 × 7.2 mm. The larger specimens are from the more northerly localities.

Habitat. — Rocks, mud, dead coral; sand, shells, algae.

Color in Life. — Of Cedros Island, Mexico, specimens: Male tan and cream mottled. Front and manus rosy red. Dactyls dark brown tipped with white. Ambulatories banded tan and cream tinged with pink. Female carapace entirely crimson, but brightest on front. Chelipeds coral red. Chelae dark brown, tipped with white. Underparts pinkish. (J. Crane, field notes).

Remarks.—Food preferences and breeding in the southern part of the Gulf of California are discussed by Crane (1937). The records from the Revilla Gigedo Islands, of which Clarion is the most remote, and from the mainland of Panama are new.

Micropanope xantusii (Stimpson)

Xanthodes xantusii Stimpson, 1871, p. 105.

Micropanope xantusii, Rathbun, 1930, p. 438, pl. 179, figs. 1-4. Crane, 1937, p. 72; 1947, p. 80. Garth, 1946, p. 457, pl. 77, fig. 6; 1948, p. 42.

Xanthias serrulata Finnegan, 1931, p. 634, text-fig. 6.

Range.—From Arena Bank, Gulf of California, Mexico, to Santa Elena Bay, Ecuador. Galapagos Islands. (Garth, 1948). Occasionally to 40 fathoms.

Material Examined.—20 specimens from 3 collections made at 2 localities:

Mexico

Sulphur Bay, Clarion Island, May 15, 1936, coral, 1 young male.

Port Guatulco, December 4, 1937, in dead pearl oyster, 1 male; December 6, 1937, Station 195, D-15, 1.5 fathoms, 8 males, 10 females (4 ovigerous).

Measurements.—Males from 3.0 × 4.2 to 7.0 × 9.8 mm., females from 4.0 × 5.6 to 7.25 × 11.0 mm., ovigerous females from 4.5 × 6.4 to 6.3 × 9.2 mm.

Habitat.—Coral obtained by diving.

Color in Life.—Variable, but majority dark red mottled with lighter and darker. Sulci on major cheliped of adult males may be almost lacking. (Crane, 1947).

Breeding.—Mexico in early December.

Remarks.—According to Crane (1947), who reported the species from the intertidal of Mexico (Clarion Island, Sihuatenejo, Acapulco) and Costa Rica (Port Parker, Culebra, Jasper Island), "always found in *Pocillopora* coral except for 3 young found at Port Parker among algae-covered stones."

Micropanope (?) maculatus (Rathbun)

Lophopanopeus maculatus Rathbun, 1898, p. 588, pl. 40, figs. 10, 11; 1930, p. 330, text-fig. 51. Garth, 1946, p. 453, pl. 78, figs. 3, 4.

Micropanope (?) *maculatus*, Menzies, 1948, p. 24.

Range.—From Magdalena Bay, Lower California, and southern part, Gulf of California, Mexico. Galapagos Islands. 2-70 fathoms. (Garth).

Material Examined.—Port Parker, Costa Rica, January 22, 1938, Station 203, D-9, 1.5-4 fathoms, 1 male, 1 female.

Measurements. — Male specimen 4.7 × 6.5 mm., female specimen 4.1 × 5.7 mm.

Habitat.—Coral bottom.

Color in Life.—Not noted.

Remarks.—The Port Parker specimens are in good condition and show the distinctive characters well. *Micropanope (?) maculatus* is now recorded from the Central American mainland.

The exclusion of the species from *Lophopanopeus* is the result of Menzies' revision of that genus. Its referral to *Micropanope* in this instance is tentative and without prejudice to systematic studies by the writer now in progress.

Paraxanthias taylori (Stimpson)

Xanthodes taylori Stimpson, 1860, p. 208, pl. 3, fig. 5.

Paraxanthias taylori, Odhner, 1925, p. 85. Rathbun, 1930, p. 466, pl. 188, pl. 189, fig. 1, synonymy.

Range. — From Monterey Bay, California, to Magdalena Bay, Lower California, Mexico. Shore to 55 fathoms. (Rathbun, 1930).

Material Examined. — SE of Cedros Island, Lower California, Mexico, November 10, 1937, Station 126, D-19, 25 fathoms, 1 male.

Measurements.—Male specimen, length 6.8 mm., width 9.8 mm.

Habitat.—Rocks, algae. From hole in rocks. (J. Crane, field notes).

Color in Life. — Pinkish tan; chelae brown tipped with white. (J. Crane, field notes).

Remarks.—A southern California—west coast of Lower California warm-temperate species.

Menippe obtusa Stimpson

Menippe obtusa Stimpson, 1859, p. 53. Rathbun, 1930, p. 478, pl. 197, pl. 198, figs. 1, 2. Sivertsen, 1933, p. 16. Garth, 1946, p. 470, pl. 82, figs. 3, 4; 1948, p. 45. Crane, 1947, p. 80.

Range.—From Corinto, Nicaragua, to La Plata Island, Ecuador. Galapagos Islands. Shore to 6.5 meters. (Garth, 1948).

Material Examined.—Passavera Island, Chamela Bay, Mexico, November 19, 1937, 1 female.

Measurements. — Female specimen, length 12.8 mm., width 18.3 mm.

Habitat.—Not given.

Color in Life.—Brown to apricot orange. (J. Crane, of Corinto, Nicaragua, specimens).

Remarks.—For a discussion of the interesting habits of this and the related *Menippe frontalis* A. Milne Edwards, including use of the stridulating ridges of the inner surface of the manus, the reader is referred to Crane (1947, p. 80). Chamela Bay, Mexico, represents a northward extension of range from Corinto, Nicaragua, an earlier reported "Zaca" record.

Pilumnus pygmaeus Boone

Pilumnus pygmaeus Boone, 1927, p. 221, text-fig. 81. Rathbun, 1930, p. 515, pl. 207, figs. 4, 5. Garth, 1946, p. 472, pl. 80, fig. 4; 1948, p. 48. Crane, 1947, p. 81.

Range.—From Port Parker, Costa Rica, to Utria Bay, Colombia. Galapagos Islands. Shore to 13 fathoms.

Material Examined.—2 specimens from 2 stations:

Mexico

Port Guatulco, December 4, 1937, in dead pearl oyster, 1 female.

Costa Rica

Port Parker, [date?], 1 male.

Measurements.—Male specimen 2.5 × 3.3 mm., female specimen 2.7 × 3.6 mm.

Habitat.—From weed in tidepools and on undersides of overgrown rocks close to low tide. (Crane, 1947, of Costa Rican specimens).

Breeding.—Eggs in January. (Crane, 1947).

Remarks.—The known range of the species is extended northward from Port Parker, Costa Rica, to Port Guatulco, Mexico.

Pilumnus limosus Smith

Pilumnus limosus Smith, 1869, p. 285. Rathbun, 1930, p. 518, pl. 208, pl. 209, figs. 1-3.

Range.—From Panama to Paita, Peru. Shore. (Rathbun, 1930).

Material Examined.—2 specimens from 2 stations:

Mexico

4 mi. SSW of Maldonado Point, November 30, 1937, Station 192, D-1, 26 fathoms, 1 female.

Tangola-Tangola Bay, December 13, 1937, Station 196, D-18, 30 fathoms, 1 male.

Measurements. — Male specimen 5.0 × 6.8 mm., female specimen 7.4 × 9.9 mm.

Habitat.—Mud.

Color in Life. — Brownish. (J. Crane, field notes).

Remarks.—A distinctive species, with a characteristic pattern of pubescence that obscures the scattered granules of the carapace. *Pilumnus limosus* is now recorded from Mexico, a northward extension from Panama, and to a depth of 30 fathoms.

Pilumnus stimpsonii Miers

Pilumnus marginatus Stimpson, 1871, p. 109. Not *P. marginatus* Stimpson, 1858.

Pilumnus stimpsonii Miers, 1886, p. 147 (name substituted for *P. marginatus,* preoccupied). Rathbun, 1930, p. 524, pl. 205, figs. 5, 6.

Range.—Cape San Lucas, Lower California, and Manzanillo, Colima, Mexico. (Rathbun, 1930).

Material Examined.—Port Parker, Costa Rica, January 22, 1938, Station 203, D-10, 2.5-6 fathoms, 1 male, 6 females (3 ovigerous).

Measurements.—Male 2.9 × 3.4 mm., females from 3.2 × 4.0 to 4.0 × 4.9 mm., ovigerous females from 3.2 × 4.0 to 3.8 × 4.5 mm.

Habitat.—Rocks.

Color in Life.—Not recorded.

Breeding.—Costa Rica in late January. Mexico in mid-July. (Rathbun, 1930).

Remarks.—Since Stimpson's type is non-extant and the material reported by Rathbun (1930) consists in its entirety of 3 specimens collected by Orcutt in Mexico, the "Zaca" series from Port Parker represents more than double the number of specimens existing in collections and reported upon to date. Orcutt's material also included an ovigerous female. The range of *Pilumnus stimpsonii* is extended southward to Costa Rica, the depth to 6 fathoms.

Heteractaea peterseni Garth

Heteractaea peterseni Garth, 1940, p. 81, pl. 22, figs. 1-5.

Range.—From San Francisco Island, Gulf of California, Mexico, to Octavia Bay, Colombia. 35-44 fathoms. (Garth).

Material Examined.—Hannibal Bank, Panama, March 20, 1938, Station 224, D-1 to D-3, 40-35 fathoms, 1 female, 2 young.

Measurements.—Female specimen 5.5 × 8.0 mm., young 3.9 × 5.7 and 4.4 × 6.3 mm.

Habitat.—Rocks, dead coral, mud; sand, shells, algae. The fact that the holotype, from Octavia Bay, Colombia, was cracked from rock makes the former habitat the more likely. The channeled meri of the walking legs are believed to represent an adaptation for breathing from a rock-bound enclosure. Unfortunately, materials from two dissimilar bottom types at Station 224 were combined.

Color in Life.—See Garth (1940, p. 83).

Remarks.—The findings by the "Zaca" of *Heteractaea peterseni* off Panama bridges the gap between the two "Velero III" stations in Colombia and the Gulf of California. The uniformity of depth for the three stations is perhaps more than coincidental: Octavia Bay, Colombia, 35-40 fathoms; San Francisco Island, Gulf of California, 43-44 fathoms; and Hannibal Bank, Panama, 35-40 fathoms.

Quadrella nitida Smith

(Text-fig. 2)

Quadrella nitida Smith, 1869, p. 288. Rathbun, 1930, p. 561, pl. 229, synonymy. Crane, 1937, p. 74. Garth, 1946, p. 494, pl. 80, fig. 6.

Range.—From off Cape San Lucas, Lower California, and Arena Bank, Gulf of California, Mexico, to Pacheca Island, Panama. Galapagos Islands. 6-75 fathoms.

Material Examined. — 15 specimens from a single station: Mazanillo, Mexico, November 22, 1937, Station 184, D-1, 25 fathoms, 1 male, 3 females, 2 young, 1 megalops; D-2, 30 fathoms, 3 males, 3 females, 2 young.

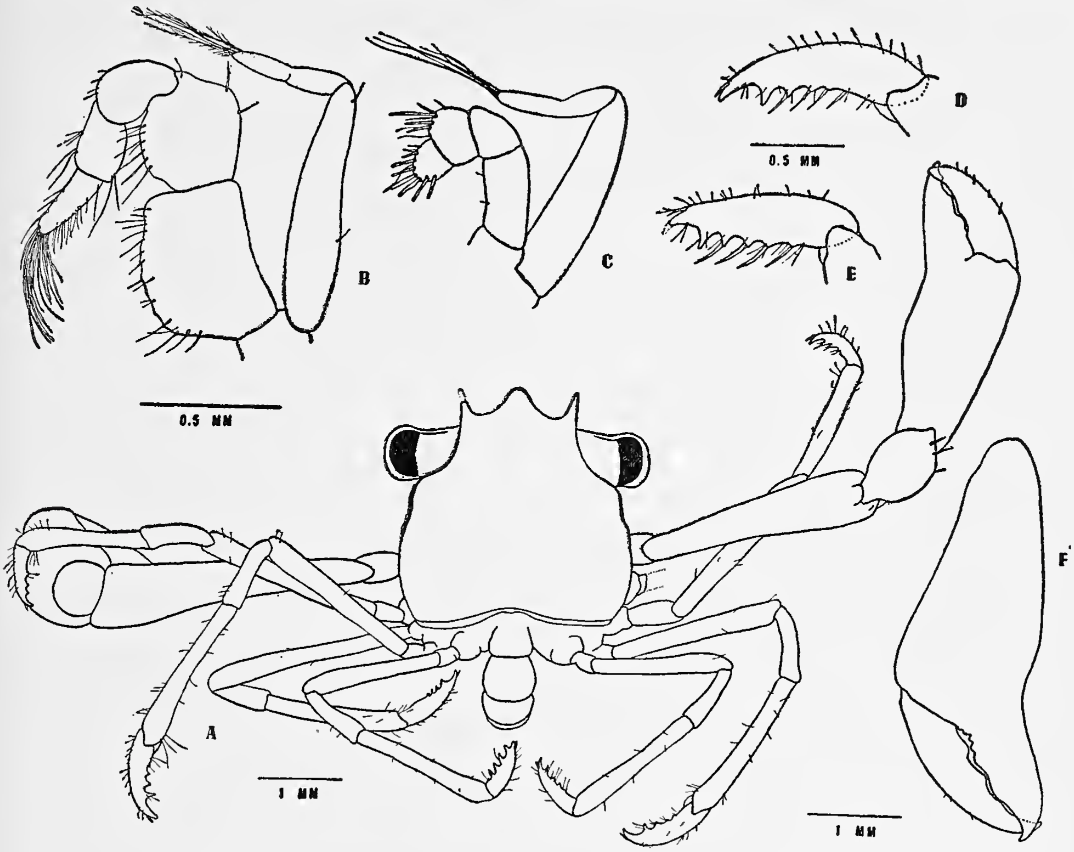
Measurements.—Males from 5.0 × 5.5 to 6.9 × 8.0 mm., females from 4.9 × 5.4 to 7.5 × 8.6 mm., young from 3.3 × 3.4 mm.

Habitat.—Gravelly sand.

Color in Life.—Two middle-sized specimens not pure white or with bold orange chelipeds like a northern form [*cf.* Crane, 1937, p. 74]. Two larger specimens all carapace mouse gray with posterolateral margins curdled white. Chelipeds light brown with a band of dark gray polkadotted with white at distal end of each segment. Rest of segment dotted with fine darker brown. Ambulatories banded gray and white. Smaller specimen white with chestnut front and chestnut chelipeds. Largest specimen curdled white; chelipeds translucent violet gray or pale rosy. Definitely matching lavender shade of some of the gorgonids. (J. Crane, field notes).

Remarks.—Although the only reference to the presence of gorgonian corals at this station is to be found in the color description above, it is a safe presumption that the specimens were taken on *Muricea miser* Verrill or a related species. The symbiotic relationship between these crabs and the corals is discussed by Crane (1937). The megalops of the species has not previously been described.

Description of the Megalops. — Carapace smooth and bare, slightly broader than long, sides convex, hepatic margins also slightly con-



Text-fig. 2. *Quadrella nitida* Smith, megalops. **A**, dorsal view; **B**, third maxilliped; **C**, second maxilliped; **D**, left third walking leg; **E**, left fourth walking leg; **F**, left chela. J. W. Knudsen, del. (B and C, same scale; D and E, same scale).

vex, posterior margin concave. Front wide, rostrum deflexed, bluntly triangular, straight-sided, broadly separated from lateral frontal spines; these spines pointing upward and not extending as far forward as rostrum. Transorbital breadth greater than breadth of carapace.

Second maxilliped with a two-segmented exopodite bearing 5 terminal setae; endopodite with four segments bearing 2, 0, 8, and 10 or more setae from merus to dactylus. Third maxilliped with a two-segmented exopodite bearing 5 or 6 plumose setae terminally; endopodite with ischium and merus broadened; ischium subrectangular, with 16 or more setae on inner margin; merus subquadrate, with 8 or more setae on inner margin and 2 at anteroexternal angle; carpus rounded distally, a few setae on outer margin; propodus with 6 or more setae arranged in tufts, one on inner, 2 on outer margin; dactylus with 12 or more setae on inner, 4 on outer margin, with a terminal brush of a dozen or more longer hairs.

Cheliped without an ischial spine; merus elongate, cylindrical, broadening distally; carpus ovate, three setae on outer distal margin; manus elongate, compressed, broadening distally, lower margin sinuous; dactylus with a row of 5 setae, closing against propodus without a gape, their tips crossing.

Walking legs slender, cylindrical; meri long and of a length equal to their respective propodi; carpi only half as long as meri and of a length equal to their dactyli; legs finely and sparingly setose. Dactyli adapted to clinging to gorgoniaceous corals, having a curved nail and superior bristle terminally, followed by a succession of teeth diminishing regularly in size from tip to base along lower margin, accompanied by stout bristles.

Abdomen with six segments and a telson; sixth segment and telson smaller than preceding segments; telson arcuate.

Family Goneplacidae

Pseudorhombila xanthiformis Garth

Pseudorhombila xanthiformis Garth, 1940, p. 86, pl. 24, figs. 1-5.

Range.—Known only from the type locality, Gorgona Island, Colombia. 40-60 fathoms.

Material Examined.—14 mi. S × E of Judas Point, Costa Rica, March 1, 1938, Station 214, D-1, D-3, 42-50 fathoms, 3 males, 1 female. Off Ballenas Bay, Gulf of Nicoya, Costa Rica, February 25, 1938, Station 213, D-12, 35 fathoms, right and left chelae, of large size; D-16, 45 fathoms, carapace fragment.

Measurements.—Males 12.6 × 17.3 and 15.6 × 22.9 mm., female 8.9 × 12.7 mm., young male 6.4 × 8.9 mm.

Habitat.—Mud and shell.

Color in Life.—Not recorded.

Remarks.—Known only from the type female, a 15 × 22 mm. specimen, *Pseudorhombila xanthiformis* is now represented by males of good size, and from a locality midway between the type locality, Gorgona Island, Colombia, and the type locality of *Oediplax granulata* Rathbun. When males of the latter species become available, it should be possible to elucidate the relationship between the two species, and to tell whether they should belong in the same or in different genera. (See discussion, Garth, 1940, p. 88).

Description of the Male Abdomen.—Segments 3-5 fused, suture lines clearly visible. Abdomen widest at base of segment 3, narrowest at middle of segment 6, sides of segments 4-6 concave. Segment 7 broader than long, sides straight and convergent, tip rounded.

Euryplax polita Smith

Euryplax politus Smith, 1870, p. 163.

Euryplax polita, Rathbun, 1918, p. 36.

Range. — Apart from a listing by Glassell (1934) as occurring in the Gulf of California, but without specific locality, the species is known only from Panama.

Material Examined. — Tangola-Tangola Bay, Mexico, Station 196, December 9, 1937, D-6, D-8, 9-7 fathoms, 2 females, 2 young; December 12, 1937, D-13, 10 fathoms, 1 male, 1 young; December 13, 1937, D-16, 16 fathoms, 1 young female; D-17, 23 fathoms, 1 female.

Measurements. — Male 8.7 × 14.8 mm., females from 4.4 × 5.9 to 5.8 × 8.8 mm., young from 3.2 × 4.4 mm.

Habitat.—Sand, gravelly sand, crushed shell, and mud.

Color in Life.—Not noted.

Remarks.—The male above is larger than the male holotype, a 6.9 × 11.2 mm. specimen. *Euryplax polita* is now known from a definite west Mexican locality. Included with the adult specimens enumerated above are a total of 11 megalopa possibly referable to this species.

Chasmophora macrophthalma (Rathbun)

Eucratopsis macrophthalma Rathbun, 1898, p. 601, pl. 43, figs. 3, 4.

Chasmophora macrophthalma, Rathbun, 1914, p. 119, text-fig. 2; 1918, p. 37, text-fig. 12.

Range.—Known only from the type locality, Bay of Panama. 51.5 fathoms. (Rathbun, 1898).

Material Examined.—32 specimens from 3 stations:

Mexico

4 mi. SSW of Maldonado Point, November 30, 1937, Station 192, D-1, 26 fathoms, 4 males, 4 females (3 ovigerous).

Santa Cruz Bay, December 7, 1937, Station 195, D-21, 18 fathoms, 2 females.

Tangola-Tangola Bay, December 13, 1937, Station 196, D-17, 23 fathoms, 12 males, 10 females (1 ovigerous).

Measurements.—Males from 2.1 × 3.1 to 4.5 × 8.8 mm., females from 2.7 × 4.6 to 4.4 × 8.3 mm., ovigerous females from 3.2 × 5.9 to 4.4 × 8.3 mm.

Habitat.—Mud.

Color in Life.—Not noted.

Remarks.—The larger males have the chelipeds tremendously developed for their rather small size. The smaller females (3.0 × 5.1 and 3.0 × 5.3) show spinulous anterolateral teeth. *Chasmophora macrophthalma* may now be reported from west Mexico, a northward range extension from Panama.

Speocarcinus granulimanus Rathbun

Speocarcinus granulimanus Rathbun, 1893, p. 242; 1918, p. 40, text-fig. 15, pl. 9.

Range.—Off Cedros Island, Lower California, and from off Consag Rock to off Point Fermin, Gulf of California, Mexico. 23-33 fathoms. (Rathbun, 1893).

Material Examined.—6 specimens from 4 stations:

El Salvador

La Libertad, December 16, 1937, Station 198, D-1 or D-2, 13-14 fathoms, 1 female.

Meanguera Island, Gulf of Fonseca, Station 199, December 23, 1937, D-1, 16 fathoms, 2 young; December 27, 1937, D-11, D-12, 5 fathoms, 1 male.

Costa Rica

Cedro Island, Gulf of Nicoya, February 13, 1938, Station 213, D-1 to D-10, 4-10 fathoms, 1 young male.

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-8, 6 fathoms, 1 female.

Measurements. — Male 11.3 × 14.3 mm., females from 6.0 × 7.9 to 7.3 × 9.6 mm., young male 3.6 × 4.3, young female 4.6 × 6.0 mm.

Habitat.—Mud; sand, mud, and crushed shell; mangrove leaves, mud, and shells.

Color in Life.—Not noted.

Remarks.—As characteristic of mud bottoms as the following *Speocarcinus californiensis,* and occurring in the same dredge haul with it at Golfito, *S. granulimanus* may now be recorded from El Salvador and Costa Rica, a southward range extension from the Gulf of California.

Speocarcinus californiensis (Lockington)

Eucrate? californiensis Lockington, 1877a, p. 33.

Speocarcinus californiensis, Rathbun, 1904a, p. 190, pl. 9, fig. 1; 1918, p. 42, text-fig. 16, pl. 10, figs. 2, 3. Schmitt, 1921, p. 249, text-fig. 148.

Range.—San Pedro to San Diego, California. (Rathbun, 1918). Also Gulf of California, without specific locality. (Glassell, 1934).

Material Examined. — 12 specimens from 3 stations:

Costa Rica

Port Parker, January 20, 1938, Station 203, D-1 to D-3, 10-15 fathoms, 1 male.

Port Culebra, January 30, 1938, Station 206, D-2, D-3, 14 fathoms, 1 male, 4 females (1 ovigerous).

Golfito, Gulf of Dulce, March 9, 1938, Station 218, D-8, 6 fathoms, 4 males, 1 female, 1 young.

Measurements.—Males from 3.9 × 5.1 to 5.8 × 7.6 mm., females from 4.1 × 5.3 to 5.6 × 7.2 mm., the latter ovigerous, young from 2.3 × 2.8 mm.

Habitat. — Sandy mud, crushed shell; mangrove leaves, mud, and shell.

Color in Life.—Not noted.

Breeding.—Costa Rica in late January.

Remarks.—Like the preceding *Speocarcinus granulimanus, S. californiensis* may now be recorded from Costa Rica, although not from El Salvador, a range extension southward from the Gulf of California, locality uncertain.

Speocarcinus ostrearicola Rathbun

Speocarcinus ostrearicola Rathbun, 1910, p. 545, pl. 48, fig. 2; 1918, p. 41, pl. 10, fig. 1.

Range.—Known only from the type locality, Matapalo (near Capon), Peru, where it occurs in oyster beds. (Rathbun, 1910).

Material Examined.—95 specimens from four localities:

Nicaragua

Monypenny Point, Gulf of Fonseca, December 24, 1937, Station 199, D-2, D-6, 4-5 fathoms, 2 males.

El Salvador

La Unión, Gulf of Fonseca, December 27, 1937, Station 199, D-14, 5 fathoms, 1 male, 1 female.

Panama

Bahia Honda, March 16, 1938, no station number, depth not given, 14 males.

Bella Vista, Panama City, date not given, 75 males, 4 females.

Measurements.—Males from 7.3 × 10.0 to 16.0 × 24.6 mm., females from 6.8 × 9.3 to 9.2 × 13.0 mm.

Habitat.—Mud.

Color in Life.—Not noted.

Remarks.—In view of the exceeding abundance of this species, both at Bahia Honda and Bella Vista, Panama (where males predominated over females, however), it is difficult to see how it has remained a rarity in collections. Known only from Peru, and from the unique type specimen, at least insofar as recorded in the literature, *Speocarcinus ostrearicola* may now be said to occur off Central America as far north as Nicaragua. Specimens show great difference as to the amount of pubescence on the carapace, some being entirely bare, others almost "furry." The interspace between the first and second anterolateral teeth is more U-shaped than V-shaped.

Chasmocarcinus latipes Rathbun

Chasmocarcinus latipes Rathbun, 1898, p. 602, pl. 43, fig. 5; 1918, p. 57, text-figs. 25, 26. Crane, 1937, p. 75. Garth, 1948, p. 52.

Chasmocarcinus ferrugineus Glassell, 1936, p. 216. Crane, 1937, p. 75, pl. 7, fig. 24.

Range.—From Cedros Island, Lower California, and Arena Bank, Gulf of California, Mexico, to off Esmeraldas, Ecuador. 20-51 fathoms. (Garth, 1948).

Material Examined.—22 specimens from 5 stations:

El Salvador

La Libertad, December 16, 1937, Station 198, D-1 or D-2, 13-14 fathoms, 1 male, 1 female.

Meanguera Island, Gulf of Fonseca, December 23, 1937, Station 199, D-1, 16 fathoms, 3 males, 5 females.

Costa Rica

Off Ballenas Bay, February 25, 1938, Station 213, D-12, D-16, 35-45 fathoms, 1 male, 1 female.

14 mi. S × E of Judas Point, March 1, 1938, Station 214, D-2, D-3, 43-50 fathoms, 5 males, 4 females.

Panama

Gulf of Chiriqui, March 13, 1938, Station 221, D-4, 38 fathoms, 1 female.

Measurements.—Males from 4.0 × 5.6 to 9.1 × 12.2 mm., females from 4.2 × 5.8 to 8.6 × 12.0 mm. A larger female, crushed, could not be measured.

Habitat.—Mud, sand, mud and crushed shell; sandy mud.

Color in Life.—Carapace and chelipeds grayish-white; center of carapace pink; basal segments of all specimens brown. Pubescence brown. (Crane, 1937, of Gulf of California specimens).

Remarks.—Specimens of the reddish-brown color associated with *Chasmocarcinus ferrugineus* Glassell occur with normally colored individuals at Judas Point, thus supporting the synonymy given above. The ruddy color is due to a reddish mud. It is noteworthy that all *Chasmocarcinus* collected by the "Zaca" were *C. latipes*, and that none were *C. longipes* Garth (1940), which has as its range the Panama Bight, from Secas Islands, Panama, to La Plata Island, Ecuador. The equal and similar chelae of the large male from Judas Point serve to distinguish *C. latipes* from *C. longipes*, the chelae of which are unequal and dissimilar.

Hexapus williamsi Glassell

Hexapus williamsi Glassell, 1938, p. 445, pl. 35, figs. 1-4.

Range.—Known only from the type locality, San José, Guatemala.

Material Examined.—7 mi. W of Champerico, Guatemala, December 15, 1937, Station 197, D-2, 14 fathoms, 1 ovigerous female.

Measurements.—Female specimen, length 9.7 mm., width 15.5 mm.

Habitat.—Mud.

Color in Life.—Not noted.

Breeding.—Guatemala in mid-December.

Remarks. — Named for M. Woodbridge Williams and described under the title "New and obscure Decapod Crustacea from the west American coasts," the species has not been taken again since its discovery in April, 1937, by the "Stranger" of Capt. Fred E. Lewis. The "Zaca" specimen is therefore the first since the type, and the only female, the holotype being a male. A slight extension of range can be reported, since Champerico is about 75 miles north and west of San José, Guatemala.

Family Cymopoliidae

Cymopolia lucasii (Rathbun)

Palicus lucasii Rathbun, 1898, p. 600, pl. 43, fig. 2.

Cymopolia lucasii, Rathbun, 1918, p. 193, text-fig. 119, pl. 44, figs. 1, 2; Crane, 1937, p. 76. Garth, 1946, p. 500, pl. 87, fig. 1.

Range.—From Arena Bank, Gulf of California, to Cape San Lucas, Lower California, Mexico. Galapagos Islands. 5-60 fathoms. (Garth, 1946).

Material Examined. — 2 specimens from 1 station:

Mexico

Sulphur Bay, Clarion Island, May 11, 1936, Station 163, D-1, 20 fathoms, 1 male, crushed.

3 mi. off Pyramid Rock, Clarion Island, May 12, 1936, Station 163, D-2, 55 fathoms, 1 male.

Measurements.—Males 5.2 × 6.0 and 11.9 × 13.4 mm.

Habitat.—At Arena and Gorda Banks on muddy and sandy bottoms. (Crane, 1937).

Remarks.—*Cymopolia lucasii* is now recorded from Clarion Island, an intermediate locality between Cape San Lucas and the Galapagos Islands.

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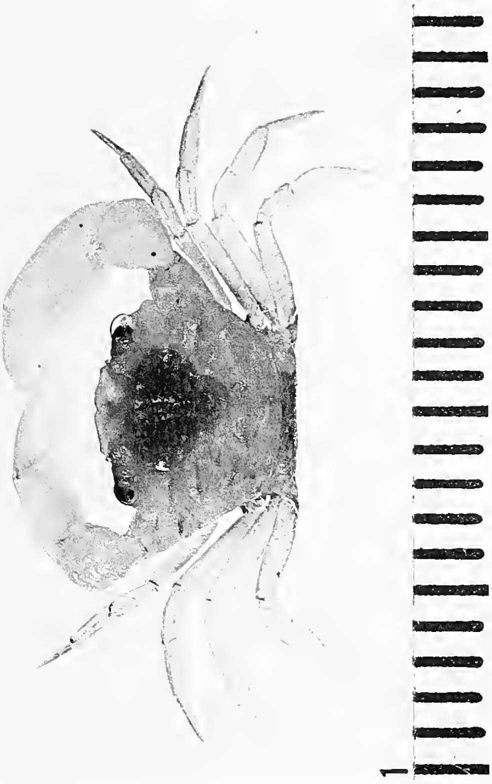
EXPLANATION OF THE PLATE

PLATE I

Hexapanopeus beebei, new species

FIG. 1. Male holotype, dorsal view.
FIG. 2. Male holotype, ventral view.
FIG. 3. Female paratype, dorsal view.
FIG. 4. Female paratype, ventral view. (Right third walking leg of female paratype missing)



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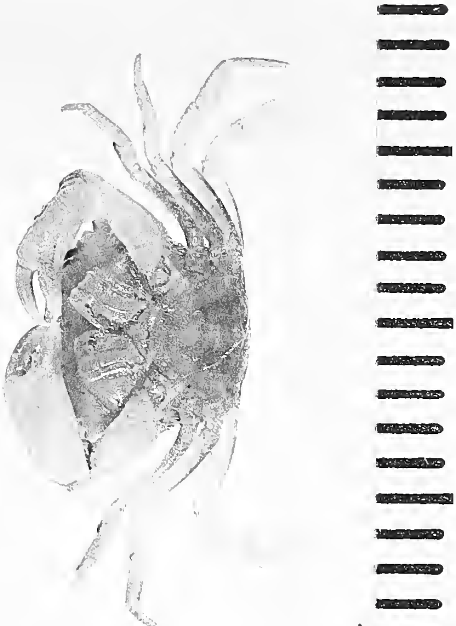

4

NON-INTERTIDAL BRACHYGNATHOUS CRABS FROM THE WEST COAST OF TROPICAL AMERICA

14

Nematodes and Cestodes from the Australian Monitor, *Varanus gouldii*

HORACE W. STUNKARD[1] & CHARLES P. GANDAL[2]

(Text-figures 1-6)

THE parasites reported here were taken from the digestive tract of a lizard, *Varanus gouldii* (Gray), which died November 10, 1959, in the New York Zoological Park. The animal, a mature male, was 1.58 meters long and weighed 7.8 kilograms. It was taken near Karumba, on the lower Norman River, North Queensland, June 21, 1959. When captured, it was eating a salt-water crocodile, *Crocodylus porosus. Varanus gouldii* is a terrestrial species which inhabits the arid interior areas of Australia.

Autopsy revealed several hundred nematodes with their anterior ends deeply embedded in the mucosa of the stomach. The stomach wall was greatly thickened and dark purple in color. The initial portion of the intestine, just beyond the stomach, contained about a dozen tapeworms with their scolices deeply embedded in the mucosa. In this area there were also several yellowish scars in the mucosa, presumably from the detached tapeworms. There was general peritonitis throughout the abdominal cavity. The liver was extremely swollen, with fibrinous deposits over the external surface. It contained numerous deep necrotic abscesses, which were light tan in color in contrast to the normal, red-colored tissue. Beginning about 10 cm. from the anus, the intestine was much distended for some 5 cm. and, on section, this region showed numerous scars in the mucosa with necrosis and sloughing.

Tanqua tiara (von Linstow, 1879) Blanchard, 1904

The nematodes are identified as *Tanqua tiara*, a species described by von Linstow (1879) as *Ascaris tiara* from *Varanus ornatus* in Natal, South Africa.

Linstow (1904a) erected the genus *Ctenocephalus* to contain *A. tiara*, and in the species he included worms from *Varanus bengalensis* taken in India and from *Varanus salvator*, a widely distributed lizard found in southeast Asia, Ceylon and the islands of the East Indies. Blanchard (1904) noted that *Ctenocephalus* von Linstow was a homonym of *Ctenocephalus* Kolenati, 1857, a genus erected to contain the siphonapterous species, *hyanae, canis, felis*, etc., and he renamed the genus *Tanqua*, with *tiara* as type species.

Meanwhile, Stiles had informed von Linstow that the name *Ctenocephalus* was preoccupied, whereupon von Linstow (1904b) substituted the name *Tetradenos* which, published later, became a synonym of *Tanqua*. The nomenclatorial combination, *Tanqua tiara*, was made by Stiles & Hassall (1905). Parona (1898) reported *Ascaris tiara* from *Varanus salvator* taken in Sumatra and from *Varanus gouldii* taken in Australia or New Guinea—the locality was not definite. Leiper (1909) identified worms from *Varanus niloticus* taken on the White Nile as *T. tiara.* Baylis (1916) suggested that the specimens described by von Linstow were from *Varanus albigularis*, rather than *V. ornatus.* He emended and amplified the earlier descriptions of *T. tiara;* to this species he assigned specimens from *V. niloticus* taken at Accra on the African Gold Coast, from *Varanus exanthematicus* taken in northern Nigeria, from *Tropidonotus quincunciatus* (= *T. asperrimus*) taken in Ceylon, and others from an unnamed species of *Varanus* taken in Zanzibar.

Baylis transferred *Heterakis anomala* von Linstow, 1904, from the stomach of *Tropidonotus piscator* taken in Ceylon, to *Tanqua* as *T. ano-*

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mala (von Linstow) and described as *T. diadema* n. sp., specimens from the South American freshwater snake, *Helicops angulatus. Tanqua diadema* was distinguished by the shorter length of the esophagus and the more anterior location of the vulva. *Tanqua anomala* is a large species and like *T. diadema,* has an anterior vulva.

The specimens from *V. gouldii* agree so completely with Baylis's definition of *T. tiara* that their allocation is readily made.

Tanqua tiara is one of the gnathostomes, spirurid nematodes, in which typically the life-cycle involves three hosts, a crustacean, a fish or amphibian and the final vertebrate host, although in certain species with hard-shelled, embryonated eggs, a terrestrial arthropod may serve as the single intermediate host. In *T. tiara* the eggs are thin-shelled. According to Mönnig (1947), juvenile worms migrate in the abdominal organs, particularly the livers of their final hosts. In passing through the liver, they destroy tissue and leave characteristic yellow mosaic markings on the surface and burrows filled with necrotic material in the parenchyma. They wander through other organs, including the diaphragm. Adult worms penetrate the wall of the stomach, producing cavities filled with sanguino-purulent fluid and a marked gastritis.

Bothridium parvum (Johnston, 1913)

The cestodes from *V. gouldii* belong to the genus *Bothridium* de Blainville, 1824, whose members are parasitic primarily in pythons and boas. The type species is *B. pithonis* de Blainville, 1824, from the intestine of *Python molurus,* although de Blainville (1828) stated that the parasite occurred also in other pythons.

Joyeux & Baer (1927) gave a historical review of the genus and the species from snakes which had been assigned to it. They suppressed *Prodicoelia* Leblond, 1836, and *Solenophorus* Creplin, 1839, as synonyms of *Bothridium. Prodicoelia* was based on *P. ditrema* Leblond, 1836, from the South American anaconda, listed as *Boa scytale* (=*Eunectes murinus* Wagl.). Joyeux & Baer recognized *B. ditremum* (Leblond, 1836) and two species from African and Asian pythons, together with a smaller variety of the Asian species. The African species was identified as *B. ovatum* (syn. *Solenophorus ovatus* Diesing, 1850) from *Constrictor hieroglyphicus* (= *Python sebae* Kuhl.). The larger Asian specimens were assigned to *B. pithonis* de Blainville, 1824, and the smaller worms from *Python reticulatus* Gray were described as *Bothridium pithonis* var. *parvum* nov. var. Joyeux & Houdemer (1928) listed both *B. pithonis* and *B. pithonis* var. *minor* Joyeux & Baer, 1926, from the intestine of *Python reticulatus.* In addition to pythons and boas, *B. pithonis* was reported from the king cobra of Malaya, *Naja hannah,* by Loewen (1945) and Yeh (1956).

Species of *Bothridium* have been described also from varanid lizards. Valenciennes (1850) recorded an unnamed species from *Varanus niloticus.* Johnston (1913) described specimens from *Varanus varius* taken in North Queensland as *Bothridium pythonis* var. *parva* var. nov. and later (1916) he listed these worms as *Bothridium parvum.* Notified of the homonomy of *B. pithonis* var. *parvum* Joyeux & Baer, 1927, and *B. parvum* (Johnston, 1913), Joyeux & Baer (1928) proposed the name *B. pithonis* var. *minor* to replace *B. pithonis* var. *parvum.* They followed de Blainville in writing the specific name *pithonis,* although they stated that correct transliteration would be spelled, *pythonis.* Certain authors including Monticelli & Crety (1891) and Johnston (1913) have used the correct orthography. Baylis (1935) noted that the name given by Joyeux & Baer (1927) was *B. pithonis* var. *parvum,* not *B. pithonis* var. *minor* Joyeux & Baer, 1926, as stated by Joyeux & Houdemer (1928). Baylis observed that the specific name, *minor,* is available to distinguish between the specimens studied by Joyeux & Baer and those of Johnston. Joyeux & Baer (1936) admitted that the specimens named *B. pithonis* var. *minor* are identical with those named earlier *B. pithonis* var. *parvum;* that the latter name disappears as a homonym and the correct designation is *B. pithonis* de Blainville, 1824; var. *minor* Joyeux & Baer, 1928.

The descriptions of *B. parvum* and *B. pithonis* var. *minor* are so brief and inadequate that satisfactory comparisons between them and the present specimens can not be made. Johnston (1913) noted differences in the form of the bothria as a result of muscular contractions and that the relative thickness of the ring of longitudinal muscles varies inversely with the elongation of the proglottid, so certain measurements that appear precise may have little value. The form of the ovary, especially its anteroposterior extension, may change as the proglottid is relaxed or compressed by contraction of the longitudinal or transverse muscles. Since the descriptions of the species named above are so inadequate, it appears worth while to present a more complete account of the worms from *V. gouldii.* They agree most closely with *B. parvum* and, although somewhat larger, are tentatively assigned to that species.

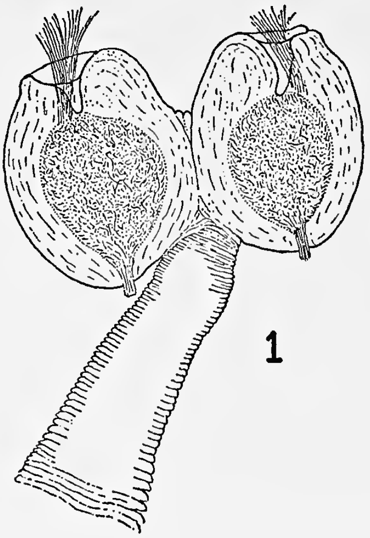
It is noteworthy that *V. varius,* host of *B. parvum,* is an arboreal species of rain-forests whereas *V. gouldii* lives in very dry interior areas and does not climb trees. The differences in locality and ecology, together with difficulties the parasite would encounter in transferring from one area to the other, suggest distinct species of *Bothridium*, but clear-cut specific differences are not apparent. Furthermore, two possibilities exist to account for the same species in different hosts and climatic regions. The species of *Varanus* are carnivorous and an intermediate host containing the larvae may migrate seasonally or otherwise between desert and rain-forest regions. Mertens (1942) reported that monitors feed on other lizards, on birds and many types of animals; also that a specimen of *V. salvator* 1.65 m. long was found in the stomach of *Python reticulatus*, 3 m. long. Thus the parasites of each species are introduced into the digestive tracts of their predators. Another possibility presupposes that a common ancestor of the two varanids may have harbored the cestode which has persisted in the existing species without specific change. The problems of specific determination and intraspecific variation in parasitic flatworms were discussed by Stunkard (1957).

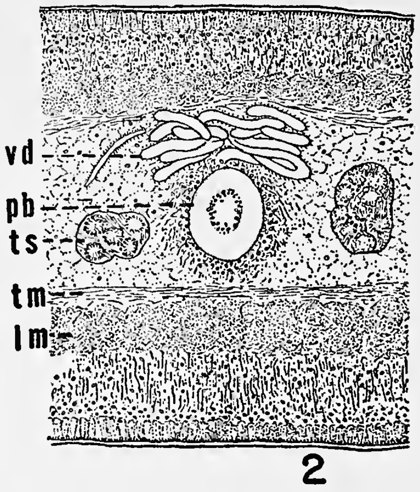
The specimens from *V. gouldii* measure as much as 75 mm. in length and 2.5 mm. in greatest width, with scolices so deeply embedded in the intestinal wall that they could be removed only when released by dissection of the surrounding tissue. When pulled free, the bothria were filled with tissue of the host and bundles of fibers protruded from the anterior openings (Text-fig. 1). The scolex measures 3.0-3.4 mm. in the dorsoventral axis; the bothria are 2.1-2.4 mm. long and 1.45-1.75 mm. in width. The strobila is craspedote and a stained, mounted specimen 68 mm. long comprises 244 proglottids. The original terminal proglottids had been lost. There is virtually no unsegmented neck region; the proglottids increase in size and after one-fifth of the total length, the sides are almost parallel. The reproductive organs mature early and by proglottid 150, one-fourth of the length from the scolex, proglottids measure 1.80 mm. in width, 0.20-0.27 mm. in length, and eggs are beginning to appear in the uterus. Further back in the strobila the proglottids become more elongate and the one before the last (Text-fig. 5) is 1.85 mm. long and 1.30 mm. wide. The reproductive organs have virtually disappeared and it is hardly more than an egg sac.

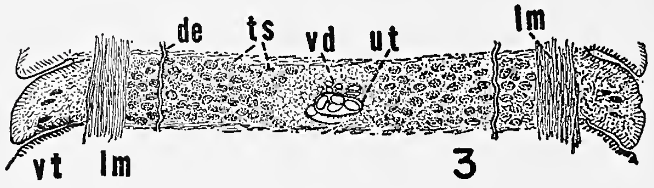
The excretory vessels and nerve trunks lie in the medullary parenchyma. The two excretory ducts on either side extend the length of the strobila; the dorsal ones are smaller and slightly lateral to the larger ventral vessels which are connected by transverse ducts near the posterior end of each proglottid. The chief longitudinal nerve trunks are situated just lateral to the ventral excretory ducts and ventral to the dorsal excretory vessels.

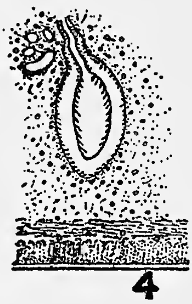
The testes are arranged in a single, staggered layer, 150-200 follicles in the medullary portion of each proglottid (Text-figs. 3, 6). They extend throughout the length of the proglottid, except in the median field, and in some proglottids there are about as many follicles on the lateral as on the medial sides of the excretory ducts. The follicles are oval, 0.032-0.055 mm. in length and 0.015-0.035 mm. in width, with their length in the transverse axis of the proglottid. Vasa efferentia fuse to form larger ducts which unite and open into a long, much coiled, sperm-filled vas deferens, 0.010-0.015 mm. in diameter (Text-fig. 2), which functions as a seminal vesicle. The vas deferens opens into a structure with thick muscular walls and lined by ciliated epithelium, formerly known as a seminal vesicle or an "Eschtrichtscher Körper," but Fuhrmann (1931) observed that it seldom contains sperm and actually functions in expulsion of spermatozoa. He called it a "Propulsionsblase," literally a propulsive bulb, and in the present worms (Text-figs. 2, 4), it is 0.10-0.15 mm. long and 0.08-0.11 mm. in diameter. The structure is continuous with and followed by the cirrus-sac, 0.15-0.20 mm. in diameter, which contains many cells that appear to be secretory, with ducts to the base of the male papilla. The common genital pore is located medially, on the ventral surface, about one-fourth of the length of the proglottid from its anterior margin and at the level of the posterior edge of the velum of the preceding proglottid. There is a transversely oval genital atrium, lined with cuticula; the opening of the vagina is immediately behind or somewhat lateral to the cirrus-sac.

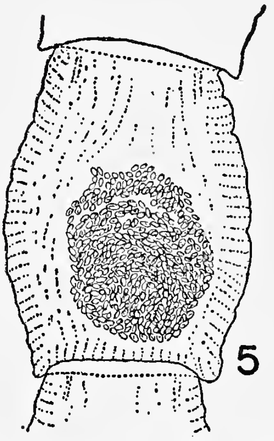
The vagina extends posteriad from the genital pore, below the uterus and either right or left of the uterine pore; it passes dorsal to the ovary where the posterior end expands to form a diagonally oriented seminal receptacle 0.08-0.12 mm. in length and 0.04-0.06 mm. in diameter. The ovary is reniform to bilobed, situated on the ventral side, near the posterior end of the proglottid. It is 0.30-0.45 mm. wide and 0.05-0.08 mm. in anteroposterior measurement. The lateral portions extend dorsally, forming a depression which is occupied by the seminal receptacle and the anterior portion of the shell-gland. The oviduct arises at the median posterior face of the ovary, curves laterally anteriorly and dorsally, where it receives a duct from the seminal receptacle; it then turns posteriorly and ventrally, receives the common vitelline duct and enters the shell-gland where it coils about and expands to form the ootype. The shell-gland in one series of

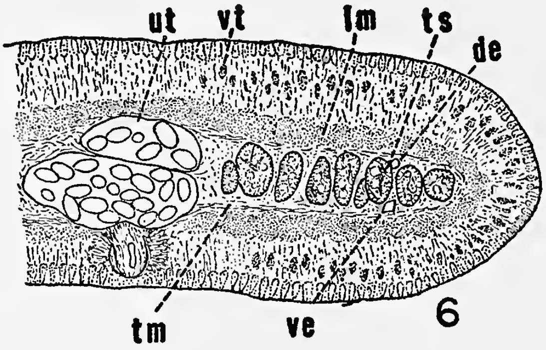












Text-fig. 1. Scolex and early proglottids; scolex turned 90 degrees in mounting so the dorsoventral aspect appears in lateral view; fibers from intestinal wall of host in the bothria. Text-fig. 2. Transverse section through the vas deferens and propulsive bulb, showing cortical and medullary parenchyma, longitudinal and transverse muscles and testicular follicles. Text-fig. 3. Frontal section, showing vitelline follicles, longitudinal muscles, dorsal excretory vessels, testes, vas deferens and uterine coils above the ovary. Text-fig. 4. Transverse section through the junction of vas deferens and propulsive bulb. Text-fig. 5. Gravid proglottid, one before the last in a strobila, with accumulation of eggs in the uterus. Text-fig. 6. Transverse section, showing vitellaria, longitudinal, transverse and dorsoventral muscles, testes, excretory ducts, uterus and male papilla. Abbreviations used in Figures: **de**—dorsal excretory duct; **lm**—longitudinal muscles; **pb**—propulsive bulb; **tm**—transverse muscles; **ts**—testis follicle; **ut**—uterus; **vd**—vas deferens; **ve**—ventral excretory duct; **vt**—vitelline follicle.

cross sections is 0.024 mm. wide and 0.010 mm. in dorso-ventral extent; frontal sections give an anteroposterior range of 0.010-0.016 mm. From the shell-gland the uterus emerges on the dorsal side of the ovary; it coils ventrally, then dorsally and anteriorly, passing forward on the dorsal side of the proglottid. As it becomes filled with eggs it forms loops (Text-figs. 3, 6), which overlie the ovary and cirrus-sac; the terminal recurrent loop is surrounded by a heavy sphincter and opens at the uterine pore, situated near the middle of the ventral surface of the proglottid. The vitelline follicles lie in the cortical area (Text-figs. 3, 6) and like the testes are interrupted in the median field. They are oval, 0.016-0.035 mm. in length and 0.010-0.022 mm. in width. On either side, ducts from the follicles unite to form larger ducts which pass mediad in the posterior part of the proglottid and join to form the common vitelline duct which opens into the oviduct just before it enters the shell-gland. As the proglottids become gravid, the reproductive organs regress and the uterus becomes a large, egg-filled sac (Text-fig. 5). The eggs are thin-shelled, 0.075-0.080 mm. in length and 0.045-0.050 m. in width.

The life-cycle is not known for any species of *Bothridium* but certain significant data are available. Solomon (1932) obtained development of *B. pythonis* to the procercoid stage in *Cyclops viridis*. Meggitt (1931) reported plerocercoid larvae from the mesentery, lung and aorta of *Naja naja* in Burma. Baylis (1933) found encysted plerocercoid larvae, believed to be those of a species of *Bothridium*, in the liver of a snake (*Bungaris fasciatus*) in Java. Baylis (1935) reported similar larvae from cysts on the external wall of the intestine and mesentery of an Australian water-rat (*Hydromys chrysogaster*) taken at Cromarty, North Queensland. On morphological grounds he assigned the plerocercoids to the genus *Bothridium*, but stated that specific identification is at present impossible. From present data it is probable that crustaceans are the first intermediate hosts and harbor procercoid stages, that fishes serve as second hosts and harbor the plerocercoids which migrate and encyst in the visceral organs of reptiles and small mammals that ingest infected fishes, and that the strobilate stage develops in the final predator.

SUMMARY

Nematodes and cestodes are reported from the digestive tract of a monitor lizard, *Varanus gouldii*, which was captured in North Queensland, Australia, and died in the New York Zoological Park. The nematodes are identified as *Tanqua tiara* (von Linstow, 1879) Blanchard, 1904, and the cestodes are referred tentatively to *Bothridium parvum* (Johnston, 1913) Johnston, 1916, although the description of that species and of *Bothridium pithonis* de Blainville, 1924; var. *minor* Joyeux & Baer, 1928, are so incomplete that final determination is equivocal.

NOTE: Specimens of *Tanqua tiara* and *Bothridium parvum* are in the collection of the American Museum of Natural History, New York.

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15

Urinary Amino Acids of Non-human Primates

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(Plates I-III; Text-figures 1-4)

I. INTRODUCTION

SMALL but measurable quantities of free amino acids are regularly excreted in the urine of all mammals. Individual patterns of urinary amino acid excretion appear to be genetically determined and related to phylogenetic position. The present paper is a comparative study of the urinary amino acid excretion patterns of non-human primates.

The urinary amino acid excretion of humans has been the subject of normative and clinical studies and of genetic research. Berry (1953), Stein (1953) and Eades & Pollock (1954) determined the amino acid concentrations of urine specimens from normal human individuals. Smith (1958) reported on the association of unusual urinary amino acid patterns with various human diseases. The extensive literature on the inheritance of human amino acid excretion patterns has been reviewed by Harris (1959).

Only two previously published papers pertain to the urinary amino acid excretion of non-human primates. Datta & Harris (1953) included one rhesus monkey in their survey of mammalian urinary amino acid patterns. Gartler, Firschein & Dobzhansky (1956) reported on the amino acid excretion of 48 apes—2 gibbons, 3 orang-utans, 37 chimpanzees and 6 gorillas.

II. MATERIALS AND METHODS

The general procedure followed in the present study is as follows. Specimens of urine were collected from 112 of approximately 200 primates kept at the Chicago Zoological Park at Brookfield, Illinois. A sample of each specimen was desalted and subjected to two-way paper chromatography. The amino acids separated on each urinary chromatogram were converted into visible spots by reaction with a color reagent. The quantity of amino acid represented by each spot was measured by comparing its optical density with the optical density of spots produced by known quantities of the corresponding pure amino acid.

As shown in Table I, the 112 animals studied belong to 7 of the 10 living families of non-human primates. Sixteen belong to 3 families of the Suborder Prosimii; 96 belong to 4 families of the Suborder Anthropoidea. The species and sex of the individuals studied are given in Table II. The classification used throughout is that of Simpson (1945), except for the generic subdivision of the Callithricidae, which follows Hershkovitz (1958).

All but two of the urine specimens were obtained by confining animals individually in a metabolism cage. Specimens from 106 of the 112 animals were collected over a 24-hour period of confinement. Specimens from the other

TABLE I. NUMBERS OF GENERA OF PRIMATES STUDIED COMPARED WITH NUMBERS OF LIVING GENERA

Family	Living Genera	Genera Studied	Individuals Studied
Suborder Prosimii			
Tupaiidae	6	1	7
Lemuridae	6	1	2
Indriidae	3	.	.
Daubentoniidae	1	.	.
Lorisidae	6	3	7
Tarsiidae	1	.	.
Total	23	5	16
Suborder Anthropoidea			
Cebidae	12	6	27
Callithricidae	3	2	8
Cercopithecidae	16	9	51
Pongidae	5	4	10
Total	36	21	96

TABLE II. SPECIES AND SEX OF ANIMALS STUDIED

Species	Males	Females	Total	Common Name
Family Tupaiidae				
Urogale everetti	2	5	7	Tree shrew
Family Lemuridae				
Lemur fulvus	1	1	2	Lemur
Family Lorisidae				
Nycticebus coucang	2	2	4	Slow loris
Perodicticus potto	1	0	1	Potto
Galago crassicaudatus	1	1	2	Galago
Family Cebidae				
Cacajao rubicundus	0	2	2	Uakari
Pithecia monacha	0	1	1	Saki
Cebus albifrons	4	1	5	Capuchin
Cebus apella	3	1	4	
Cebus capucinus	1	1	2	
Cebus nigrivittatus	1	0	1	
Saimiri sciureus	2	1	3	Squirrel monkey
Ateles belzebuth	0	3	3	Spider monkey
Ateles geoffroyi	1	1	2	
Lagothrix cana	0	1	1	Woolly monkey
Lagothrix infumata	1	0	1	
Lagothrix poppigii	2[1]	0	2	
Family Callithricidae				
Callithrix jacchus	0	4	4	Marmoset
Saguinus leucopus	0	1	1	Tamarin
Saguinus nigricollis	1	1	2	
Saguinus oedipus	1	0	1	
Family Cercopithecidae				
Macaca irus	0	3	3	Macaque
Macaca maura	1	1	2	
Macaca mulatta	0	2	2	
Macaca nemestrina	0	3	3	
Macaca nemestrina × *M. silenus*	1	0	1	
Macaca radiata	2	4	6	
Macaca silenus	1	0	1	
Cercocebus torquatus	1	1	2	Mangabey
Papio cynocephalus	7	1	8	Baboon
Comopithecus hamadryas	1	0	1	Hamadryas baboon
Mandrillus leucophaeus	0	2	2	Drill
Mandrillus sphinx	1	1	2	Mandrill
Cercopithecus aethiops	1	2	3	Guenon
Cercopithecus diana	0	1	1	
Cercopithecus l'hoesti	0	2	2	
Cercopithecus mitis	0	1	1	
Cercopithecus neglectus	1	2	3	
Cercopithecus talapoin	1	0	1	
Erythrocebus patas	1	0	1	Patas monkey
Presbytis entellus	2	1	3	Langur
Presbytis obscurus	1	1	2	
Colobus polykomos	1	0	1	Guereza
Family Pongidae				
Hylobates lar	1	2	3	Gibbon
Pongo pygmaeus	1	1	2	Orang-utan
Pan troglodytes	1	3	4	Chimpanzee
Gorilla gorilla	1	0	1	Gorilla
Total	51	61	112	

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[1] One, unavailable for re-examination, may be *L. infumata*.

6 individuals—1 orang-utan, 4 chimpanzees and 1 gorilla—were collected from a single urinary discharge. To assess individual constancy of amino acid excretion, a second urine specimen was obtained from 12 of the 106 animals originally represented by 24-hour specimens. Eleven of the 12 subsequent specimens were 24-hour collections; the other was from a single urination. A third 24-hour specimen was obtained from 1 animal.

Following the collection of each specimen its volume was measured, and after thorough mixing a sample was taken for amino acid determination. Because it was necessary to store the samples for a time between collection and analysis, isopropyl alcohol was added routinely as a preservative. The amount added was such that the original volume of urine equaled 90% of the final volume. The samples were stored at 4°C. for an average of approximately 8 months. The effect of storage on the amino acids of human urine has been studied by Stein (1953). He detected no change resulting from storage except for an increase in glutamic acid concentration. Stein attributed this increase to the presence in urine of a labile conjugate of glutamic acid which during storage is converted to the free amino acid.

Before chromatography each of the stored samples was desalted in a Research Specialties Co. electrolytic desalter in order to improve chromatographic resolution (Smith, 1958). A measured quantity, ranging from 20 to 200 μl., of each desalted sample was applied as a 7-10 mm. spot centered 2.5 cm. from one corner of a 23 × 28 cm. sheet of Whatman No. 1 filter paper. Five or six sheets spotted with urine were mounted on a chromatography rack together with 5 or 6 sheets spotted with various concentrations of a known mixture of commercially-obtained amino acids. In each instance 11 sheets, including unknowns and standards, were chromatographed simultaneously on one rack. Two chromatograms were made of each urine sample, usually on separate racks.

The first chromatographic solvent, which was run the length of the sheet, was a mixture (30:10:10:1) of 2,6-lutidine (Eastman Practical, 95%), absolute ethanol, distilled water and diethylamine. The second solvent, run at right angles to the first, was a mixture (100:20:0.6) of liquefied phenol (Mallinckrodt Gilt Label, 88%), distilled water and ammonia. As recommended by Block, Durrum & Zweig (1958), a beaker containing 1% sodium cyanide in water was placed in the tank during phenol development. Following phenol development the sheets were allowed to air-dry thoroughly. Color was developed by dipping the dried sheets in a 0.2% solution of ninhydrin in acetone and subsequently heating them in a water-saturated atmosphere for 15 minutes at 76°C. Results of the technique are illustrated by Pl. I, Fig. 1, which shows the typical resolution of a known mixture of amino acids.

Within 15 hours after ninhydrin treatment, maximum optical densities of the developed spots were determined by means of a Welch Densichron transmission densitometer. Quantitative estimates of urinary amino acid concentrations were derived by interpolation from logarithmically plotted curves for standards developed on the same rack as the urine samples. Concentration values reported in this paper are the average of duplicate determinations. The distribution of differences between pairs of determinations is shown in Table III.

TABLE III. DISTRIBUTION OF DIFFERENCES BETWEEN DUPLICATE URINARY AMINO ACID DETERMINATONS

Concentration Log Difference between Duplicate Determinations[1]	Cumulative frequency in percent										
	Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxyproline	Average
<0.1	44	61	57	47	62	50	60	55	66	68	57
<0.2	70	90	82	76	80	67	69	75	93	78	78
<0.3	85	97	89	84	85	77	77	84	97	85	86
<0.4	94	100	94	93	88	84	85	89	99	88	91
<0.5	95	100	94	94	91	86	89	92	100	92	93
<1.0	100	100	100	100	99	97	94	98	100	97	99
<1.5	100	100	100	100	100	100	100	100	100	100	100

[1]Concentration unit = 10^{-5} mmoles/ml.

III. RESULTS

A total of 10 urinary amino acids were chromatographically identifiable in the specimens studied. Traces of unidentifiable ninhydrin-positive substances were detected in some specimens. The clearly identified amino acids are: glutamic acid, aspartic acid, serine, glycine, glutamine, lysine, taurine, threonine, alanine and hydroxyproline. Table IV gives urinary concentrations of these 10 amino acids in specimens from the 112 individuals included in the study. The body weight of each animal and the volume of its 24-hour specimen are also given.

Concentrations shown in Table IV are expressed logarithmically based on a concentration unit of 10^{-5} mmoles of amino acid per ml. of urine. In the interpretation of differences in concentration, a difference of 1.0 between concentration logs shown for two specimens obviously represents a tenfold difference in arithmetically expressed concentration. As indicated in a footnote to Table IV, concentration logs of zero are assigned to amino acids absent from a particular specimen or present in concentrations insufficient to yield a detectable chromatographic spot with the volume of urine applied.

TABLE IV. AMINO ACID CONCENTRATION OF URINE SPECIMENS FROM 112 NON-HUMAN PRIMATES

Individual	Species	Sex	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml.)	Log of concentration in 10^{-5} mmoles/ml.[1]									
					Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy-proline
33	*Urogale everetti*	♀	0.27	13	1.9	2.0	1.7	2.3	0	2.0	3.3	1.8	2.4	0
34	*U. everetti*	♀	0.27	23	1.6	2.3	1.6	2.1	0	1.5	2.9	1.6	2.0	0
35	*U. everetti*	♂	0.34	37	1.3	2.4	1.4	2.3	0	1.1	1.7	1.5	1.9	1.7
38	*U. everetti*	♀	0.28	12	1.6	1.4	1.1	2.0	0	0	3.0	0	1.9	0
39	*U. everetti*	♂	0.28	11	1.6	1.8	0.7	2.0	0	1.1	2.3	1.0	2.2	0
40	*U. everetti*	♀	0.35	9.4	1.3	2.0	1.4	1.8	1.5	1.6	3.3	1.5	1.3	0
41	*U. everetti*	♀	0.41	9.0	0	1.8	1.4	1.6	1.2	1.4	3.4	0	1.2	0
37	*Lemur fulvus*	♀	2.9	37	0.7	1.6	1.7	2.1	0	1.1	1.0	0.9	1.0	0
42	*L. fulvus*	♂	3.2	96	0.4	1.1	0	3.2	0	0.7	0	0	1.5	0
43	*Nycticebus coucang*	♂	1.1	22	0.6	0.6	0.6	2.1	1.0	0.8	3.0	1.5	1.1	0
45	*N. coucang*	♀	0.56	37	1.0	1.3	1.3	2.4	1.7	1.3	0	2.5	1.6	0
46	*N. coucang*	♂	0.42	11	1.3	1.7	0	2.9	0.7	0	0	0	1.3	0
53	*N. coucang*	♀	1.5	30	0.9	1.7	1.3	3.5	0.6	0	2.8	1.0	1.4	0
36	*Perodicticus potto*, 8 Sep. 58	♂	0.76	37	0.6	1.6	1.1	1.5	0.4	0	2.9	0	0.9	0
139	(Indiv. No. 36), 24 Oct. 58	♂	n.d.[2]	(20)[3]	1.0	1.3	1.0	1.6	0	1.0	2.6	1.3	1.0	0
132	*Galago crassicaudatus*	♀	1.1	16	1.3	2.7	2.0	2.5	1.4	1.3	2.3	2.1	1.5	1.4
133	*G. crassicaudatus*	♂	1.0	19	0.8	1.6	1.2	2.1	0	1.2	2.4	0.6	1.1	0
47	*Cacajao rubicundus*[4]	♀	3.5	180[4]	1.3	2.3	2.3	3.5	3.5	1.9	1.9	2.2	2.1	2.3
44	*Pithecia monacha*, 16 Sep. 58	♀	2.7	83	1.3	2.8	2.0	3.3	3.2	0.6	0	2.3	2.0	2.3
138	(Indiv. No. 44), 24 Oct. 58	♀	n.d.[2]	125	0.9	1.7	1.9	2.6	3.0	0.6	0	1.2	1.9	2.1
94	*Cebus albifrons*	♂	2.3	92	0	1.9	1.8	2.7	2.0	1.4	2.3	1.7	1.8	2.3
96	*C. albifrons*	♂	2.3	56	1.0	1.4	1.8	2.2	1.6	1.3	0	1.6	1.4	1.8
104	*C. albifrons*[5]	♂	3.2	91	1.5	1.7	1.8	1.3	1.7	1.5	0.8	0.9	1.3	1.5
106	*C. albifrons*[5]	♂	2.9	67	1.4	1.9	1.8	2.5	1.9	0	2.1	1.3	1.7	1.7
109	*C. albifrons*[5], 8 Oct. 58	♀	1.1	165	1.0	1.8	1.1	1.9	0.9	0.8	0	0.8	1.3	0
158	(Indiv. No. 109)[5], 12 Dec. 58	♀	n.d.[2]	n.d.[2]	1.6	2.1	1.0	2.1	1.3	0.6	1.9	0.7	2.0	1.3
64	*C. apella*	♂	2.9	165	0.8	2.1	0.7	1.6	0	0.9	2.5	1.1	1.3	1.0
67	*C. apella*	♀	2.3	116	1.0	2.4	1.2	2.2	1.3	1.1	1.6	1.5	1.8	1.9
93	*C. apella*	♂	4.1	63	1.0	1.7	2.0	2.7	2.3	1.5	2.9	1.9	1.9	2.2
108	*C. apella*[5]	♂	5.6	36	2.3	2.4	2.0	1.9	2.0	1.5	2.0	1.6	1.9	2.2
95	*C. capucinus*	♂	4.8	90	1.1	2.1	2.1	2.8	1.8	1.2	0.7	1.4	1.9	1.6
137	*C. capucinus*	♀	1.6	116	1.4	2.3	2.2	2.8	2.1	1.0	1.8	1.9	2.4	2.0
97	*C. nigrivittatus*	♂	3.2	86	1.1	1.8	1.9	2.7	1.5	1.5	2.5	1.6	1.6	1.6
55	*Saimiri sciureus*, 12 Sep. 58	♀	0.58	23	1.0	2.0	1.7	1.9	1.8	1.5	0	1.5	1.5	1.5
157	(Indiv. No. 55), 18 Dec. 58	♀	n.d.[2]	9.5	1.6	1.7	0	2.0	2.0	0.7	1.3	1.3	1.6	1.0
56	*S. sciureus*	♂	0.86	20	1.2	1.7	1.6	2.2	1.7	1.3	2.7	0	1.4	2.1
140	*S. sciureus*	♂	0.42	9.0	1.6	2.6	1.0	2.7	0	1.4	1.9	1.5	2.4	3.1
66	*Ateles belzebuth*	♀	5.9	105	1.0	1.8	1.2	2.8	1.4	0	2.4	1.3	1.3	1.6
118	*A. belzebuth*[5]	♀	7.7	225	1.4	1.8	0.2	2.8	0	0.7	2.5	0.5	1.7	2.3

TABLE IV. AMINO ACID CONCENTRATION OF URINE SPECIMENS FROM 112 NON-HUMAN PRIMATES (*cont.*)

Individual	Species	Sex	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml.)	Log of concentration in 10^{-5} mmoles/ml.[1]									
					Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy-proline
119	*A. belzebuth*[5]	♀	5.0	98	0.7	1.4	1.4	2.3	1.2	0.6	2.1	1.0	1.0	1.9
122	*A. geoffroyi*[5]	♀	10.9	193	0.3	2.0	0.6	2.1	1.2	0.5	0	0	1.1	1.9
136	*A. geoffroyi*	♂	3.6	147	1.6	1.7	1.9	2.4	1.7	1.1	1.9	1.6	1.7	1.9
58	*Lagothrix poppigii*	♂	3.6	147	1.1	1.7	1.2	3.0	0	1.4	0	1.1	1.5	2.0
63	*L. poppigii* (or *infumata*)	♂	5.8	110	1.3	1.6	0.8	2.9	0	1.1	1.9	0	1.7	1.5
65	*L. cana*	♀	6.4	65	1.0	1.3	1.2	2.5	1.0	0	1.8	1.2	1.0	1.1
117	*L. infumata*	♂	6.1	42	2.2	2.3	2.3	3.3	2.3	1.7	2.4	2.1	1.9	1.9
48	*Callithrix jacchus*	♀	0.23	34	0	0.9	1.2	1.5	0	0	2.0	0.9	0.8	0
49	*C. jacchus*	♀	0.32	2.9	1.1	3.0	1.6	2.8	1.6	0.9	1.6	2.0	1.3	0
50	*C. jacchus*	♀	0.23	4.0	0.9	1.9	1.1	2.1	0.9	0.8	1.6	1.1	1.1	0
52	*C. jacchus*	♀	0.25	16	1.1	1.7	0.6	1.7	1.1	1.0	2.9	0.9	1.0	0
60	*Saguinus leucopus*	♀	0.44	27	0.9	2.6	0	2.6	1.0	1.5	1.9	1.8	1.7	0
128	*S. nigricollis*	♂	0.38	19	1.8	2.1	1.0	2.4	0	1.4	2.1	1.9	1.8	1.4
129	*S. nigricollis*	♀	0.36	36	0.8	1.7	0	2.2	0.5	0.5	1.1	1.3	1.2	1.2
59	*S. oedipus*	♂	0.39	20	0.4	2.0	1.2	2.9	1.4	0	0	1.6	1.5	0
62	*Macaca irus,* 16 Sep. 58	♀	4.0	207	1.0	1.2	0	2.9	0.8	0.5	0	0	1.3	0
112	(Indiv. No. 62), 8 Oct. 58	♀	3.6	146	1.7	2.4	0.6	2.6	1.2	1.3	0.7	0.7	2.3	0
135	(Indiv. No. 62), 22 Oct. 58	♀	4.1	105	1.3	1.4	0	2.9	1.1	1.0	0	0	1.0	0
103	*M. irus*	♀	3.8	120	1.4	1.5	1.8	2.5	2.2	0.8	0	1.6	1.2	1.5
105	*M. irus*	♀	2.9	41	1.5	2.0	1.9	2.3	2.9	1.4	0	1.7	1.5	0
83	*M. maura*	♀	3.6	310	1.1	1.8	0	3.0	1.3	0	1.4	0.8	1.3	0
84	*M. maura*	♂	10.4	485	1.4	1.6	0	2.9	1.3	0	0	0	1.4	0
100	*M. mulatta*	♀	6.1	228	1.1	1.3	1.0	2.0	1.0	0.8	1.7	0.4	1.1	0.3
101	*M. mulatta*	♀	8.6	123	0.4	2.0	1.3	1.9	1.5	1.5	1.4	1.4	0.9	0.4
75	*M. nemestrina*	♀	11.3	133	1.6	2.3	0	2.6	0.5	0	0	0.7	1.9	0
77	*M. nemestrina*	♀	8.6	n.d.[2]	1.4	1.8	1.1	1.9	1.0	1.1	0	0.9	1.5	0
92	*M. nemestrina*	♀	7.3	298	1.2	1.9	0.6	2.0	1.3	0.8	0	0.6	1.9	0.4
99	*M. nemestrina* × *M. silenus*	♂	10.7	188	1.3	2.1	0.9	1.8	0	1.0	1.3	0.9	0.9	0.3
68	*M. radiata*	♀	5.4	103	1.2	2.2	1.4	2.2	1.8	1.2	0	1.4	1.5	0
69	*M. radiata*	♂	5.2	120	1.3	2.0	1.1	2.7	1.4	1.6	0	1.2	1.5	0
70	*M. radiata*	♀	4.5	65	0	1.9	0.7	2.5	0.8	0.4	0	1.0	1.0	0
71	*M. radiata*	♀	5.8	100	1.0	2.0	1.1	2.3	0	0	0	0.9	1.0	0
72	*M. radiata*	♀	5.4	125	0.9	1.4	0	2.3	1.1	0	0	0	1.5	0
91	*M. radiata,* 29 Sep. 58	♂	6.8	372	0.9	1.2	0.3	2.2	1.5	0.4	1.0	0.7	0.9	0
163	(Indiv. No. 91), 22 Dec. 58	♂	7.3	425	0.1	1.2	0.8	1.8	1.0	0.6	0	0.5	0.7	0
98	*M. silenus*	♂	7.7	145	1.0	2.1	1.0	1.8	0.8	0.7	0	1.2	0.9	0
88	*Cercocebus torquatus*	♀	2.3	141	1.2	2.2	0.9	2.7	2.0	0.9	0.7	1.0	1.3	1.3
102	*C. torquatus*	♂	10.4	435	1.0	2.1	0.7	2.4	1.3	0.8	2.1	0.4	1.2	0.6
141	*Papio cynocephalus*	♂	16.8	450	0.2	1.1	0.9	2.1	1.2	1.1	2.1	0.9	0.8	0
142	*P. cynocephalus*	♂	12.2	327	0.7	1.2	1.0	2.0	1.4	0.9	2.6	1.4	0.8	1.0
143	*P. cynocephalus*	♂	16.7	61	0	1.6	0	2.2	1.4	0	2.9	0	1.2	0
144	*P. cynocephalus*	♂	15.9	210	0.7	1.4	0	2.4	0.9	0	2.3	0	1.1	0
145	*P. cynocephalus*	♀	6.8	(550)[3]	0.9	1.1	0.3	1.9	1.1	0.4	2.3	0.2	0.9	0.3
147	*P. cynocephalus,* 21 Nov. 58	♂	8.1	690	1.0	1.2	0.7	1.6	1.5	0.8	0	0	0.9	0
156	(Indiv. No. 147), 18 Dec. 58	♂	n.d.[2]	132	1.3	2.0	1.3	2.0	1.3	1.0	0	0	1.5	0.5
148	*P. cynocephalus*	♂	13.6	300	0.9	1.4	1.1	2.1	1.4	0.8	2.5	0	1.0	0
149	*P. cynocephalus*	♂	18.6	560	0.9	1.1	1.0	1.9	1.7	0.9	1.9	1.0	1.0	0
121	*Comopithecus hamadryas*	♂	18.8	>310	0.9	1.9	0.6	2.8	1.3	0.3	0.6	1.0	1.2	0.5
116	*Mandrillus leucophaeus*	♀	14.5	310	1.8	2.5	0.7	2.4	0.5	0.4	2.1	0.8	1.5	0
166	*M. leucophaeus*	♀	2.3	275	1.4	1.7	1.1	1.9	2.8	0.9	1.0	0.7	1.0	1.3
110	*M. sphinx*	♀	4.5	521	1.2	2.5	1.1	2.7	2.2	0	0	0.7	1.6	0
111	*M. sphinx*	♂	4.5	n.d.[2]	1.2	2.0	1.5	2.4	2.3	0	0	0	1.4	1.4
78	*Cercopithecus aethiops*	♀	3.4	59	0.5	1.4	0	2.9	0.8	0.8	0.8	0.3	1.1	0
79	*C. aethiops*	♀	3.9	250	1.0	1.7	0.2	2.4	0.7	0.8	0	0	1.0	0
86	*C. aethiops*	♂	7.9	250	0.5	2.1	0	2.8	1.2	1.1	0	1.0	1.0	0

TABLE IV. AMINO ACID CONCENTRATION OF URINE SPECIMENS FROM 112 NON-HUMAN PRIMATES (*cont.*)

Individual	Species	Sex	Body wt. (kg.)	Vol. of 24-hr. Specimen (ml.)	Log of concentration in 10^{-5} mmoles/ml.[1]									
					Aspartic	Glutamic	Serine	Glycine	Glutamine	Lysine	Taurine	Threonine	Alanine	Hydroxy-proline
87	*C. diana*, 25 Sep. 58	♀	2.7	193	1.4	3.0	0	2.8	2.2	0.5	0	1.3	2.3	0
130	(Indiv. No. 87), 21 Oct. 58	♀	2.7	202	2.0	2.8	1.3	2.9	2.8	0	0	1.6	2.3	1.1
73	*C. l'hoesti*	♀	2.3	280	1.1	1.2	0	2.7	1.1	1.1	0	0.5	1.2	0
74	*C. l'hoesti*	♀	4.3	190	1.2	1.5	0.3	2.8	1.7	0.4	0	0	1.5	0
81	*C. mitis*, 23 Sept. 58	♀	4.3	400	0.7	1.1	0	3.0	1.2	0	0.5	0	1.1	0.6
131	(Indiv. No. 81), 21 Oct. 58	♀	n.d.[2]	250	1.1	1.2	0	3.5	2.2	0.7	2.1	0.8	1.5	0.8
80	*C. neglectus*	♂	6.6	290	1.0	2.6	0	2.7	1.2	0.9	0.6	0.4	1.9	0
82	*C. neglectus*	♀	3.9	275	1.3	1.9	0	2.5	0	0	0	0	2.0	0
85	*C. neglectus*, 25 Sep. 58	♀	4.3	175	0.4	2.7	0	2.5	1.8	1.1	0	1.5	2.2	1.4
164	(Indiv. No. 85), 31 Jan. 59	♀	5.4	s.u.[6]	0.8	2.4	0.9	2.0	0	1.0	1.7	1.1	1.3	0.5
107	*C. talapoin*, 6 Oct. 58	♂	1.4	76	1.1	1.4	0	2.2	1.0	0.3	0.7	0.3	1.3	0
127	(Indiv. No. 107), 20 Oct. 58	♂	n.d.[2]	92	1.1	1.5	0	2.3	1.3	0	0	0	1.3	0
120	*Erythrocebus patas*	♂	13.6	86	1.4	1.5	0.9	2.0	0.6	1.3	1.6	1.2	0.8	0
113	*Presbytis entellus*	♂	11.7	188	0.7	1.8	2.3	2.8	2.4	1.4	2.0	1.9	1.2	0.5
114	*P. entellus*	♀	10.4	468	0.9	1.5	1.0	2.5	0	0	2.1	0.7	1.0	0.6
115	*P. entellus*	♂	15.4	(650)[3]	0.6	1.8	2.0	3.1	2.0	1.2	1.9	1.5	1.3	0.5
89	*P. obscurus*	♀	4.8	134	1.2	1.4	2.0	2.6	2.7	1.1	2.2	2.1	1.4	1.2
90	*P. obscurus*	♂	7.2	200	1.5	2.5	0.6	2.7	2.7	0.9	2.1	1.4	1.7	0
54	*Colobus polykomos*, 12 Sep. 58	♂	13.6	425	0.4	1.3	0.3	2.4	0.3	0	0	0	0.9	0
126	(Indiv. No. 54), 20 Oct. 58	♂	n.d.[2]	283	0.7	1.6	0.6	1.9	1.1	0.9	0.8	0.9	0.7	0.7
150	*Hylobates lar*	♀	4.5	50	1.0	1.5	1.4	2.0	1.2	1.4	1.3	0.8	1.6	0
151	*H. lar*	♂	6.4	172	0.9	1.0	1.2	2.3	1.3	0.9	0	0	1.3	0
152	*H. lar*	♀	5.0	153	0.8	0.9	1.1	1.7	1.2	0.6	1.0	0.6	0.9	0
123	*Pongo pygmaeus*	♀	15.9	n.d.[2]	1.1	1.6	0.4	2.4	0.9	0.3	1.2	0.5	1.5	0.9
153	*P. pygmaeus*	♂	24.9	s.u.[6]	0.5	0.4	0.6	1.5	0.1	0.6	1.2	0.3	0.7	0
159	*Pan troglodytes*	♀	12.7	s.u.[6]	0.5	0.5	0.3	1.3	0.9	0	2.0	0.1	0.1	0
160	*P. troglodytes*	♂	14.5	s.u.[6]	0.3	0.6	0.3	1.4	1.3	0.1	1.5	0.8	-0.1	0.6
161	*P. troglodytes*	♀	15.9	s.u.[6]	0.5	0.8	0.4	1.6	0	0.2	2.1	0.2	0.7	0.1
162	*P. troglodytes*	♀	10.4	s.u.[6]	1.2	1.3	1.0	1.5	1.3	0.9	2.2	0.9	0.9	0
154	*Gorilla gorilla*[7]	♂	n.d.[2]	s.u.[6]	1.0	1.6	0.9	2.2	1.4	0.8	1.0	0.8	1.0	0

[1] Zero values are assigned to amino acids whose concentration was insufficient to yield a detectable chromatographic spot with the volume of urine applied.

[2] Not determined.

[3] Approximate volume.

[4] Specimen from this species is composite collection of 24-hour output of two female uakaris.

[5] Cebid individual fed meatless diet.

[6] Specimen represents single urinary discharge.

[7] Specimen from this animal siphoned off previously scrubbed floor of exhibition cage.

IV. DISCUSSION

A. Urinary Output and Body Weight

In Text-fig. 1 generic mean logs of 24-hour urinary output in ml. ($U_{24\text{ hr.}}$) are plotted against corresponding mean logs of body weight in grams (B). Superimposed on these points is the line

$$U_{24\text{ hr.}} = 0.1536\ B^{0.82},$$

which is equivalent to

$$U_{1\text{ hr.}} = 0.0064\ B^{0.82},$$

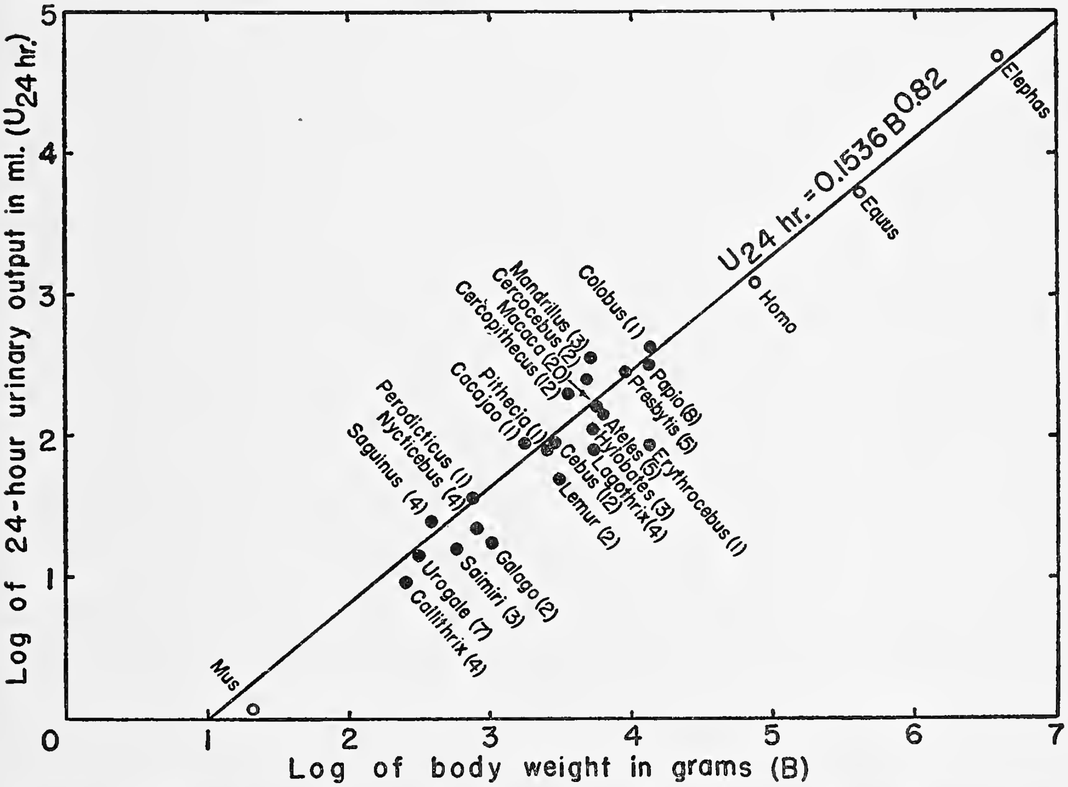
the equation found by Adolph (1943; 1949) to express the constant relationship between urinary output and body weight in mammals of diverse orders. Also shown on the graph are values from Adolph for man and for three non-primate genera. The primate data apparently conform to the general mammalian relationship. This casts doubt on the assumption of Gartler, Firschein & Dobzhansky (1956) that the low urinary creatinine concentration of gibbons, orang-utans, chimpanzees and gorillas is compensated for by a high daily volume of urine per unit of body weight.

B. Successive Specimens from Individuals

The amino acid concentrations of urine specimens from the 12 individuals sampled more than once are compared graphically in Text-fig. 2. These graphs show the successive specimens of each individual to be generally similar in urinary amino acid pattern. Taurine concentrations, however, do vary considerably in many cases, for 5 of the 12 re-sampled individuals taurine concentration log differences between successive specimens are 1.0 or greater. The general constancy of amino acid pattern in successive specimens from these non-human primates is in accord with the constant urinary amino acid patterns reported for human individuals by Berry (1953) and Harris (1953). Plates I and II, Figs. 2-4, are photographs of chromatograms comparing the successive specimens of 3 re-sampled individuals.

C. Effect of Diet

The results of previous studies (Sutton, 1951; Gartler, Firschein & Dobzhansky, 1956; Smith, 1958) indicate that the urinary amino acid excretion of humans is generally unaffected by normal variations in diet. In the course of the present study an opportunity to observe the effect of certain dietary factors on the urinary amino acid excretion of non-human primates was afforded by the housing and feeding arrangement at the Brookfield Zoo. Prosimians and most South American monkeys are kept in the Small Mammal House, and old world monkeys are kept in the Primate Building. Primates in the Small Mammal House are fed fruit, vegetables, bread and raw ground horsemeat fortified with a vitamin-mineral supplement. The basic diet in the Primate Building includes only fruit, vegetables and bread. Meat is not given in the Primate Building, although animals of the following species do receive a raw egg daily: *Cebus albifrons, Cebus apella, Macaca nemestrina* × *M. silenus, Macaca silenus, Cercocebus torquatus, Comopithecus hamadryas, Mandrillus leucophaeus, Mandrillus sphinx, Cercopithecus diana, Erythrocebus patas.* The phylogenetic separation of housing facilities outlined above is not maintained completely. Of the South American monkeys, 4 of 12 capuchins (*Cebus*) and 3 of 5 spider monkeys (*Ateles*) are kept in the Primate Building. The 7 cebids housed in the Primate Building receive a diet identical with that of the old world monkeys; these animals are individually identified in Table IV.



TEXT-FIG. 1. Urinary output in relation to body weight. Closed circles=means for primate genera studied (number of specimens in parentheses); open circles=values for man and non-primates, from Adolph (1943). Superimposed straight line also from Adolph.

In Table V the capuchins and spider monkeys fed meat and those not fed meat are compared with respect to their frequency distributions of urinary amino acid concentrations. The two groups differ consistently only in distribution of threonine concentrations. The feeding of fortified meat thus appears to have no general effect on the urinary amino acid excretion patterns of these cebids. A similar comparison of urinary amino acid concentrations of species fed eggs and of those not fed eggs reveals that this dietary factor also is without apparent effect on urinary amino acid excretion.

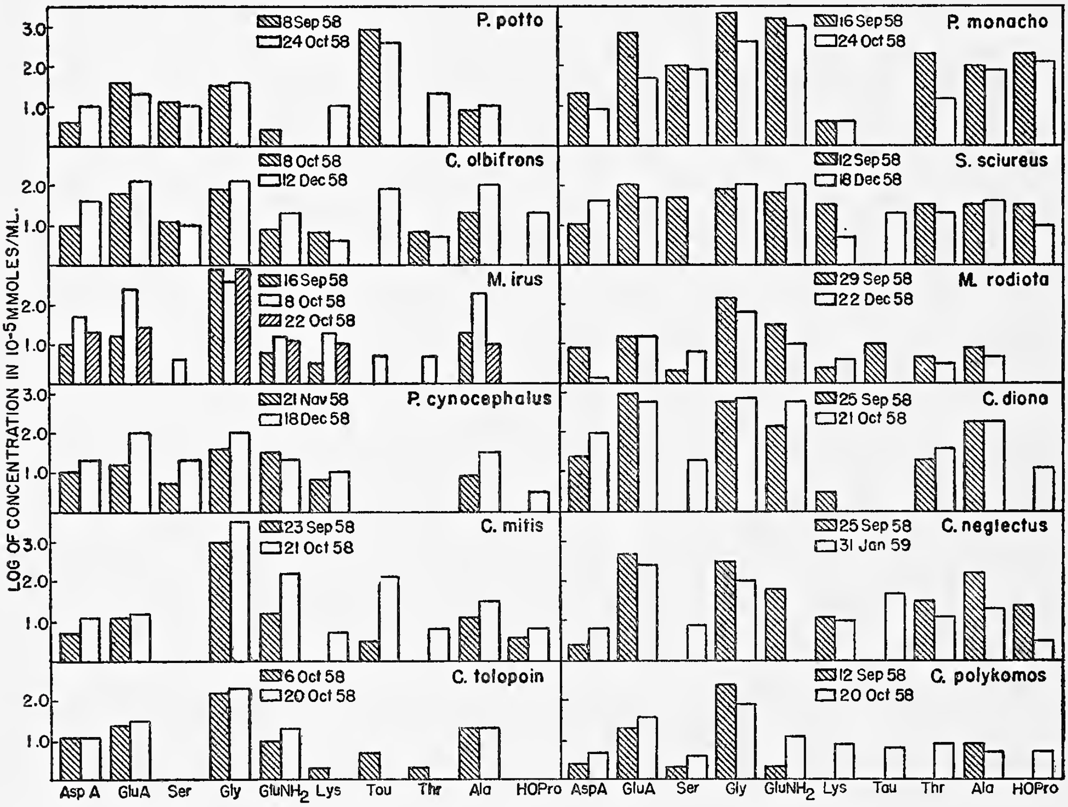
D. Phylogenetic Comparisons

Examination of Table IV reveals no consistent differences in concentration between species within a given genus. Accordingly, for purposes of phylogenetic analysis, attention is centered on comparison of the urinary amino acid patterns of genera and families.

Generic means and standard errors of urinary amino acid concentrations are represented graphically in Text-fig. 3. These means are based on data in Table IV for the 106 animals represented by 24-hour specimens. As there appear to be no sexual differences in urinary amino acid concentrations, data for both males and females are included in the computation of each generic mean. Data for the 6 animals represented only by specimens passed at a single urination are not included; the concentration level of these specimens may differ from that of the 24-hour specimens. Individuals from which two or more specimens were collected are represented by average concentrations.

Inspection of Text-fig. 3 reveals that the 10 amino acids identified differ considerably in inter-generic uniformity of concentration. Aspartic acid, glutamic acid, glycine and alanine maintain relatively constant concentrations in the urine of the 24 genera studied. Inter-generic variations of mean concentration of these 4 amino acids are small relative to intra-generic variations, represented by the standard errors. The other 6 amino acids show a relatively greater degree of inter-generic variation of mean concentration.

For the 4 amino acids of relatively constant concentration in primate urine, over-all concen-



TEXT-FIG. 2. Comparison of amino acid concentrations in successive specimens from 12 non-human primate individuals.

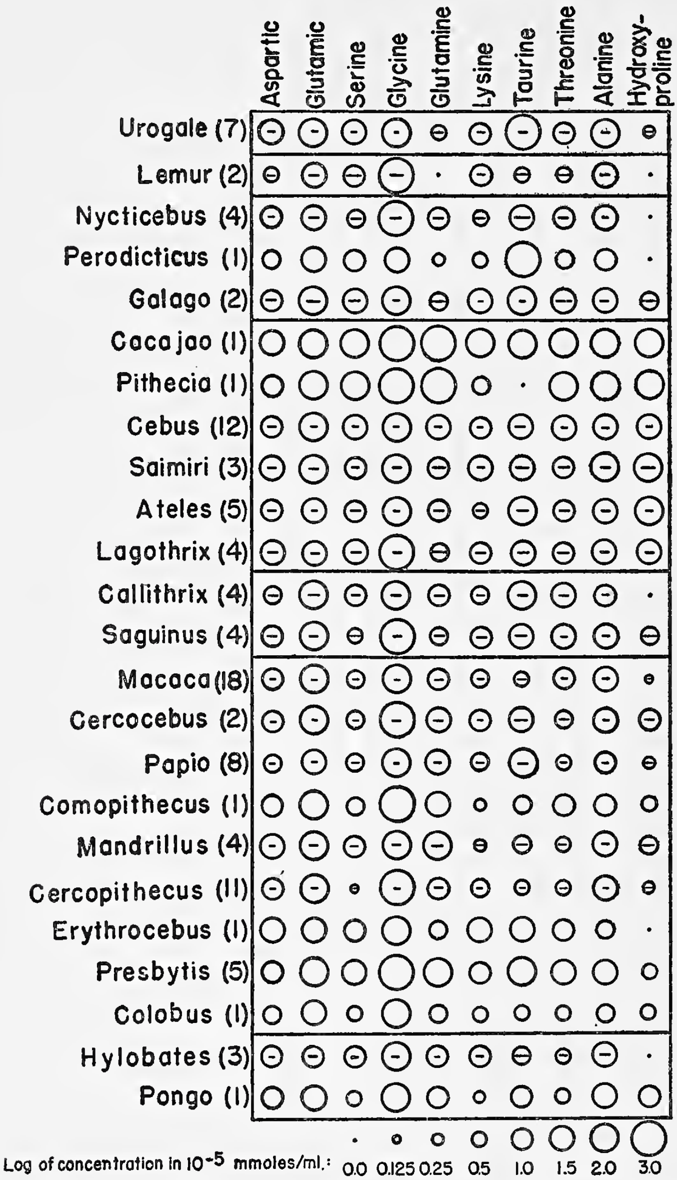
TABLE V. EFFECT OF DIET ON URINARY AMINO ACID EXCRETION OF 17 CAPUCHINS AND SPIDER MONKEYS

	Log of amino acid concentration in 10^{-5} mmoles/ml.											
Diet	<0.5	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9	<0.5	0.5 to 0.9	1.0 to 1.4	1.5 to 1.9	2.0 to 2.4	2.5 to 2.9
	Cebus						Ateles					
Aspartic Acid												
Meat	1	1	6	.	.	.	.	.	1	1	.	.
Meatless	.	.	2	1	1	.	1	1	1	.	.	.
Glutamic Acid												
Meat	.	.	1	3	4	.	.	.	.	2	.	.
Meatless	.	.	.	2	2	.	.	.	1	1	1	.
Serine												
Meat	.	1	1	3	3	.	.	.	1	1	.	.
Meatless	.	.	1	2	1	.	1	1	1	.	.	.
Glycine												
Meat	.	.	.	1	2	5	.	.	.	.	1	1
Meatless	.	.	1	1	1	1	.	.	.	.	2	1
Glutamine												
Meat	1	.	1	3	3	.	.	.	1	1	.	.
Meatless	.	.	1	2	1	.	1	.	2	.	.	.
Lysine												
Meat	.	1	5	2	.	.	1	.	1	.	.	.
Meatless	1	1	.	2	.	.	.	3	.	.	.	.
Taurine												
Meat	1	1	.	2	1	3	.	.	.	1	1	.
Meatless	.	1	1	.	2	.	1	.	.	.	1	1
Threonine												
Meat	.	.	2	6	.	.	.	.	1	1	.	.
Meatless	.	2	1	1	.	.	1	1	1	.	.	.
Alanine												
Meat	.	.	2	5	1	.	.	.	1	1	.	.
Meatless	.	.	1	3	.	.	.	.	2	1	.	.
Hydroxyproline												
Meat	.	.	1	4	3	.	.	.	.	2	.	.
Meatless	.	1	.	2	1	.	.	.	.	2	1	.

tration means may be taken as approximations of values for the order as a whole. These over-all means are given in Table VI. Glycine, with an average concentration of 2.40 μmoles per ml., is the most prominent amino acid in primate urine. Glutamic acid has the next highest average concentration, 0.63 μmoles per ml., about one-fourth the mean glycine concentration. It should be noted, however, that, because of the previously mentioned effect of storage, all of the glutamic acid measured may not originally have been present as the free amino acid. Urinary alanine and aspartic acid are much lower in mean concentration. The average alanine concentration is about one-tenth that of glycine; the average aspartic acid concentration, about one-twentieth.

The urinary glutamic acid and aspartic acid concentrations reported above for non-human primates are much higher than human urinary concentrations of these two amino acids. This agrees with one of the principal findings of Gartler, Firschein & Dobzhansky (1956), who found glutamic acid and aspartic acid concentrations in ape urine to be significantly higher than in human urine. The high urinary glycine concentrations found in the present study also agree with the results of Gartler, Firschein & Dobzhansky; they do not agree with the apparent absence of glycine from the urine of the single rhesus monkey studied by Datta & Harris (1953).

The 6 amino acids showing relatively great inter-generic variation of concentration—namely, serine, glutamine, lysine, taurine, threonine



Text-fig. 3. Comparison, by genera, of mean amino acid concentrations of urine specimens from 106 non-human primates. Area of each circle proportional to generic mean; bar within circle represents diameter of standard error of mean; horizontal lines separate data for each family.

and hydroxyproline—are those among which evidence is to be sought concerning phylogenetic trends in primate urinary amino acid excretion. Generic frequency distributions of concentrations of these 6 amino acids are compared graphically in Text-fig. 4, from which are omitted genera represented by only a single urine specimen. Generic excretion tendencies indicated by the frequency distributions are presented in Table VII. Phylogenetically these may be summarized as follows:

At the subordinal level, prosimian genera all tend to be low in urinary glutamine. At the family level, tupaiids, as represented by the single species available for study, appear to be high in urinary serine, lysine and taurine. Cebids as a family are uniquely high in urinary hydroxyproline; among cebid genera, *Cebus,* represented by 12 individuals of 4 species, clearly tends to be high in all 6 generically variable urinary amino acids. The two callithricid genera studied tend slightly to be high in urinary taurine. Cercopithecids generally are low in the generically variable urinary amino acids other than glutamine; most extreme in this respect is the genus *Cercopithecus. Papio* and *Presbytis,* however, are exceptional; *Papio* is high in urinary taurine, and *Presbytis* is high in serine, lysine, taurine, and threonine.

TABLE VI. MEAN CONCENTRATION OF ASPARTIC ACID, GLUTAMIC ACID, GLYCINE AND ALANINE IN URINE SPECIMENS FROM 106 NON-HUMAN PRIMATES

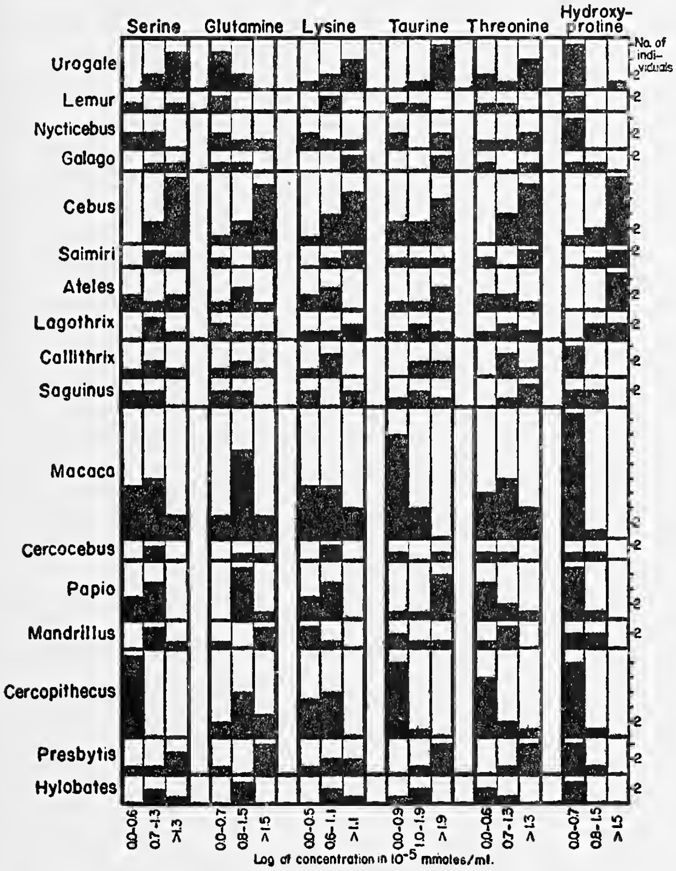
Amino Acid	Mean and Standard error of Concentration Logs[1]	Mean Concentration in μmoles/ml.
Aspartic acid	1.05 ± 0.04	0.11
Glutamic acid	1.80 ± 0.04	0.63
Glycine	2.38 ± 0.04	2.40
Alanine	1.41 ± 0.04	0.26

[1] Unit of concentration = 10^{-5} mmoles/ml.

The hydroxyproline content of cebid urine is distinctive. Twenty-two of 26 cebid specimens have hydroxyproline concentration logs of 1.5 or greater; only 2 of 79 non-cebid specimens have hydroxyproline concentrations this high. The mean urinary hydroxyproline concentration log for cebids is 1.83 ± 0.11 (0.66 μmoles per ml.); the corresponding mean for non-cebids is 0.27 ± 0.08 (0.02 μmoles per ml.). The hydroxyproline spot characteristic of cebid urinary chromatograms is illustrated in Pl. III, Fig. 5, which shows photographs of one chromatogram from each of the 6 cebid genera studied. These chromatograms also illustrate the dietary independence of cebid urinary hydroxyproline excretion. Although only the *Ateles* chromatogram is from an individual not fed meat, its hydroxyproline spot is not different from the others.

V. SUMMARY

Urinary amino acid excretion patterns have been studied chromatographically in specimens from 112 primate individuals representing 7 of 10 non-hominid families. Analysis of successive specimens from 12 animals indicates that urinary amino acid patterns of primate individuals



TEXT-FIG. 4. Distributions of urinary serine, glutamine, lysine, taurine, threonine and hydroxyproline concentrations in 16 non-human primate genera.

Table VII. Urinary Amino Acid Excretion Tendencies of Primate Genera

Suborder Family Genus	Amino Acid Concentration Level[1] of Genus					
	Serine	Glutamine	Lysine	Taurine	Threonine	Hydroxyproline
Prosimii						
Tupaiidae						
Urogale	+	0	+	+	(+)	0
Lemuridae						
Lemur		0		(0)	(0)	0
Lorisidae						
Nycticebus	0	(0)	(0)		(+)	0
Galago	(+)	(0)	+	+		(0)
Anthropoidea						
Cebidae						
Cebus	+	+	+	+	+	+
Saimiri	(+)	(+)	+		(+)	+
Ateles			0	+	(0)	+
Lagothrix	(+)	(0)	(+)			+
Callithricidae						
Callithrix			(0)	+	(+)	0
Saguinus	0	0			+	0
Cercopithecidae						
Macaca	0		0	0	(0)	0
Cercocebus		(+)			(0)	(0)
Papio	0	(+)	0	+	0	0
Mandrillus	(+)	+	0	(0)	(0)	0
Cercopithecus	0	(+)	0	0	0	0
Presbytis	+	+	(+)	+	+	0
Pongidae						
Hylobates	(+)		(+)	(0)	0	0

---

[1] + = high; O = low; ( ) = weak tendency.

remain relatively constant through time. Dietary variations appear in general to be without effect on urinary amino acid excretion. Daily urinary output bears a constant exponential relationship to body weight.

The primate genera studied tend to be uniform in urinary concentration of glycine, glutamic acid, alanine and aspartic acid. Average concentrations of these 4 amino acids, expressed in μmoles per ml. of urine, are as follows: glycine, 2.40; glutamic acid 0.63; alanine, 0.26; aspartic acid, 0.11. Inter-generic variations of concentration are relatively great for glutamine, serine, lysine, taurine, threonine and hydroxyproline. Prosimians in general are low in urinary glutamine. Tupaiids, represented in the study by one species, appear to be high in urinary serine, lysine and taurine. Cebids are uniquely high in urinary hydroxyproline; the genus *Cebus* also is high in serine, glutamine, lysine, taurine and threonine. Cercopithecids tend to be low in inter-generically variable urinary amino acids other than glutamine; *Papio,* however, is high in taurine, and *Presbytis* is high in serine, taurine and threonine.

Acknowledgments

During the course of this investigation the author was a National Science Foundation predoctoral fellow in the Department of Zoology, University of Chicago. The research was conducted under the supervision of Dr. H. H. Strandskov, University of Chicago, to whom the author wishes to express grateful appreciation. Sincere thanks also are extended to Dr. H. H. Strain, Argonne National Laboratory, for advice concerning chromatographic technique, and to Mr. Philip Hershkovitz, Chicago Natural History Museum, for taxonomic assistance. Special thanks are due Mr. Robert Bean, Director of the Chicago Zoological Park at Brookfield, Illinois, and his staff. Without the generous cooperation of officials and staff of the Brookfield Zoo, the present study would not have been possible.

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EXPLANATION OF THE PLATES

Plate I

Fig. 1. Typical chromatographic resolution of known mixture of amino acids.

Fig. 2. Chromatographic comparison of amino acids in successive urine specimens collected on indicated dates from male *Perodicticus potto.*

Plate II

Fig. 3. Chromatographic comparison of amino acids in successive urine specimens collected on indicated dates from female *Macaca irus.*

Fig. 4. Chromatographic comparison of amino acids in successive urine specimens collected on indicated dates from female *Cercopithecus neglectus.*

Plate III

Fig. 5. Urinary chromatograms representing each of six cebid genera studied. Arrows indicate hydroxyproline spots.

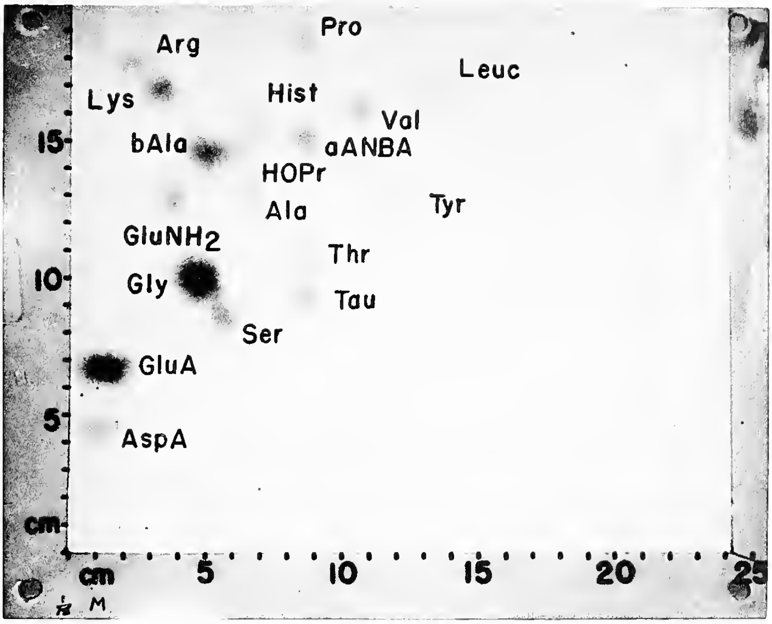

FIG. 1

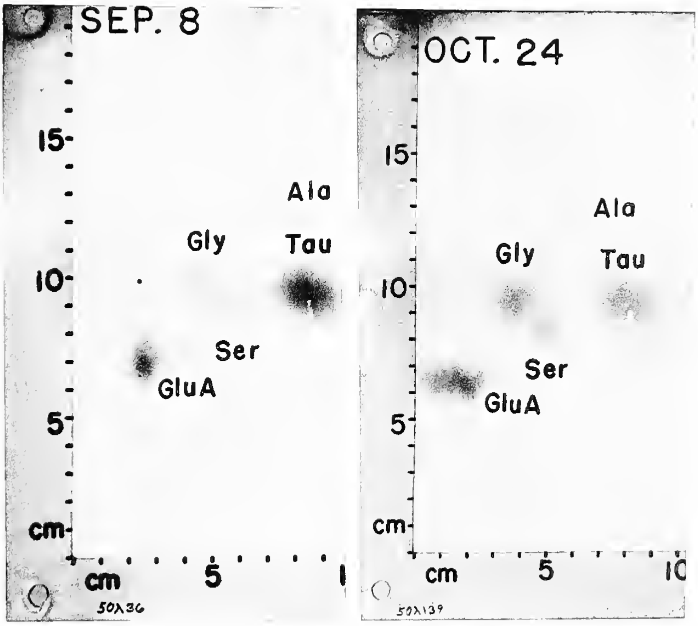

FIG. 2

URINARY AMINO ACIDS OF NON-HUMAN PRIMATES

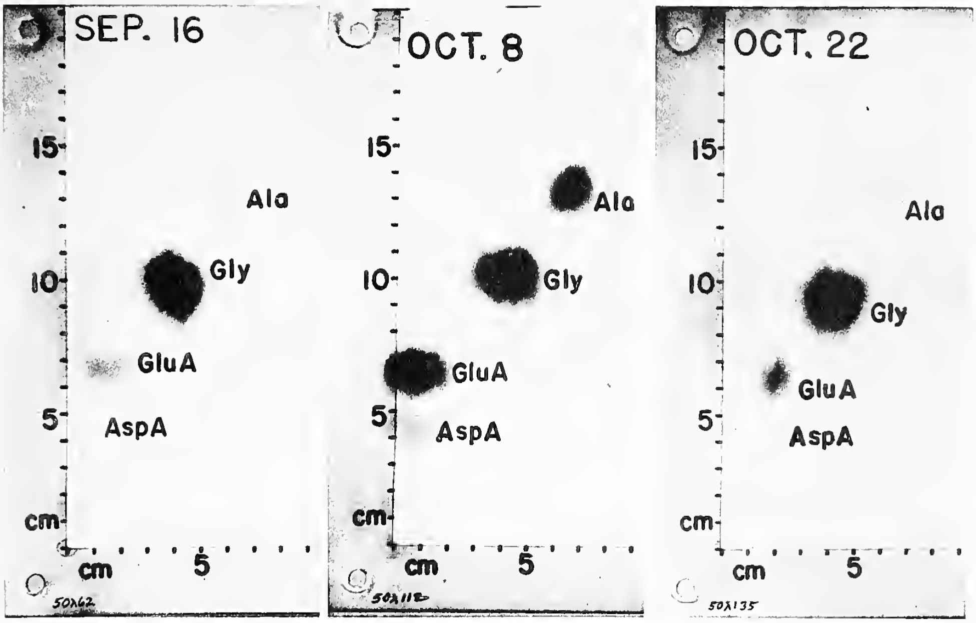

FIG. 3

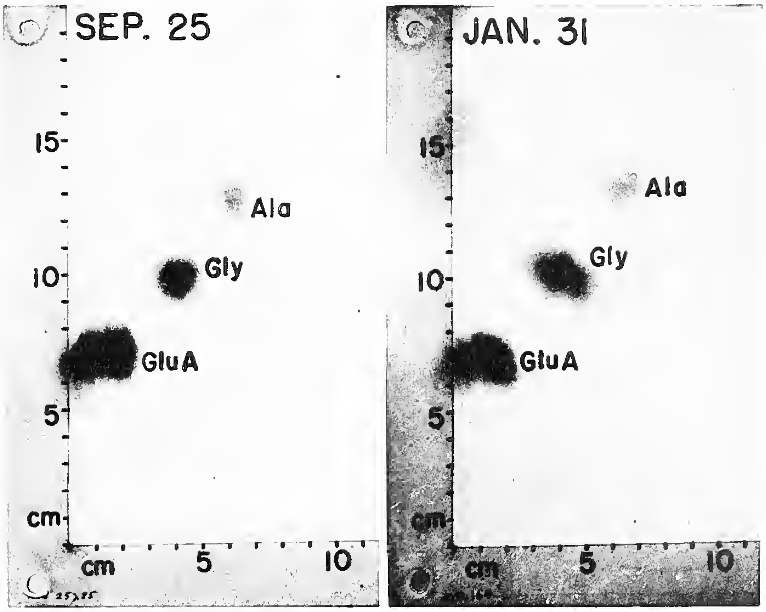

FIG. 4

URINARY AMINO ACIDS OF NON-HUMAN PRIMATES

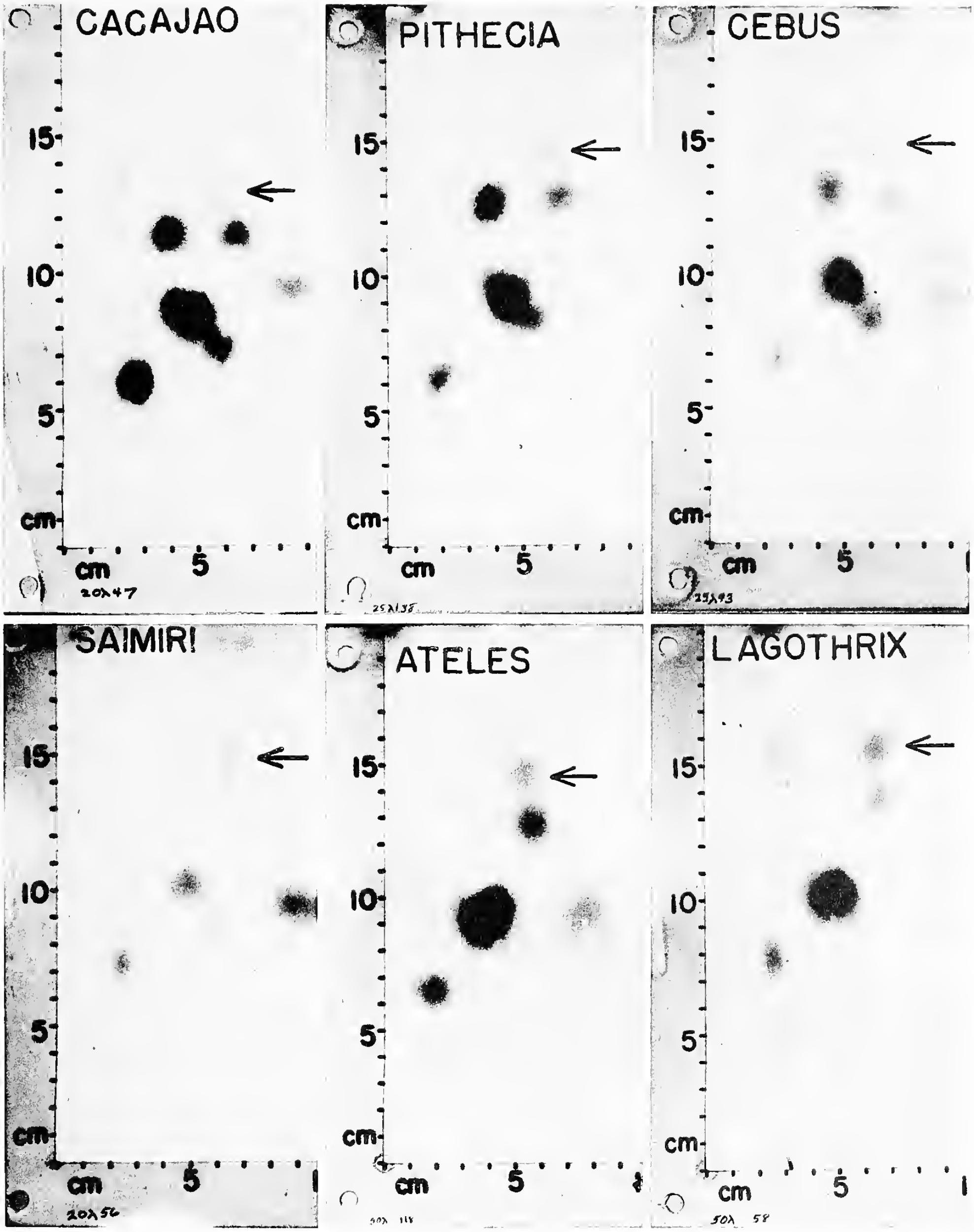

FIG. 5

URINARY AMINO ACIDS OF NON-HUMAN PRIMATES

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Contents

16

The Role of the Thyroid in the Development of Platyfish[1]

K. France Baker-Cohen[2]

Genetics Laboratory of the New York Aquarium, New York Zoological Society

(Plates I-IX; Text-figures 1-4)

THE role of the thyroid in the physiology of fishes has been a subect of controversy for years (Lynn & Wachowski, 1951; Hoar, 1957; Pickford, 1957). Although a considerable body of evidence has accumulated in support of the importance of the thyroid in the growth and maturation of teleosts, there also have been many conflicting reports. Until recently, work in this field was hampered by the impossibility of thyroidal extirpation in most teleosts, owing to the diffuse nature of the tissue and its intimate relation to major blood vessels. Observations were limited to the study of effects of antithyroid drugs ("chemical thyroidectomy") and of thyroid preparations that were administered to fish with autogenous thyroids (Table 12). The results of such experiments were always subject to criticism, because of the possibility of toxic effects exerted independently of the effect on thyroid hormone production, and because non-physiological hormonal excesses may have led to abnormalities.

Recently, radioactive iodine (I^{131}) has been employed as a thyroidectomizing agent in teleosts, with varying effectiveness (Table 11). However, the effects of replacement therapy in athyroid fish have not been reported. With proper controls, the use of I^{131} may permit analysis of thyroidal function in fish in a manner more nearly comparable to that made possible in higher animals by thyroidectomy.

This paper presents observations made, through the use of radioiodine treatment, on thyroidal function in the growth and sexual development of platyfish, *Xiphophorus maculatus.* The work presented here is an outgrowth of a series of experiments in which radiothyroidectomy of very young platyfish was attempted in an effort to obtain evidence relating to the origin of heterotopic thyroid tissue in this species (Baker, 1958a). Many of the radioiodine-treated fish and their controls described here were included in data reported in that publication, but only a few incomplete observations on their anatomy and pathology were given at that time.

Materials and Methods

1. *Radioiodine Treatment of Young Platyfish.*—The origins of the strains of platyfish used in these experiments have been described in Baker *et al.* (1955). Most of the experiments were made on fish of the *BH* strain, which was very susceptible to pharyngeal goiter and to thyroid tumors in the kidneys and other organs (Baker, 1958a).

a. *General Procedures*: (Table 1) Groups of platyfish, ranging in age from 17 to 70 days, were treated with radioiodine (I^{131}) by immersion in 200 ml. of aquarium water containing 4.5-5.02 mc of carrier-free I^{131}. The size of the treated groups ranged from 10 to 24 fish. Their standard length (tip of nose to end of caudal peduncle) was no more than 6 to 8 mm. and thus no deleterious overcrowding existed on a short-term basis. Young fish to be treated were carefully selected by eye, so that in treated and control groups the sizes of the fish were as evenly matched as possible. Exact measurements of length or weight were not made. The exposure time was varied between 24 and 72 hours, but in all but two of the 13 experiments, the exposure time was 48 hours or more.

Experiment 1 was a preliminary test of the efficiency of the treatment. These fish were of the 20th inbred generation of the *30* strain, which was highly resistant to goiters of all types.

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TABLE 1. EXPERIMENTS IN WHICH RADIOTHYROIDECTOMY OF YOUNG PLATYFISH WAS ATTEMPTED BY IMMERSION OF FISH IN WATER CONTAINING LARGE AMOUNTS OF RADIOIODINE

Experiment Number[1]	I^{131} conc. —mc./200 ml.	Hours Exposed	Age (days) at Exposure	Initial Fish Exposed	Exposed Fish Harvested		Initial Controls	Controls Harvested[2]	
					♀	♂		♀	♂
1.	5.5	48	60	12	0	8	.	.	.
2.	4.5	24	50	17	7	1	.	.	.
3.	5.0	48	63	21	4	3	.	.	.
4.	4.6	48	36	12	3	5	13	1	1
5.	5.0	48	37	12	7	5	11	3	8
6.	5.0	60	17	14	5	3	12	5	7
7.	5.01	72	66	12	2	2	11	1	1
8.	5.0	25.5	52	10	2	3	9	4	2
9.	4.82	60	58	13	0	0	8	0	0
I.	5.02	55.75	42	12	0	5	5	1	4
II.	4.99	52	56	12	0	6	6	2	3
III.	4.99	52	35	13	7	6	6	3	3
IV.	5.02	50	42	24	8	8	8	3	3
			Total:	184	100		89	55	

[1] Experiment 1 was performed on strain *30* fish, experiments 2 and 3 on strain *Fu* fish, and the remaining experiments on strain *BH* fish. No controls were kept in experiments 1 to 3.

[2] Discrepancies between initial controls and harvested controls in experiments 4 to 9 do not necessarily show final death rates, as controls were harvested in small groups at varying ages, for comparison with I^{131}-treated fish, and unused controls sometimes were returned to the breeding stock. See Table 4 for correct death rates among control groups.

Four of the fish in this experiment were sacrificed immediately post-exposure to determine radioiodine uptake per fish; the remainder were sacrificed at intervals of 6, 17 and 24 days post-exposure to determine the condition of the thyroid tissue. In all subsequent experiments, fish were not killed for examination until at least 40 days post-exposure.

Radioiodine uptake per fish was determined by counting aliquots of a 1N NaOH hydrolysate of the head, and aliquots of the radioactive water; per cent. uptake was obtained by the ratio of total counts in each. Total uptake was then obtained by multiplying per cent. uptake by the total original activity known to be in the water.

No specific controls were maintained in experiments 1 to 3; comparisons made were between treated fish and age-matched members of the same strain. In all other experiments, a closely similar number of broodmate controls was maintained. The controls were subjected to a "sham exposure," i.e., they were confined without feeding in a container in 200 ml. of water for the same period of time as the treated fish. The trip to the treatment site (Department of Zoology, Columbia University) was also taken by the controls, so that any effects of various shocks, such as cold, concussion, sudden darkness or light, would be the same.

In experiments 1 to 9, the treated fish, after exposure, were washed in radioiodine-free aquarium water and transferred to 4-gallon stock tanks, containing gravel, growing plants and snails, and were fed and maintained in the manner used throughout the Genetics Laboratory (Gordon, 1950). Control groups were maintained in adjacent tanks under similar conditions.

b. *Replacement Therapy:* In experiments I to IV, the radioiodine-treated fish were subsequently divided into two or three groups. One of these received no further treatment, while the others received potassium iodide or were fed desiccated thyroid. Non-treated broodmates were kept in equal initial number to each subdivision of the radioiodine-treated group. Further treatments of the I^{131}-exposed fish were begun 5-9 days after removal from the radioisotope. Fish that died during this interval are not included in figures comparing the variously treated groups, but only in total mortality data for radioiodine treatment.

In experiment I, the three groups of fish (control, I^{131}-treated, and I^{131}-treated subsequently given potassium iodide) were kept in stock tanks, and one group was given extra KI by the addition of 10 ml. of a 4 mg./ml. stock solution to its 4-gallon tank every two weeks. Therefore, until the tank water was changed, the concentration of remaining KI was unknown. In experiments II to IV, each subgroup was maintained in a specified volume of water (4-7 fish in two liters, or 8 fish in three liters) in all-glass aquaria, without gravel, plants or snails. These tanks were cleaned and the water changed weekly, and a known concentration of KI, where used, was employed (1 mg./l. final volume). Thyroid was administered by feeding ½ tablet of desiccated thyroid[3] weekly at the start of the experiments; other food was withheld on that day. This was increased to a whole tablet weekly as the fish grew (1 to 3 months after the start of thyroid-feeding, depending on size and number of fish in the group). On all other days, the fish were fed, together with the rest of the fish in the Laboratory, on dried shrimp or liver-pablum (Gordon, 1950). General observations on growth, body shape, coloration, secondary sexual development and "health and temperament" were noted during the weekly aquarium cleaning, when each group was transferred to a small observation vessel while its tank was washed and refilled. Whenever cleaning was done, the water was replaced from the Laboratory breeding tanks in such manner that each subgroup in an experiment received the same mixture of various tank waters.

Of 100 radioiodine-treated fish examined in these experiments, 87 were radioautographed, using tracer doses of I^{131} (Baker, 1958a), and all fish were serially sectioned for complete examination of internal organs.

2. *Radioiodine Treatment of Adult Platyfish.* —(Table 2). In these experiments, radioiodine was given by intraperitoneal injection to fully mature fish of both sexes. Since male playfish are smaller than females, and thus more difficult to inject, fewer males were used. Four groups, totalling 46 fish, were injected; each fish received 48.2 to 100 μc. of I^{131} in 0.012 to 0.05 ml. of distilled water. The first group was made up of 5 wild-type females of the *Fu* strain, the members of which were highly susceptible to thyroid tumors. One fish of this group gave birth to a few young 19 days after the injection; two of these young were radioautographed with the mother 22 days after birth and are described with the group. Groups 2 and 3 included females and males of the *163* strain, which was at least moderately susceptible to thyroid tumors. Group 4 consisted of 12 females of the *30* strain; these fish all died within 5 months post-injection and are included only in the mortality figures.

After injection, these fish and their broodmate controls were maintained under ordinary stock conditions. Twelve of the 18 radioiodine-treated fish that were harvested were also radioautographed, and all were serially sectioned for complete examination of internal organs.

3. *Radiophosphorus Treatment of Young Platyfish.*—As radiation controls for the I^{131}-treated young fish, 16 platyfish of the *BH* strain were placed in 200 ml. volumes of aquarium water containing 1.7 mc. of P^{32} for 48.5 to 50.75

Table 2. Experiments in which Radiothyroidectomy of Adult Platyfish Was Attempted by Intraperitoneal Injection of Radioactive Iodine (Starred (*) fish were radioautographed)

Group	Pedigree	Number of Injected Fish ♀	♂	μc. I^{131} Injected	Age (months) when Injected	Post-injection Period (months)	Number of Controls ♀	♂
1.	*507 Fu*							
	Fish started	5	0	Various	8.4		0	0
	Fish harvested	1*	0	80		1.4	0	0
		2*	0	50		6.5	0	0
2.	*163*[8]							
	Fish started	11	.	96.4	8.7-9.5		10	.
		.	6	48.2	9.0-9.8		.	No record
	Fish harvested	1*	.			1.3	0	.
		2*	1*			3.5	2*	1*
		1	.			19.5	1	.
3.	*163*[8]							
	Fish started	12	0	50	8.8-11.5		12	0
	Fish harvested	5*	0			8.5	0	0
		5	0			18.75	3	0

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[3] Burroughs-Wellcome & Co., "Tabloid" thyroid, U.S.P. Each tablet contained 0.065 gm. desiccated thyroid. These tablets were tested for metamorphic activity on tadpoles (three years after the fish experiments were completed). In three tests, ¼-2 tablets, fed to 4 or 5 three-centimeter *Rana pipiens* tadpoles, produced tail resorptive processes, hind and fore limb and foot development, modification of mouth parts, lung breathing, pigmentary differentiation, raised eyes and lack of further growth. Controls were unchanged within the period (6-11 days) of treatment.

hours. These were groups of 4-7 fish, aged 30 to 54 days. The amount of P^{32} used was calculated to give the same roentgen dose of whole-body irradiation within the 200 ml. volume in 48 hours (720 r.) as would 5.0 mc. of I^{131} (Glasser *et al.,* 1952).[4] Fourteen untreated broodmate controls were kept. These fish were maintained in stock tanks after treatment and, when 4.7 to 7 months old, were fixed in formalin and serially sectioned for examination.

4. *Analysis of Pituitary Development.*—A ratio of pituitary to body size was obtained in several experiments, using counts of consecutive 10μ sections of the pituitary as a measure of hypophyseal size, and standard length of the fish, taken from the specimen itself or its photograph, as the measure of body size. The average ratios obtained by this method were often inconsistent between experiments, when variously treated groups were compared. A better measure of hypophyseal development was obtained by making a series of outline drawings of each section of the pituitary by means of uniformly magnified projections. The tracings from each fish were then cut out and weighed on an analytical balance. These weights were then averaged within each treatment group and divided by the average standard length within the same group. The ratios obtained by this method proved to be satisfactorily consistent between experiments with reference to the relations between the variously treated groups. Volume or weight measurements were not taken on any of the fish before sectioning, so that only length was available as a measure of body size. Objection to a ratio of volume: length, as involving two different types of measurements, may be countered, in these experiments, with the following observations: Had the proportions of all of the fish been identical, the use of this ratio would have been justified without doubt, but if not, it might have led to misinterpretation. However, the differences in proportion observed among the treated groups of fish usually would have *enhanced* the differences found by using the pituitary-volume-to-body-length ratio. For example, radioiodine-treated fish tended to be dumpy and pot-bellied in shape, which would have increased their weight or volume in proportion to their length, but if such an increased volume had been used to calculate the ratio, the difference between these fish and the controls would have become *greater* than indicated at present. This would also hold true for KI-treated, radioiodine-exposed fish, for the same reason. On the other hand, thyroid-fed fish, if slenderer than their controls, would have had their ratios shifted slightly towards those of the controls. The interpretation of the results obtained, in any case, could not have been affected seriously.

5. *Histological Methods.*—All fish were serially sectioned at 10μ and stained with haematoxylin-eosin or occasionally with Masson's trichrome. Fixation was routinely in Bouin's fluid with formic acid as a decalcifier, with the exception of the P^{32}-treated fish which were fixed in 10% formalin. Radioautography was carried out as described in Baker, 1958a.

6. *Gonopodial Development.*—In experiments I to IV, the anal fins of all fish were removed, after fixation of the whole fish in Bouin's fluid, dehydrated in alcohols and xylol, and whole-mounted on slides in Permount, unstained, for examination. This was also done with the anal fins of some animals from experiments 2-8 and with those of all P^{32}-treated fish.

RESULTS

1. *The Efficacy of Radiothyroidectomy in Platyfish.*

a. *Criteria for Thyroidectomy:* Thyroidectomy was judged to be complete when I^{131} radioautographs of serial sections through the thyroid and kidney regions of the fish were blank for thyroid tissue. Radioautographic spotting usually was found also over the auditory region and portions of the pharynx and intestines. In the latter two locations, the spots were produced by I^{131} that was bound to food particles in the lumen of the alimentary tract or adhering to its lining (Pl. II, Fig. 1). These non-thyroidal sources of spotting were readily differentiated by their location from spots produced by thyroid tissue, even when crude contact radioautography was employed. Location of spots within a section was often aided by the occurrence of a "shadow picture" of the whole section, sometimes including definition of organ areas contrasting with open areas within the section.

In addition to the radioautography, all serial sections were searched histologically in a methodical fashion for thyroid tissue in the pharyngeal and renal areas. In some cases, when radioautography was not feasible, as in case of preservation after death, histological search was accepted as sole criterion for thyroidectomy. This was used in relatively few cases, however, and was related to the total morphological picture of the individual before any acceptance of

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[4] I wish to thank Dr. Edith Quimby, Department of Radiology, Columbia University College of Physicians and Surgeons, for performing the initial calculation of the P^{32} activity required and for references to the relevant published material.

total thyroidectomy was made. In many instances, especially in younger fish, radioautographic speckling was noted, but no thyroid tissue could be found histologically. The probable presence of undestroyed thyroid tissue in these cases was revealed by later study of fish from the same group; in these older fish, visible thyroid tissue was often found. Thus, histological criteria for total thyroidectomy were not found to be completely reliable on a short-term basis.

b. *Radioiodine Treatment by Immersion:* On the whole, this method was very successful. In 92 fish examined 40 or more days after treatment, the frequency of complete radiothyroidectomy, by all criteria, was 68.5%. With the omission of two less successful experiments (Nos. 2 and 5), however, this incidence was 86% (62 of 72 fish harvested).

The results appeared to be affected both by exposure time and by the strain to which the fish belonged, when dose was nearly constant. For example, no fish were completely thyroidectomized in experiment 2, when strain *Fu* fish were exposed for 24 hours to 4.5 mc. I^{131}, but all fish were thyroidectomized in experiment 8, when strain *BH* fish were exposed for 25.5 hours to 5.0 mc. I^{131}. On the other hand, in experiment 3, most strain *Fu* fish *were* thyroidectomized by 48 hours exposure to 5.0 mc. I^{131}. The *BH* strain of platyfish was particularly highly sensitive to low iodine concentration in its environment, as judged by the frequency of thyroid tumors and heterotopic thyroid tissue (Baker, 1958a, b), and the fish probably concentrated iodide in the thyroid tissue, when supplied in excess, to a greater degree than platyfish of other strains.

The poor results of experiment 5 perhaps were related to an addition of $NaHCO_3$ to the radioiodine solution. This was the only apparent difference between this and later experiments using fish of the *BH* strain of similar age as well as closely similar amounts of radioiodine and exposure times. Although none of these fish was completely thyroidectomized, as judged by spotting on radioautographs, the amount of thyroid tissue was severely reduced, and none could be found histologically in many cases.

Among the group of 29 fish accepted as *incompletely* radiothyroidectomized, thyroid follicles were unquestionably seen in 18 animals (Table 3). In the remaining 11 fish, radioautographic spotting, together with visualization of a few doubtfully identified thyroid follicles in some fish, comprised the evidence for incomplete thyroidectomy. These doubtful cases were largely confined to animals that were examined within three months after I^{131} treatment and were no more than 4.5 months old. Among fish

TABLE 3. COMPARISON OF CRITERIA FOR COMPLETENESS OF THYROIDECTOMY IN PLATYFISH TREATED WITH RADIOIODINE BY IMMERSION[1]

Experiment Number[2]	Total No. Fish Harvested	Days Post-treatment when Examined	Number of Incomplete Thyroidectomies, Judged from: Radioautograph Spots, No Follicles Seen	Follicles Seen Histologically
2	8	41	0	3
		150	0	2
		176	0	1
		539	0	2
3	7	47	1	1
		171	0	1
5	12	79	2	2
		95	5	0
		168	1	1
6	8	60	1	1
		277, 289	0	1
I	5	159	1	1
IV	16	140	0	2[3]
Total of incomplete thyroidectomies:			11	18

Fraction of incomplete thyroidectomies: 29 out of 92 I^{131}-treated fish harvested in all experiments, except No. 1.

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[1] *All* fish radioautographed 40 or more days after treatment. The number of *incomplete* thyroidectomies that could be shown by radioautographic criteria exceeded by a large increment the number that could be demonstrated by histological examination alone.

[2] In experiments 4, 7, 8, II and III all fish were totally thyroidectomized. Therefore these experiments are not listed here.

[3] One of these had only a single thyroid follicle, but in the kidney.

more than 6 months old there rarely was any question about the presence of undestroyed thyroid tissue. Not only did the radioautographic spotting per follicle become unusually intense, but the amount of thyroid tissue often became considerable (Pl. II, Figs. 3, 4). Whenever visualized and in whatever quantity, thyroid tissue in the non-thyroidectomized fish was hypertrophied and the colloid was granular and small in amount. The two oldest fish were 19.6 months of age, and in them the thyroid tissue had assumed almost goitrous proportions, although each follicle still remained distinct and slightly separated from others and did not form a continuous mass of follicular and afollicular tissue as seen in most goiters among platyfish (Pl. II, Fig. 4).

In the pilot experiment (No. 1), 12 young fish of a strain insensitive to low concentrations of environmental iodide were used. Four of these fish were killed immediately after exposure and the radioiodine uptake per fish was determined. This proved to be 20-50 μc. Two fish were examined on the 6th day after treatment, two on the 17th day and four on the 24th day. On the 6th day, the number and distribution of thyroid follicles appeared to be normal. The morphology of the follicles was normal, but the colloid often stained blue with haematoxylin-eosin. Normally, thyroid colloid stained bright red with this stain combination. The blood vessels in the thyroid area were normal, and no other anatomical changes were noted. By the 17th day, the radioautographs were "spotty" and the number of thyroid follicles was severely reduced. The cells of these follicles were heightened, often puffy, with very indefinite boundaries and pale cytoplasm. The colloid was pale pink and coarsely granular. No follicles had normally dense colloid or colloid stained blue. The aorta and walls of the gill chamber in the thyroid area were somewhat thickened. No other pathological effects were noted. On the 24th day post-treatment, one fish was completely lacking thyroid by all criteria, one had a single spot on the autograph but no visible thyroid follicles, and two retained a few identifiable follicles. The latter were often very indistinct and had pale, enlarged cells and pale-pink granular colloid. In one fish, the thyroid may have begun to recover as there were two or more follicles with dense vacuolated colloid and flat cells, as well as several pathological follicles. In all of these fish, the aorta was very shrunken, its walls were thickened, and it was surrounded by a thick layer of gelatinous-appearing connective tissue which attached it to the walls of the anterior pericardial space (Pl. II, Fig. 6). One fish had some enlarged tubules containing concretions in the kidneys (the kidneys of 2 of the 4 fish were not sectioned). The positive results obtained in this trial, with fish of a goiter-resistant strain, provided a basis for repetition of the method, using the goiter-prone strains, which might be expected to take up radioiodine more avidly, or release it more slowly, and therefore be more effectively treated.

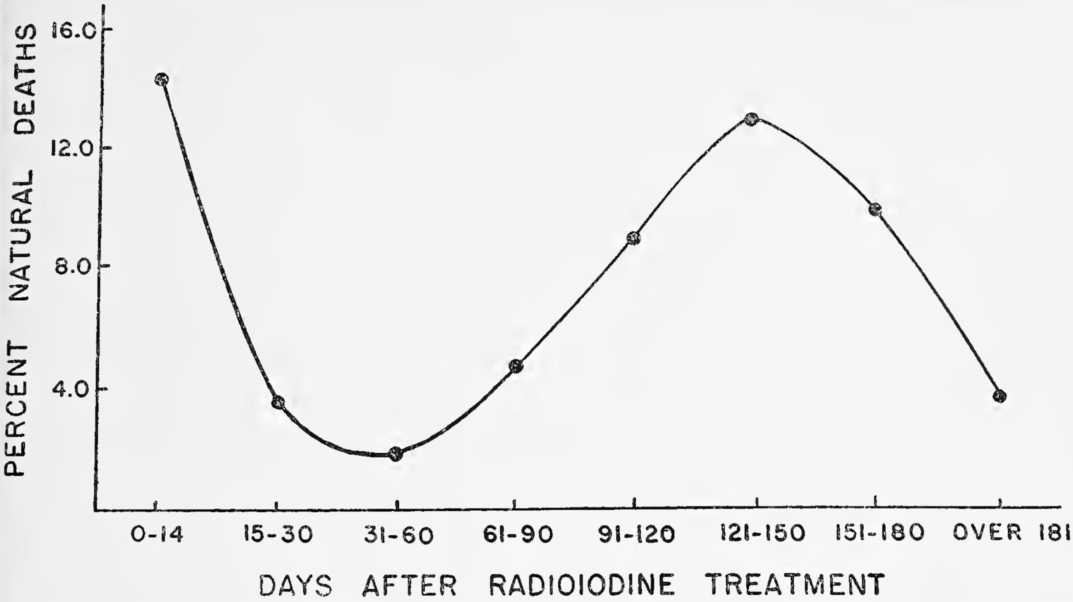
c. *Radioiodine Treatment by Injection:* (Table 2). This method was only moderately successful in producing thyroidectomized fish, although it produced severely hypothyroid animals in all cases. The chief difficulties encountered were the necessity for keeping the injected volume very small, especially for male fish, which are smaller than females, and the tendency for post-injection leakage to occur. Since relatively few fish were treated by this means, and because of the lesser importance of these experiments to the analysis of the chief problem in hand at the time, these difficulties were never fully dealt with. Nevertheless it is likely that successful administration of the dosages intended for the fish would not have brought about much different results since various amounts of I^{131} in various volumes were injected, with fairly similar effects.

Here again, strain differences were noted. The three female *Fu* fish of Group 1 were quite successfully thyroidectomized by injections of 50-80 μc. of I^{131}, whereas in Group 2, none of the *163* female fish was completely thyroidectomized by injections of 96.4 μc. of I^{131}. Fish of the *163* strain produced renal thyroid tumors, but with a much lower frequency than did the *Fu* strain. From this it might be assumed that the thyroids of *163* fish had a lower avidity for, or retention of, iodine than those of *Fu* fish.

Among the 18 fish harvested (Table 2), total *histological* thyroidectomy was obtained in 9 animals, but two of these had died in I^{131} tracer solution before fixation, so that the preservation was poor. Radioautographic total thyroidectomy was obtained in only one instance. Two fish were examined 38-41 days post-injection. From Group 1 (strain *Fu*), one fish was lacking thyroid histologically, although it retained a faint radioautographic speckling in the thyroid area. From Group 2 (strain *163*), one fish showed thyroid tissue histologically, although extremely reduced. The thyroid cells were high and the colloid was pale and granular. All other fish were examined 3.5 or more months after the injections.

2. *The Effects of Radioiodine Treatment on Young Platyfish.*

a. *Mortality:* The death rate among treated fish in these experiments was very high. Of 172



TEXT-FIG. 1. The distribution in time of deaths among radioiodine-treated platyfish. The percentages are based on corrected totals for remaining fish, after subtraction of those killed for examination in each time period. Therefore, the figures do not add up to 100%. The analysis is based on an initial 113 fish, from those experiments in which all deaths were recorded with respect to the time periods (experiments 2, 5, 8, 9, and I-IV). Of these fish, 59 were killed after the 30th day post-treatment, and 54 died "naturally."

radioiodine-treated fish, in all experiments except No. 1, 89 (52%) died during maintenance periods that ranged from 128 to 290 days. These deaths were largely concentrated in the periods between 0 to 2 weeks post-exposure, and between 3 to 5 months post-exposure (Text-fig. 1). A more accurate picture of survivorship, uncomplicated by early sacrifices and lack of control data, is obtained by the analysis of experiments 7-9 and I-IV only, in which all fish were maintained untouched until the conclusion of the experiment. Survivorship to the 109th and 130th day post-exposure for these experiments is shown in Table 4. (The 109-day survivorship is presented for comparison with mortality data on P^{32}-treated fish, most of which were kept for only 109 days post-treatment. See below, part 4). Comparison of the 109-day with the 130-day survivorship further illustrates the heavy loss of treated fish between three and five months after treatment. The death rate among the untreated controls in these experiments was almost negligible.

b. *Growth:* Among the most prominent effects of radioiodine treatment was a striking reduction in the growth of the treated fish in comparison with untreated broodmates. Examples of differences in average standard length between treated and control fish, at various ages, are given in Table 5. These differences may be also seen in Pl. I, Fig. 2. There was little or no difference between the 2.5-month-old treated and control fish of experiment 6, but in older fish differences of more than 10% were found in 5 out of 6 groups. It must be borne in mind that mortality among the treated fish was invariably highest among the smaller fish, so that the final survivors were a selected group of the largest size. Among the controls, deaths were also most frequent among the smallest fish, but very few of any size died. No measurements of growth rate were made, only the size attained at fixation being recorded.

Radioiodine-treated fish were frequently observed to develop a hunched, "cretinous" body shape, with a pot belly. This may be seen in Pl. I, Figs. 1 and 3, in both living and fixed specimens. The pot-bellied appearance was found to be related to the condition of the liver and the abdominal tissues (see below). Treated fish also were noticeably darker in color than the controls in most cases, but this difference was not further investigated.

c. *General Pathology:* The ventral aorta and the bases of the afferent branchial arteries, the bulbus arteriosus and other arteries passing through the thyroid area were usually shrunken in radioiodine-treated fish. The walls of these

TABLE 4. SURVIVAL RATE IN PLATYFISH TREATED BY IMMERSION IN WATER CONTAINING LARGE AMOUNTS OF RADIOIODINE[1]

Experiment	Fish Treated	Survivors	Controls Kept	Control Survivors
A. Survivorship to the 109th day after treatment:				
7	12	9	(11)	Incomplete record
8	10	10	9	9
9	13	8	8	8
I	8	3	5	5
II	5	3	6	5
III	7	7	6	6
IV	8	6	8	7
Total:	63	46	42	40
Percent. Survival:		73[2]		95
B. Survivorship to the 130th day after treatment:				
7	12	4	(11)	Incomplete record
8	10	10	9	9
9	13	2	8	8
I	8	3	5	5
II	5	2	6	5
III	7	5	6	6
IV	8	6	8	6
Total:	63	32	42	39
Percent. Survival:		50		93

[1] These figures do not include fish that were additionally treated with potassium iodide or fed thyroid material.

[2] Percent. survival would be 70 if two fish in experiment 7, whose deaths were inexactly recorded, had died before the 109th day.

vessels, particularly the aorta, were thickened, and connective tissue "adhesions" between the aorta and the pericardial lining frequently were present and developed to a considerable extent (Pl. II, Fig. 5). In some fish, this material formed a thick, structureless, gelatinous-appearing mass around the aorta (Pl. II, Fig. 6). The heart was never visibly affected. The area of the thyroid often was filled with fibrous stroma, and in some fish the caudal portion of the area, above the aorta, disappeared through the collapse of the walls of the gill chamber into the vacant space.

Most I^{131}-treated fish suffered a loss of lymphoid tissue in the kidneys to some extent. These are the chief blood-forming organs in teleosts, with the spleen second in importance. In most of the fish, this loss was not severe, but definitely was noticeable upon histological examination. In general the spleen seemed to be normal. No lymphoid cell counts or blood counts were made on these fish. In a few treated fish, almost complete loss of lymphoid elements was found. This was first apparent from the empty appearance of the kidneys (Pl. III, Fig. 2), and was accompanied by a severely shrunken spleen (Pl. III, Fig. 7) and an absence of thymus tissue.

The thymus was found to persist as a pair of large lymphoid organs in all fish that were totally radiothyroidectomized, regardless of sex, with the exception of those specimens just described. The thymus glands of normal platyfish are very large and solid structures in late embryonic and immature post-natal stages. As the fish mature, these glands often develop a few large internal cysts, sometimes filled with an eosinophilic colloidal material. In some instances, these cysts closely resembled thyroid follicles, but they were not found to produce I^{131} radioautographs. They evidently correspond to the cysts and follicular or alveolar structures described in the thymus of *Necturus* by James (1939). As the male gonad becomes fully mature and full of sperm, the thymus glands shrink and may disappear entirely. In female fish, however, large thymus glands were found at all ages up to 20 months, but they more often were cystic in females 9 or more months of age. Pregnancy had no noticeable effect on the thymus. In fish that were not totally radiothyroidectomized, the thymus glands changed in the normal, characteristic way, as sexual maturation took place.

Although the glomeruli and the bulk of the kidney tubules were not visibly affected, the latter structures in the kidneys of I^{131}-treated fish

TABLE 5. DIFFERENCE IN GROWTH BETWEEN RADIOIODINE-TREATED FISH AND THEIR BROODMATE CONTROLS[1]

Experiment	Age (months)	Number of Treated Fish	Mean Length: Treated Fish	Range in Length: Treated Fish	Number of Controls	Mean Length: Controls	Range in Length: Controls	Difference: Control Mean Minus Treated Mean	Difference: as % of Control Mean
5	4.4	5	17.4	16.5-17.5	5	21.9	20 -24.0	4.5	20.5
6	2.5	4	10.5	10.0-11.0	3	9.9	9 -10.8	−0.6	−6.1
8	9.6	5	15.5	15.0-16.0	6	24.0	20 -26.0	8.5	35.4
I	6.8	2	28.8	27.5-30.0	5	32.1	29 -35.5	3.3	10.3
II	6.3	2	20.8	19.5-22.0	5	21.4	15 -26.0	0.6	2.8
III	6.3	4	17.8	15.0-22.0	6	23.2	20.5-27.0	5.4	23.3
IV	6.1	4	14.5	11.0-16.5	6	16.9	10.5-20.5	2.4	14.2

[1] Standard length (tip of nose to end of caudal peduncle) was measured in millimeters. In experiments 5-8, true measurements were taken just after fixation of the fish; in experiments I-IV, measurements were made on group photographs taken for each experiment after the fish were fixed (see Pl. I, Figs. 2, 3) and do not represent the true lengths of the fish. The measurements made on photographs are not comparable between experiments, because the magnifications are not identical.

were in part pathological. Many tubules were swollen and their lumens contained unstained masses of material best described as "concretions" (Pl. III, Figs. 3, 4). Tubules also were seen to be degenerating, and sometimes small wormlike structures which were very darkly basophilic were seen (Pl. III, Fig. 6). These are believed to be regenerating tubules.

The liver of radioiodine-treated fish was usually the most noticeably and severely affected internal organ. The pot-bellied appearance of these fish resulted from an enlargement of the liver, which was often extreme, coupled with greatly increased abdominal "vacuolation." In these enlarged livers, "vacuolation" was found to pervade the entire organ to a remarkable extent (Pl. IV, Figs. 2, 4). The term "vacuolation" will be used throughout this paper to describe the condition of the liver and abdominal tissue in many radioiodine-treated fish. Although it is believed that this condition probably represented fatty deposits, the presence of fat could not be demonstrated because of the routine processing of all material in fat solvents, such as xylol. No frozen sections stained for fat were prepared, as the liver condition was not analyzed at the time when fresh experimental material was available.

Vacuolated livers were found in a large proportion of the treated fish; severity ranged from small vacuolated areas in the antero-central portion of the liver to complete change of the entire organ. Severity was found to be correlated with age (or to time elapsed after treatment) in a remarkably linear fashion (Text-fig. 2). Vacuolated livers were found in incompletely thyroidectomized fish with nearly as high a frequency as in totally thyroidectomized fish (Table 6), but never was found among the controls, even in the presence of thyroid hypertrophy.[5]

Included among the fish with vacuolated livers are several specimens whose livers contained large amounts of ceroid (Pl. IV, Fig. 3). The ceroid found in the I^{131}-treated platyfish occurred in globules in the liver cells themselves, and also in large masses of large, dark-nucleated cells, which were solidly filled with it. This observation concurs with the distribution in experimentally cirrhotic rats reported by Endicott & Lillie (1944), who described ceroid as occurring largely in large round phagocytes which formed broad sheets and trabeculae. In the I^{131}-treated

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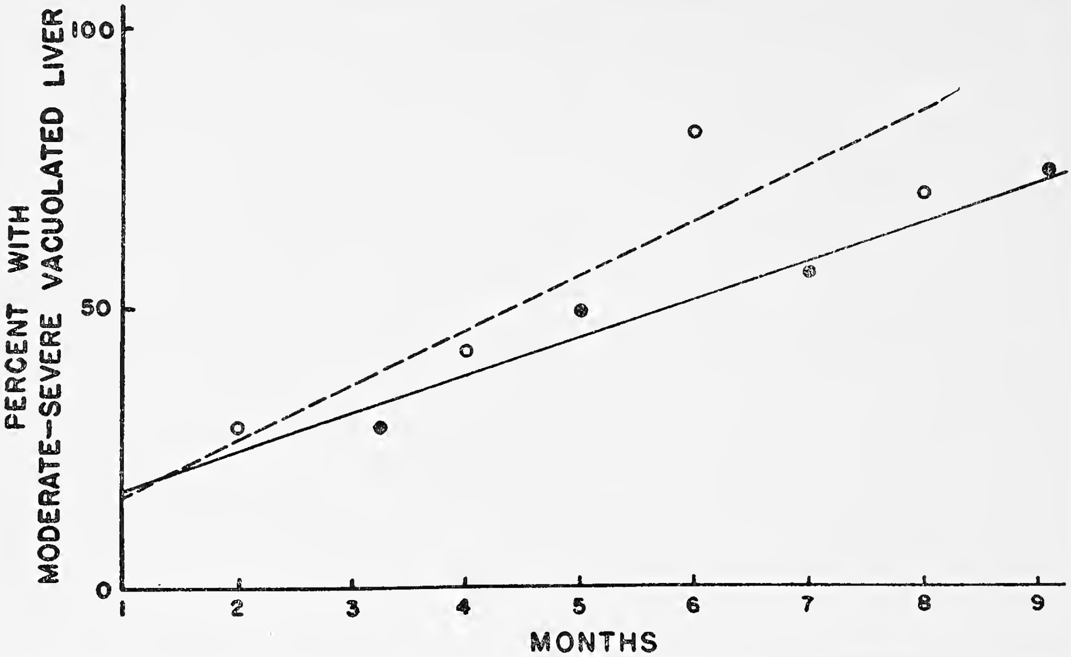
[5] Vacuolated livers were found in many platyfish that had thyroid tumors in their kidneys, although not in all specimens. Most of these fish were relatively old animals.



TEXT-FIG. 2. Increase with time in severity of liver vacuolation (see text) among radioiodine-treated platyfish. The fish were classified into four groups of increasing severity from their histological descriptions, and then subgrouped by age and by the period elapsed after treatment. When the two most severely affected groups were compared with the two least severely affected, a straight line relationship to age emerged, and a similar, though less regular, relationship to post-treatment time appeared. These curves show that long-continued hypothyroidism had a progressive deleterious effect on liver function. Pathological effects of radiation, on the other hand, show recovery in time, if the animal is able to survive. Solid circles and continuous line represent age, and open circles with dashed line represent time post-treatment, both on the same time scale.

fish, broad masses of ceroid-filled cells also were found near hepatic blood vessels and among the pancreatic tissue outside of the liver. In some fish, vacuolated areas and extensive ceroid deposits were found together in the liver.[6] In the case of the radioiodine-treated platyfish, it was supposed that the conditions of vacuolated liver and "ceroid liver" were related, and, if the assumption is made that vacuolation does represent fat, available information appears to support this view (see Discussion). In normal fish, ceroid also was seen in small aggregations of macrophages next to blood vessels, but never was seen in liver cells.

In fish with extensive vacuolation in the abdomen, the pancreatic tissue was severely compressed and may best be described as wispy (Pl. IV, Fig. 6). In most cases, however, the amount of pancreatic tissue appeared to be relatively normal.

d. *Gonadal Development:* No completely radiothyroidectomized fish ever attained sexual maturity, even at an advanced age (there were three possible exceptions, which will be described below, but these fish were not conclusively athyroid). Gonadal development in treated fish and controls is shown in Tables 7 and 8. From the youngest fish sampled, including incompletely thyroidectomized ones, it was apparent that gonadal development was retarded in all I^{131}-treated fish (Pl. V, Figs. 1, 2). Among completely athyroid animals, this retardation frequently was extreme. As shown in Table 8, many males had developed no primary spermatogonial cysts (Wolf, 1931) by the age of 6 months or more (Pl. V, Fig. 4), when controls were producing young, and many treated females had developed only a few early basophilic oocytes (Pl. V, Fig. 6). Although some thyroidecto-

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[6] A re-examination of the livers of 8 thiourea-treated platyfish and their 9 controls (originally described in Baker, 1958b) was made. Ceroid-filled macrophages were found massed in the livers of all treated fish and to an extreme degree in 7 of them. Ceroid deposits, besides those in macrophages, were found in the liver cells themselves. Prominent vacuolization, coupled with ceroid deposition, occurred in 4 fish, and in two of these cases there was severe cellular degeneration in the central parts of the liver. In the controls, ceroid was absent from liver cells and scant in macrophages, while vacuolated livers did not occur.

Table 6. Incidence of Vacuolated Livers (see text) in Radioiodine-treated Platyfish and Their Broodmate Controls

	Number of Fish	Number with Vacuolated Liver	Percent. with Vacuolated Liver
Fish 5 or less months old:			
Complete thyroidectomy	7	3	43
Incomplete thyroidectomy	8	5	63
Doubtful	9	7	78
Total I^{131}-treated	24	15	63
Controls	11	0	0
Fish 5.1 to 7 months old:			
Complete thyroidectomy	24	17[1]	71
Incomplete thyroidectomy	5	3	60
Total I^{131}-treated	29	20	69
I^{131} + KI treatment	9	6	67
I^{131} + thyroid feeding	15	0	0
Controls	30	0	0
Fish more than 7 months old:			
Complete thyroidectomy	9	8	89
Incomplete thyroidectomy	3	2	67
Total I^{131}-treated	12	10	83
Controls	13	0	0
	—	—	—
Grand total solely I^{131}-treated:	65	45	69
Grand total controls	54	0	0

[1] One additional fish with other abnormalities of the liver not included.

Table 7. Gonadal Development in Radioiodine-treated Platyfish and Their Broodmate Controls

Experiment	Age (months)	Radioiodine-treated			Controls		
		Total	Mature	Immature	Total	Mature	Immature
A. Total Thyroidectomy							
3	6.8	2	0	2	.	.	.
	8.4	1	0	1	.	.	.
4	6.0	4	0	4	2	2	0
6	7.5	2	0	2	.	.	.
	10.2	1	0	1	7	7	0
7	6.5	4	0	4	2	2	0
8	9.7	5	0	5	6	5	1
I	6.8	2	0	2	5	5	0
II	6.3	2	0	2	5	3	2
III	6.3	6	0	6	6	4	2
IV	6.1	5	0	5	6	1	5
		—	—	—	—	—	—
Total:		34	0	34	39	29	10
Percent. Mature:			0%			74%	
B. Incomplete Thyroidectomy							
2	6.6	2	2	0	.	.	.
	7.5	1	1	0	.	.	.
3	7.8	1	1	0	.	.	.
5	5.7	1	1	0	.	.	.
	6.8	2	2	0	1	1	0
6	9.8	1	1	0	.	.	.
		—	—	—	—	—	—
Total:		8	8	0	1	1	0
Percent. Mature:			100%			100%	

TABLE 8. DEGREE OF SEXUAL DEVELOPMENT AMONG TOTALLY RADIOTHYROIDECTOMIZED PLATYFISH MORE THAN 5 MONTHS OF AGE[1]

Experiment	Females with:		Males with:	
	≦ 5 oocytes	> 5 oocytes	Spermatogonial Cysts Absent	Spermatogonial Cysts Present
3	2	1	.	.
4	1	0	2	1
6	.	.	3	0
7	3	0	1	0
8	0	2	1	2
I	.	.	2	0
II	.	.	0	2
III	2	2	0	2
IV	0	2	3	0
Total:	8	7	12	7

[1] None of these fish was mature: females showed no signs of yolk deposition, while males possessed no germ cells in late stages of meiosis or in sperm formation. Some of the males in which spermatogonial nests or cysts were found exhibited a few cysts with cells in early spermatogonial division. Radioiodine-treated fish that were treated secondarily with potassium iodide or were fed thyroid have been excluded.

mized fish were maintained until 8 to 10 months of age, gonadal development proceeded no further than the enlargement of a few oocytes, without yolk deposition, in females (Pl. VI, Fig. 1), and the beginning of germinal cyst development, with some early meiotic cysts, in males (Pl. VI, Fig. 2). On the other hand, *all* fish with incompletely destroyed thyroid tissue were sexually mature by the age of 5 months, although no females were found to be gravid (note, however, that only in experiment 2 were treated females and males, that were maturing normally, living together).

Development of the gonopodium (the structurally specialized anal fin found in the males of this live-bearing species of fish, which is used for internal fertilization of the female) in thyroidectomized male fish was inhibited along with the testes, the anal fin remaining juvenile in form and undifferentiated. In one case, where an extremely infantile testis was present, there was elongation of the anal fin, but no segmental differentiation of the rays or of the specialized structures at the tip of the fin had occurred (Text-fig. 4; Pl. VII, Fig. 4). This type of anal fin development was described in gonadless hybrids between *Xiphophorus helleri* and *X. maculatus* by Rosen (1960).

Two of the exceptional I^{131}-treated fish mentioned above were males that developed large mature testes, with spermatophore production, in the apparent absence of thyroid tissue (Pl. VI, Figs. 3, 4). One, aged 6 months (experiment 5), unfortunately died and was not radioautographed, but the sections were remarkably good and were stained in a most striking way for thyroid delineation (Masson's trichrome). The other had been treated with potassium iodide (see below, experiment I) and was radioautographed, but KI is known to interfere with the uptake of further (radioactive) iodide (Baker, 1958b). Both of these fish had anal fins that were elongated but completely undifferentiated (Pl. VII, Fig. 4). Both also had among the most extremely vacuolated livers seen (Pl. VI, Fig. 4), with large aggregations of vacuoles compressing the pancreatic tissue in the abdomen, and with concretions in the kidneys. The KI-treated fish had developed the bright red color characteristic of most males of the *BH* strain (females and young were more yellowish or brownish).

The third possibly exceptional fish was a female, also from experiment 5, aged 7 months, with maturely yolked oocytes in the ovary. Although there were a few spots on the radioautographs, thyroid tissue was not positively identified histologically. This fish also had some vacuolization in the liver, extremely large abdominal aggregations of vacuoles, and concretions and degenerating tubules in the kidneys.

Anomalous development of the testes was found in 4 fish of the radioiodine-treated groups. Two of these were KI-treated fish of experiment I (see below), the others fish of experiments 5 and II, treated only with radioiodine. Three of these presented the same picture: an anterior testicular mass suspended solitarily from the dorsal peritoneum and unconnected by any discernible duct to the cloacal area or to a slightly posterior second testicular mass. The latter continued with a normal spermatic duct, opening close to the anus. In one case, both masses appeared to be bipartite. In the 4th fish (experiment 5), both testes appeared normal, but no spermatic duct could be found. None of these testes were near maturity. Similar anatomical peculiarities were not found among the controls.

e. *Pituitary Development:* No cytological studies were made on the pituitaries of I^{131}-treated fish because the fish were serially sectioned with the utmost rapidity in order to make radioautographs, and they therefore were cut relatively thickly (10μ). Staining also was routine; special methods were not used. The only cytological distinction that could be made was between a darkly basophilic cell type in the most anterior portion, a paler basophilic type in the mid-region, and the neural hypophysis. Eosinophils were not clearly recognizable because of the thickness of the sections and irregularities in staining.

A volumetric study of the hypophysis was attempted, however, and this produced rather clear-cut results (Table 9). It may be seen that the radioiodine-treated fish had significantly smaller pituitaries per unit of body length than did their controls. The pituitary:body size ratio remained constant at a low level as the I^{131}-treated fish grew older (in Table 9, experiments 5 and 8 may be compared), while the pituitary size of the controls increased in relation to their body length. It had been believed that the hypophysis of severely hypothyroid or completely athyroid fish might have been enlarged, owing to stimulation of thyrotrophs by the lack of thyroid hormone, but this was not the case. Instead, the size and appearance of the pituitaries suggested exhaustion, and the absence of any cellular increase in response to the environment.

The hypophyses of the control fish became larger per unit of body length as the fish grew older and at the same time that thyroid hypertrophy appeared in many of them. Both features presumably resulted from continued exposure to a low-iodine environment. The large relative size of the glandular portion of the hypophysis in the controls may be seen in Text-figure 3. This was particularly marked in female fish; which is in accordance with the observed higher incidence of goiter in females of the *BH* strain (Baker, 1958a) . The sex ratios among the treated and control fish were sufficiently alike to preclude any strong bias on a sex basis. Among the control fish, several had developed a marked overgrowth of the paler, intermediate basophiles, so that these overlapped the darker anterior basophiles ventrally, causing the appearance of a sharp line of demarcation in the anterior region and a distortion of the usually round cross-section of the hypophysis at that point (Text-fig. 3; Pl. VI, Figs. 6, 7). This appearance of the hypophysis was usually found in the presence of thyroid hypertrophy.

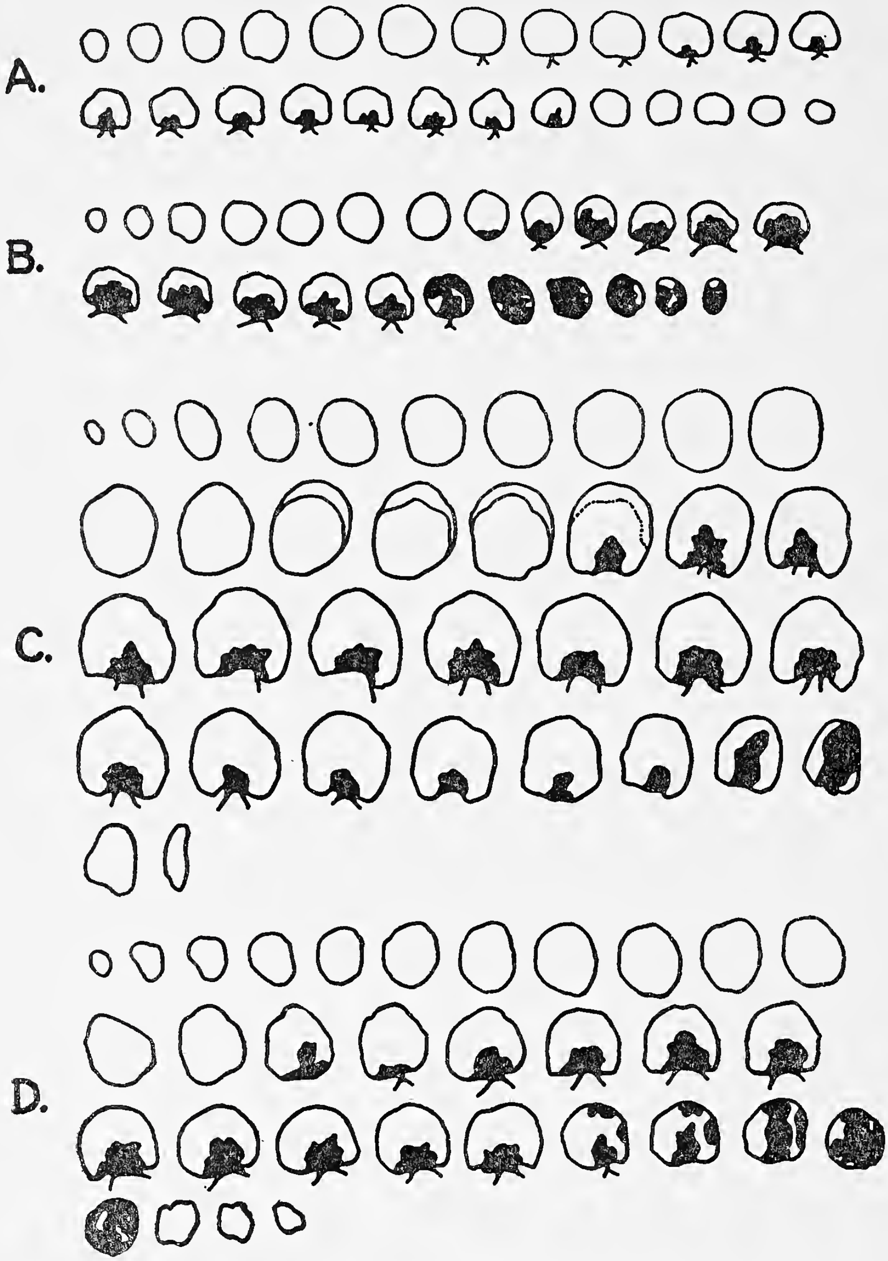
f. *Behavior and Fragility:* Although these observations are somewhat subjective and were not measured in a controlled manner, it is believed worth recording that the radioiodine-treated fish were very sluggish. They never were seen darting about the tank, and when caught for tank changing, were listless in avoiding the net. From general observation and two specific incidents, it was concluded also that they were easily killed by the shocks of handling and other disturbances. The first incident was an attempt to run two treated and two control fish in a Warburg apparatus. This also entailed transportation in a small container by 'bus over a considerable distance. The two treated fish died within an hour of being individually set up in Warburg vessels, although the rate of shaking used was very low. The two controls, on the other hand, remained alive and healthy for two days under identical conditions and were removed apparently none the worse for the experience. The second incident was the subjection of a treated fish to photography, together with a pair of normal broodmates (Pl. I, Fig. 1). This fish was manipulated for some time to obtain two

Table 9. Pituitary versus Body Size in Radioiodine-treated Platyfish, Their Controls, and in Radioiodine-treated Fish Fed Thyroid[1]

Experiment	Age (months)	Solely I^{131}-treated	I^{131}, plus Thyroid-feeding	Control Fish
5	4.4	16.6 (3/2)	....	27.9 (0/5)
II	6.3	14.5 (0/2)	19.9 (0/4)	29.4 (2/2)[2]
III	6.3	13.1 (2/2)	11.7(3/3)	24.5 (3/3)
8	9.6	16.9 (2/3)	....	35.8 (2/4)

[1] Pituitary size was measured volumetrically and body size was measured as standard length. A ratio of the averages of the two parameters is tabulated here, for each group of fish. The number of males and females in each group is given in parentheses, as females/males.

[2] One fish of unknown sex not included. Its gonad may have been torn out when the anal fin was removed for mounting.



TEXT-FIG. 3. Projection drawings of serial sections of the hypophyses of representative strain **BH** platyfish from different experimental groups, all to the same magnification. The hypophyses were cut in cross-section, and are drawn with the ventral aspect uppermost. A comparison of over-all size and neural versus glandular development may be made. **A,** Radioiodine-treated immature female; **B,** Thyroid-fed radioiodine-treated mature female; **C,** Control (pregnant) female; **D,** Control mature male. It may be seen that the neural hypophysis (solid black areas) in the thyroid-fed fish appears to be nearly as large as that in the controls, but the glandular portion is much smaller. The glandular hypophysis in the normal females usually was much larger than in the normal males, although the male represented here had a slightly larger than average glandular portion; this may be related to the higher incidence of goiter in females of the strain. The hypophyses shown here were selected both for their representation of their respective groups, and for the most complete and undamaged series of sections.

photographic exposures. The animal was found to be dying the following morning and had to be fixed without autography to prevent its loss for examination.

3. *Replacement Therapy Experiments with Young Radioiodine-treated Platyfish.*

a. *Thyroid-feeding Experiments:* In these experiments, 18 radioiodine-treated fish were fed on desiccated thyroid once a week. The effects of this treatment were striking (Table 10). Mortality was reduced to a figure insignificantly different from that of the untreated controls, and the growth in body length equaled that of the controls, being significantly greater than that of the fish treated only with I^{131}. Body shape was normal, with a tendency towards a more streamlined appearance than shown by the untreated controls. This is enhanced in the photographs by the greater length of the caudal fin (Pl. I, Fig. 2) but the growth of the fins was not noted in the living fish and consequently the tails were not saved or measured. Examination of the photographs taken of each of these groups of fish suggests that the pectoral fins were also elongated, but these are often twisted from fixation and one cannot be sure. No sign of exophthalmia was found in the thyroid-fed fish.

These fish produced blank or nearly blank radioautographs, and the most meticulous histological search revealed no thyroid tissue. This also was true of the radioiodine-treated controls.

Most of these thyroid-fed fish showed a moderately to severely shrunken ventral aorta, with thickening of its walls and fibrous adhesions to the pericardium. In other respects they differed but slightly from the untreated controls. Very little lymphoid loss was seen in the kidneys or elsewhere, no degenerating tubules or concretions were found in the kidneys, and no vacuolization of the liver or abdominal tissue was present.

The sexual development in the thyroid-fed fish was most striking. Not only did fish of both sexes reach maturity by the end of the experiments, in equal or greater proportion to the untreated controls, but external male secondary sexual characters were often apparent considerably earlier than in the untreated controls (Table 10). Gonopodial structure was perfectly normal in the thyroid-fed males that matured before termination of the experiments (Text-fig. 4; Pl. VII, Fig. 2). At the close of the final experiment (IV), one of the thyroid-fed females was found to be gravid (in these small treatment groups, the sexes were not separated) (Pl. VIII, Figs. 3, 4).

TABLE 10. REPLACEMENT THERAPY OF RADIOIODINE-TREATED PLATYFISH. A SUMMARY OF THE RESULTS OF 4 EXPERIMENTS IN WHICH RADIOIODINE-TREATED FISH WERE SUBSEQUENTLY TREATED WITH POTASSIUM IODIDE OR WERE FED THYROID TABLETS[1]

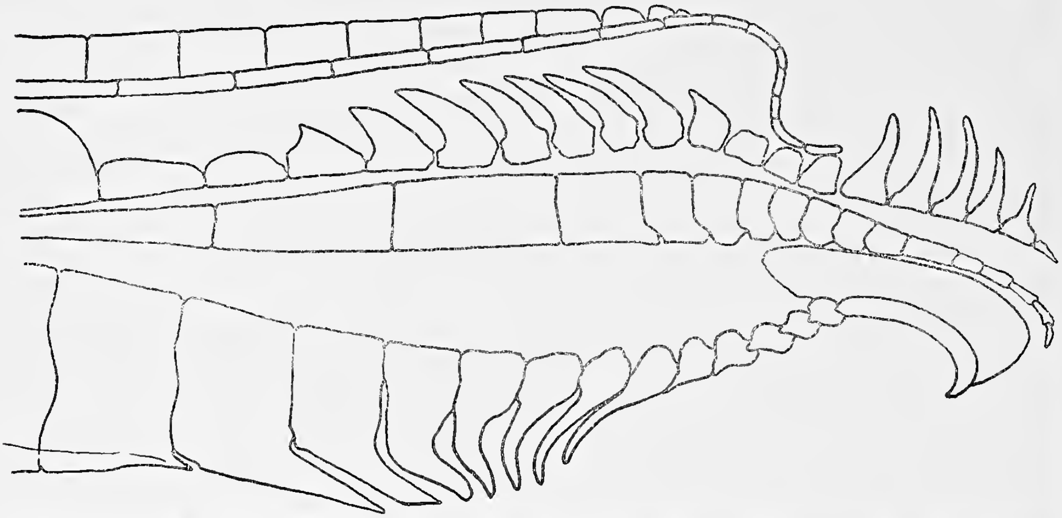
	Radioiodine-treated Fish			Untreated Controls
	Not Treated	KI-treated	Thyroid-fed	
Fish at beginning of treatments	25	12	18	25
Survivors at end of experiments[2]	11	9	15	22
Percent. Survival	44	75	83	88
Average age (days) first male seen	.	.	114	159
Males with sperm	0	1[3]	6	7
Males with mature gonopodium	0	0	6	7
Females with yolked oocytes	0	0	3	5
Pregnant females	0	0	1	0
Percent. histologically mature fish	0	11[3]	60	55
Average standard length (mm.)[4]	17.7	.	20.1	20.5
	21.8	22.0	.	24.5

[1]Each experiment included radioiodine-treated controls and non-treated controls, and the fish in each experiment were broodmates.

[2]Age of these fish at the end of the experiments was 6.1 to 6.8 months.

[3]This exceptional fish had a mature testis, but the anal fin was undifferentiated (see text).

[4]Two sets of comparative figures are given because all the measurements for the four groups could not be averaged and then compared, since the measurements were taken on photographs which varied slightly in magnification. Instead, means were taken for each of the groups within each experiment and these means were averaged for the groups shared by two or more experiments. For example: only experiments I and IV included KI-treated fish, but experiment I did not include thyroid-fed fish. Therefore the mean lengths of the KI-treated, the solely I^{131}-treated, and the control fish were averaged for these two experiments, but the thyroid-fed fish were omitted. This is represented in the second line of the comparison given for average standard length. The same method was used with experiments II, III and IV, excluding the KI-treated fish, and this comprises line 1. By this procedure, each experiment received equal weight, but the varying numbers of differently magnified fish in each treatment group in the experiments pooled was not allowed to distort the result.



Text-fig. 4. The structure of the normal gonopodium of the male platyfish. In mature thyroid-fed, radioiodine-treated fish, in P^{32}-treated fish, and in controls, no deviation from the arrangement and numbers of the elements shown here was found (compare with photographs of gonopodia of variously treated fish in Pl. VII). Drawing by Dr. Donn E. Rosen.

Among the immature fish in these experiments, the thyroid-fed group included 4 fish with extremely undeveloped gonads, that is, males with no spermatogonial cysts and females with less than 5 differentiated ova. One such fish was found in the control group. These were among the smallest fish in each group. All other immatures in the thyroid-fed and control groups appeared to be progressing normally towards eventual maturity.

The pituitary glands of some of the thyroid-fed fish were included in volumetric analyses made on radioiodine-treated and untreated fish (Table 9). In two experiments which included thyroid-fed fish, the hypophyses of these proved to be as small, relative to the fish's body length, as those of I^{131}-alone-treated fish, and much smaller than those of the untreated controls. There was a morphological difference, however, from the radioiodine-treated controls: the neurohypophysis, although not measured other than by eye, appeared to be as large in the thyroid-fed fish as in their untreated controls, but the glandular portion was very much smaller (Text-fig. 3). In the solely I^{131}-treated fish the whole pituitary seemed to be smaller, just as the whole fish was smaller. Accuracy in projection and drawing was not good enough to make a sufficiently defined separation of the neural and glandular elements of the hypophyses to quantitatively verify this distinction. It seems reasonable to suppose that the thyroid-fed fish were receiving sufficient thyroid hormone to cause the thyrotrophic cells in their pituitaries to be reduced to a minimum in numbers and/or size, and activity, and thus for the glandular volume of the hypophysis to be considerably reduced.

The thyroid-fed fish were healthy and vigorous and as lively, or more so, than the untreated controls.

b. *Potassium Iodide Treatment:* The development of these fish was not noticeably improved over that of the fish treated only with I^{131}. There was, however, a definite improvement in the survival rate (Table 10), and they appeared to be healthier and livelier than the sluggish I^{131}-treated controls. Retardation of growth was not improved over the I^{131}-treated controls, and none became sexually mature, with the exception of one anomalous male (described above). Internal pathology was similar to that of the treated controls; vacuolated liver with its accompanying abdominal rotundity was found with almost equal frequency. Gonadal development was improved slightly, as judged by counts made of oocytes in females (average of 45+ in 4 KI-treated with a range of 20 to 84+; 14 in each of 2 radioiodine-treated controls) and by the number of males which had developed spermatogonial cysts (3 of 5 KI-treated; 1 of 4 radioiodine-treated controls) in experiments I and IV.

No evidence for the existence of thyroid tissue was found in 8 of 9 KI-treated, and in 5 of 6 radioiodine - treated controls. Because radioautography of KI-treated fish had been found to be unsuccessful, producing totally blank film strips (Baker, 1958b), only three of the present KI-treated fish were radioautographed. Some slight spotting was produced on these radioautographs, but only in the pharyngeal lining and gut areas. The remaining fish were examined section by section with particular care, and in all except one case no thyroid tissue was found in the pharyngeal, renal, splenic and other areas. The exceptions were a male KI-treated fish with a few regenerating pharyngeal follicles and a testis with the bare beginnings of spermatogonial cysts (experiment I), and a radioiodine-treated female with a single thyroid follicle in the kidney (Pl. II, Fig. 2) and an immature ovary containing 14 oocytes (experiment IV). The amount of thyroid tissue present in these two fish was apparently insufficient to improve their gonadal and visceral condition appreciably over that of other members of their groups.

The latter exceptional specimen was the only instance of finding renal thyroid tissue after the destruction of the pharyngeal thyroid at an early age. This represents an incidence of one out of 79 radioautographed fish, with or without regenerating pharyngeal thyroid tissue (experiment 1 excluded). With the exclusion of fish given replacement therapy, the incidence is one out of 42 radioautographed fish, or 2.4%; the incidence of renal thyroid tissue among untreated controls was 37 out of 50, or 74% (experiments 4-IV, in which sibling controls were kept). These results corroborate the ones previously presented by Baker (1958a) in support of an hypothesis that renal thyroid tissue in the platyfish arises as the result of cell migration from the pharyngeal area, rather than endogenously in other parts of the body.

4. *Radiation Control Experiments with Young Platyfish.*

Sixteen strain *BH* platyfish, in two groups, were treated with the amount of phosphorus-32 calculated to deliver whole-body irradiation equivalent to the average dose of radioiodine used. The conditions of exposure were similar.

Nine of the 16 treated fish survived until termination of the experiments, 109-178 days after treatment, and all of the 14 broodmate controls survived. One group of 5 fish was exposed to a P^{32} solution whose pH was not quite physiological (6.6), which may help to explain the high death rate seen among these fish, although the deaths did not occur until a month after treatment. As the single surviving P^{32}-treated fish in this group was a male, three female controls were discarded.

The over-all survivorship of the P^{32}-treated fish was 56%, but that of the 109-day, pH 7.5 group was 73% (8 out of 11 fish). This is not different from the 109-day survivorship shown for I^{131}-treated fish. Because of the small numbers of fish, no pattern of death distribution was recognizable, but it seems significant that there was no evidence for a bimodal mortality distribution such as that found for radioiodine treatment.

The average length attained by the P^{32}-treated fish did not differ from that of their controls (Pl. I, Fig. 4), *viz.*, 20.4 mm. for the P^{32}-treated (range 13.0 to 25.0 mm.), 21.1 mm. for the controls (range 18.5 to 24.5 mm.). Means were not corrected for numbers of fish of each sex, as these were as close to equality as possible in odd-numbered groups, being 5 males, 4 females among the treated fish and 6 males, 5 females among the controls. Upon fixation, at ages between 4.7 and 7 months, all except one fish in the treated and one in the control group appeared to be fully mature by external criteria. Moreover, the P^{32}-treated fish did not differ from the controls in body shape, coloration or general health and activity.

All of the fish were studied histologically. No abnormalities were found in the viscera of the treated fish, *i.e.*, liver, kidney, spleen and thymus were normal. Goitrous thyroids were found in 3 out of 9 of the treated specimens, and in 5 out of 11 of the controls. Renal thyroid tissue occurred in at least 6 of the treated fish and in 7 of the controls. It should be noted that the kidneys were not carefully searched; follicles were discovered in spot checks of the kidneys, implying that they were quite numerous in the specimens in which they were seen.

The only difference found between the P^{32}-treated fish and the controls was in gonadal development, primarily among males. Five of the 6 control males had fully mature testes, full of spermatophores, as well as completely differentiated gonopodia. In the testes of the 6th, spermatogenesis had begun but no spermatophores had yet appeared; the gonopodium was partially differentiated and was at a normal incomplete stage (Grobstein, 1953). Of the 5 treated males, 4 had fully normal mature gonopodia (Pl. VII, Fig. 3), but *none* had any testicular germ cells. Only the ducts were present, but these were complex in structure (Pl. VII, Fig. 5). One treated male had juvenile, simple ducts and an undifferentiated anal fin. Among the 5 control females, all had fully mature ovaries and two were gravid (Pl. VIII, Fig. 1); all 4 P^{32}-treated females also appeared to have completely mature normal ovaries containing fully yolked ova and young yolkless oocytes in all stages of maturation (Pl. VIII, Fig. 5). No counts of ova were made, but from the general microscopic appearance of the ovaries, there appeared to be no fewer in the P^{32}-treated female fish.

From these data it is concluded that P^{32} irradiation, equivalent to that received in the I^{131} treatment, produced none of the pathological or developmental effects noted after radioiodine treatment. The only effects were a very specific and distinctive action on the male gonad, which did not affect secondary sexual characters, and a similar total mortality rate to radioiodine treatment, up to 109 days post-treatment. The specific causes of mortality resulting from the two treatments is probably not similar, when the lower mortality rate of radioiodine- treated fish fed thyroid or KI-treated is taken into consideration.

5. *The Effects of Radioiodine Treatment in Adult Platyfish.*

a. *Survival:* Forty-six fish, all under one year of age, were injected with I^{131}. Ten of these died within two days after the injection; this is attributed to shock or infection and is not considered in relation to radiation damage or to thyroid condition. Two additional fish may also have died this way, but they were not recorded and are not considered here. Eleven more fish died within 8.5 months after injection; that is, ⅓ of the 34 remaining fish. Unfortunately, data on the mortality of the controls was poorly kept, but in Group 3, 3 out of 12 controls were recorded as dying within 8.5 months after the injection of their siblings. This suggests a somewhat higher death rate among the I^{131}-treated fish, but is by no means conclusive. Six out of 23 radioiodine-injected individuals (omitting those sacrificed when younger and those dying from the immediate effects of injection survived to an age of 27 months or more. At death, histologically visible thyroid was present in some of these old fish and absent in others.

b. *Gonadal and General Pathological Effects:* It is doubtful whether radiothyroidectomy (or near-thyroidectomy) had any profound effect on the gonads of these mature fish, at least up to 6.5 months after treatment. In Group 1, two of the three fish had fully normal mature ovaries. The third fish had given birth 22 days before she was radioautographed and contained no fully yolked ova; normally these should have been present soon after parturition (Tavolga, 1949). Thus a slight delay may be noticeable in this instance. In Group 2 (females and males kept together) young were born in the tank up to three months after treatment. Three females, fixed within 3.5 months of the injection, had normal ovaries and one was gravid; the single male sacrificed within this period had normal testes.

Among the female fish examined at longer intervals after injection (no males survived), there appeared to be some effect on the ovaries. These fish include 10 females from Group 3 and one from Group 2, sacrificed 8.5 to 19.5 months post-injection. The youngest 5 were noted to be "thin" when radioautographed. Only one of these had any functional-looking ova; this fish had a single basophilic, pre-yolk-deposition oocyte, and primitive gonial cells appeared to be proliferating from the lining of the ovarian cavity, in small balls resembling primary spermatogonial cysts in young males (Pl. VIII, Figs. 7, 8). Although the future function of these cells is unknown, the condition is suggestive of Essenberg's (1926) description of the development of testes from the epithelium of the ovarian cavity in sex-reversing females of *Xiphophorus helleri.* Essenberg's suggestion of a relation between depressed metabolism and sex reversal is also of interest in the case of the hypothyroid platyfish. In the other 4 fish, only fibrous atretic follicles were present. The 5 older fish from Group 3, which were 27.5 to 30 months old, included two with yolked oocytes, but only one of these had ova in earlier stages of development. The other three fish had ovaries with atretic follicles only (Pl. VIII, Fig. 6). Although the three sibling controls for these last 5 fish also had atretic follicles in their ovaries, they had many ova in various early stages of maturation as well. The single female from Group 2 was 28-29 months old and had an almost completely degenerate ovary with practically no visible germ cells. This fish exhibited several other pathological conditions (see below).

Vacuolated liver in varying degrees was found in 4 of the 18 I^{131}-injected fish, and ceroid-filled liver cells and/or ceroid-filled macrophages in the liver were seen in three additional fish. In one fish that died while in radioiodine tracer, the liver was decomposed too badly for its condition to be judged. Excessive peritoneal vacuolation was seen in 9 of the 16 fish that were freshly preserved.

Concretions were seen in the kidney tubules of 5 fish, and degenerate tubules in two, one being the extreme case described below. Lymphoid tissue in the kidneys appeared normal in all but the latter, and likewise the thymus glands appeared normal.

The pituitary glands of 11 of the 16 well-preserved fish showed a prominent overgrowth of the pale intermediate basophiles (Pl. VI, Fig. 6). This was found in all fish of the *Fu* strain. It also was found in all strain *163* fish fixed more than 8.5 months after injection, but not in strain *163* fish 38 days to 8.5 months post-injection.

This was a markedly higher incidence than in the young fish radiothyroidectomized by immersion, and is believed to be related to the incomplete degree of thyroidectomy in the injected mature fish, rather than to their age.

c. *Anomalies Noted:* From Group 2, in one male and one female, aged 12-13 months, a large mass of pathological tissue was found in the abdomen among the pancreatic and fatty tissue. In the male, this mass was attached to the normal testis (Pl. IX, Fig. 1). These masses were encapsulated and contained collections of whorled fibrous nodes. These nodes resembled, in part, the fibrous "nests" seen in various organs of other fish, but did not occur elsewhere in the bodies of these two specimens. A similar anomaly occurred in one 27.5-30-month-old female from Group 3.

In Group 1, a 15-month-old female had "nests" of fibrous tissue scattered throughout its body, including the dorsal pericardium and transverse pharyngeal muscles, the kidneys, liver, spleen and abdominal fatty tissue. None occurred in the ovary, heart and thymus areas. These "nests" of cells have been described in KI-treated platyfish with thyroid tumors in their kidneys by Baker (1958b) and in *Astyanax* by Rasquin & Rosenbloom (1954). They probably represent a reaction to injection or other bodily insult. It is unlikely that they are directly related to the I^{131} injection or to processes of thyroid degeneration, as the fish in which they were seen had been treated 3.5 months or more earlier.

The oldest fish in Group 2 (a 28-29-month-old female) was an extreme specimen. Its pathology included an almost total lack of lymphoid tissue (no thymus, shriveled kidneys and spleen) and an *extremely* highly vacuolated liver and abdomen, with excessive deposits of pigment in the spleen and kidneys, large necrotic cysts in the liver and a pituitary overgrowth of such proportions that it might well be classed as a tumor (Pl. IX, Figs. 3-7). There was no sign of hypertrophied thyroid, as might have been expected from the condition of other organs; instead the thyroid area was filled with fibrous tissue and masses of material which suggested thyroid, but which had flat margins and was stained a pale bluish color. The exact nature of this material is unknown, but the presence of thyroid cells is most unlikely. This fish appears to be the most extreme case of pathological hypothyroidism met with in this study. This fish also contained some of the "nests" of cells mentioned above, especially in subcapsular edematous spaces in the shriveled kidneys and in the necrotic cysts of the liver. The kidneys were deficient in glomeruli; many tubules seemed excessively small, others contained large concretions and had clear, "blown-up" cells that were unquestionably degenerate.[7]

None of these anomalies was found among the controls, but because of the small number and the relatively advanced ages of these fish, the peculiarities cannot definitely be related to hypothyroidism, except to surmise that such animals are very likely more prone to infection than normal similarly aged fish. A study of general senile degeneration has not been made on normal untreated *Xiphophorus maculatus,* but it is suspected that some of the effects noted here may be the result of a slightly premature appearance of such degenerative processes. Other effects appear to be definitely related to thyroid function, irrespective of age, *e. g.,* vacuolation of the liver.

In addition to the pathological anatomy, it should be noted that renal thyroid tissue was seen, without use of radioautographs, in all of the 6 control fish and in the spleens of three of them, but such tissue was not found or radioautographically suggested in any of the I^{131}-injected platyfish.

d. *Young Born after I^{131} Injection of Mother*: One female of Group 1 gave birth to young 19 days following the injection of approximately 80 μc. of I^{131}. Two of these young were radioautographed with the mother when they were 22 days old. One was found to be totally thyroidectomized, both by autographic and histological criteria. This was a female; the other was a male that had many thyroid follicles. These follicles, as is typical in very young fish, were made up of high basophilic cells. Both young seemed normal, except that the thyroidectomized female showed some vacuolization of the liver, and an aorta that seemed shrunken. Both young had undifferentiated gonads, although they definitely were sexable, and the female appeared to have large, clear oogonia at the cranial end of the oviduct. The testes of the young male fish were almost as well developed as those of some of the totally radiothyroidectomized fish 6-9 months old (Pl. VII, Figs. 6, 7). Since no normal young of equal age were sectioned, gonadal development was not controlled in these young fish.

According to the accepted gestation period of platyfish, *i.e.,* 22 days (Tavolga, 1949), the mother fish was injected when the young were

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[7] The kidneys of this specimen were in an even more pathological condition than those of fish with the most extreme renal thyroid tumors described by Baker (1958b).

only three days in development. Since the thyroid gland does not appear until about the 8th day (Tavolga, 1949), enough radioiodine must have been retained by the yolk or the maternal tissues to affect the young thyroid when it first began to concentrate iodine, 5 days later. Another possibility is that the treatment slowed down the development of the young, so that they were born belatedly and were actually at the thyroid development stage when the mother was injected. It is known that most non-thyroid tissue and the yolk of platyfish ova do not retain iodine in protein-bound form (Baker, 1958a), but it is quite possible that *iodide* may be retained in such tissues for considerable periods. This instance suggests that sufficient I^{131} could be retained in the body of an adult fish to thyroidectomize both the adult, and later, young fish developing in its body. There was no possibility of any appreciable amount of I^{131} remaining in the aquarium by the time these young were born because of the number of times that the water had been changed since the time of injection. Thus, it seems probable that embryonic platyfish can be thyroidectomized *in ovarum* and still develop to a normal birth.

Discussion

1. *Radiothyroidectomy.*—Table 11 summarizes published attempts, to date, to extirpate the thyroid of teleosts using radioiodine. These few attempts have met with mixed success. It is possible that the differences between the activities of the thyroid found in various species of fish are responsible for the variety of results obtained (as an example of such differences, see the three species of *Fundulus* compared by Gorbman & Berg, 1955). It seems likely that less active thyroid glands, which both concentrate and release iodine and thyroxine or its relatives less rapidly, will receive more irradiation from a relatively prolonged dose of I^{131} than will very active thyroids. It is believed that the differences in the efficiency of radiothyroidectomy in the various strains of platyfish described here were at least partly due to genetic differences in iodine requirements and metabolism. Those strains that were more goiter-prone (or had less actively secreting thyroids?) seemed to be more readily radiothyroidectomized. Differences in the radioresistance of thyroid cells might also be a factor among different species or populations of teleosts.

The platyfish discussed here were probably

Table 11. Summary of Reports of the Use of Radioactive Iodine in Thyroidectomy of Teleost Fish

Author and Date	Species and Stage used	Degree of Thyroidectomy Obtained	Observations Made
LaRoche & Leblond, 1954	Atlantic salmon (*Salmo salar*), parr	Complete	No effect on growth; reduction of skin pigmentation
Baker *et al.*, 1955	Platyfish (*Xiphophorus maculatus*), young	Some complete	No effect on growth and sexual maturation[1]
Arvy *et al.*, 1956	Rainbow trout (*Salmo gairdneri*), parr	Incomplete	No effect on O_2 consumption
Fromm & Reineke, 1956	Rainbow trout, fingerlings	Complete	No effect on O_2 consumption
Fontaine, Y.-A., 1957	Eel (*Anguilla anguilla*), silver adult females	Complete	Decreased hypophyseal thyrotropin; no size difference in hypophysis; no TSH in plasma
Fontaine, M. *et al.*, 1957	Eel, adult silver	Presumed initially complete	Fall in O_2 consumption after 48 hours; later recovery presumed correlated with thyroid regeneration
Olivereau, 1957	Eel, small males; large females	Incomplete	Thyroid destruction and regeneration studied
Baker, 1958a	Platyfish, young	Many complete	Some pathological effects noted; effect on heterotopic thyroid development studied
Harris, 1959	Killifish (*Fundulus heteroclitus*), adult males	Mostly incomplete	No effect on salinity tolerance nor on growth or gonads

[1] These early published conclusions were mistaken and were corrected in the oral presentation of the material (see also Pickford, 1957). Complete results are embodied in the present paper.

more readily thyroidectomized by I^{131} than some of the fish used by other workers because they had been bred in an especially iodine-poor environment and because their iodine-to-thyroxine turnover was relatively slow. Despite the administration of thyrotropin prior to each of several I^{131} injections, Harris (1959) was unable to obtain complete thyroidectomy in most male *Fundulus*. Perhaps a more protracted period of exposure to I^{131} would be required if the turnover rate of iodine in these fish were relatively high within each post-injection period.

2. *Thyroid and Development.*—Various aspects of the function of the thyroid in fish have been thoroughly reviewed recently by Lynn & Wachowski (1951), Hoar (1957), Pickford (1957) and Leloup & Fontaine (1960). Papers that present evidence for thyroidal involvement in development and sexual function of teleosts, based on observations of non-experimentally treated fish, are included in these reviews. Some of these papers are omitted from discussion here. A synopsis of exclusively experimental observations on the effects of thyroid preparations and antithyroid compounds in the development of teleost fish is presented in Table 12. Many of the entries in this table have been more extensively summarized by Pickford (1957). Therefore the substance of the table will not be discussed here, except in a general way.

Among the more striking features to be noted in Table 12 is the heterogeneity of the results obtained with thyroid preparations and the relative homogeneity of those obtained with antithyroid drugs. One reason for this is probably the uniformity of dosage employed in the latter groups and the chemical uniformity of the drugs employed. Nearly all investigators agree that treatment with thiourea or thiouracil retards both growth and sexual differentiation in young fish, and brings about regression of gonads and loss of weight in adults. The effects of radiothyroidectomy of young platyfish observed by the author agree with this consensus, except that gonadal effects on adults were doubtful. LaRoche & Leblond (1954) reported that radiothyroidectomy had no effect on growth of salmon parr, but since histological criteria alone were used to determine the extent of thyroidectomy, it is suspected that the fish may not have been totally thyroidectomized. In some of the platyfish described here, it was found that a barely visible amount of thyroid tissue was sometimes sufficient to support sexual maturation and growth to normal adult size, and this barely visible tissue was found only with the aid of radioautography.

The results of treatment with thyroid derivatives run the gamut from retardation to enhancement of almost every feature of development. This variety in response is probably the result of the different methods of administration, types of preparations and dosages that were employed. In all instances, the fish undoubtedly were receiving abnormally high amounts of thyroid hormones, especially as their own thyroids were also present, even though their activity may have been inhibited. The occurrence of exophthalmia, abnormal gonopodial development, vascular abnormalitites and deformations of the head certainly cannot be considered as physiological, and it is hard to assess such peculiarities in terms of the normal function of the thyroid hormone. The most commonly described findings seem to be: slenderization in body shape; increased fin, skin and connective tissue development; silvering (increased guanine deposition) and reduced pigmentation in salmonoids; and exophthalmos. Yet even among these, there are reports describing the exact opposite, or the absence, of these features—sometimes in the same species. The effects on sexual development and over-all body growth are even more conflicting. Some of this disagreement may be related to the age of the fish tested, as well as to species differences in the coordination of thyroid function with developmental stages. Observations reported here suggest that reproductive processes in fully adult platyfish are relatively independent of thyroid function, just as growth, once completed, no longer can be generally influenced. Histological evidence suggested a sharp falling off of thyroid activity with age, after full attainment of mature body size, in this species.

Among young poeciliid fishes (including the guppy, platyfish and mosquito fish, *Gambusia*), increased growth rate, early male secondary sex differentiation, abnormal gonopodial development, increased fin length, and exophthalmia have usually been produced by treatment with thyroid derivatives. The results of thyroid-feeding of athyroid platyfish, reported here, concur with increased growth (increased to *normal,* that is), slightly accelerated male secondary sexual development, and some elongation of the caudal fin. They disagree in that gonopodial differentiation was completely normal in all cases and that no exophthalmia occurred. In comparison with controls, the proportion of mature fish was slightly higher at the conclusion of the experiments and only among the thyroid-fed, athyroid fish was a pregnancy found. It is believed that the normal development of these fish took place because they were given very little thyroid material. For example, the amount of dried

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1]

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
A. *Effects of treatment with thyroid derivatives:*			
Terao, 1922	Goldfish (*Carassius auratus*)	Sheep thyroid powder fed, 1/25 of food	Depigmentation; stimulation of head growth
Blacher, 1927	Goldfish Crucian carp (*C. carassius*)	Thyroid gland fed	Depigmentation; exophthalmia
Sklower, 1927	Trout (species not given), fry	Thyroid fed in food (source not specified)	Slenderization of body shape
Zarski, 1927	Loach (*Misgurnus fossilis*) *Tinca vulgaris*	Beef thyroid fed or injected I.P.	No effect on histology of skin
Herzfeld *et al.*, 1931	Brook trout (*Salvelinus fontinalis*), hatchlings	Thyroxine by immersion, 0.002 gm./250 ml.	Acceleration of growth rate; darkening; spasms
Baumann & Pfister, 1936	Rainbow trout (*Salmo irrideus*), embryos	Thyroxine by immersion, solution 1:100,000	Acceleration of growth; vascular abnormalities, causing death
Krockert, 1936	Guppy (*Lebistes reticulatus*) young	Dried beef thyroid fed, 1.5-2.0 mg./day/fish	Enlargement of all fins; exophthalmia
Grobstein & Bellamy, 1939	Platyfish (*Xiphophorus variatus*), young	Desiccated thyroid fed, a "pinch" daily	Decreased growth rate; slender shape; all fins elongated; exophthalmia; precocious male sex differentiation, with abnormal gonopodial development
Landgrebe, 1941	Atlantic salmon (*Salmo salar*), parr	Ox thyroid extract injected 2× weekly (= 0.5 gm. fresh tissue)	Silvering; no gonadal effect
	Brown trout (*S. trutta*), adults	Same (= 1 gm. fresh tissue)	Silvering
	Eel (*Anguilla anguilla*), immature, yellow	Same, weekly	No effect on color or gonads
Hasler & Meyer, 1942	Goldfish, adults	1-6 mg. thyroxine injected I.P.	No effect on spawning
Svärdson, 1943	Guppy, young males	Thyroxine (method not specified)	Retardation of growth; earlier male maturation
Smith & Everett, 1943	Guppy, newborn	Thyroid powder fed; Synthetic thyroxine by immersion (8-15 drops/day of 2 mg./ml. sol. to 5-gal. tank)	No effect on growth in length or on male sex differentiation
Albert, 1945	Killifish (*Fundulus heteroclitus*), adults	Thyroxine; desiccated thyroid: various (unspecified) single doses injected	Exophthalmia not produced
Nigrelli *et al.*, 1946	Guppy, young, thiourea-treated	Mammalian thyroid powder fed, 0.2 gm. 2× weekly	Protected against retardation of growth and of male secondary sex differentiation, caused by thiourea

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Vilter, 1946	Eel, leptocephalus ("glass eel")	Synthetic thyroxine by immersion, 1:1,000,000-1:50,000	Growth of stomach; increased pigmentation—but only when thyroxine concentration was raised progressively
Gerbilsky & Saks, 1947	Sturgeon (*Acipenser stellatus*), larvae	Thyroxine by immersion, 2×10^{-7}; 1×10^{-7}	Increased rate of development of bony plates
Zaks & Zamkova, 1947 (as summarized by Chambers, 1953)	Loach, embryos	Thyroxine by immersion, 1×10^{-7}; same plus 0.033% thiourea; thiourea alone	Increased bony scute development; increased resorption of yolk & external gills; change to adult-shaped head; gut secretory processes increased; retraction of pigment cells; iris pigmentation like adult. Thiourea plus thyroxine: *increased* the effects of thyroxine
La Roche, 1949 (abstract)	See La Roche *et al.*, 1950		
Robertson, 1949	Rainbow trout (*Salmo gairdneri*), parr, 2 years old, 9-10 inches long	Hog thyroid powder extract injected 3× weekly, doses increasing from 6.6 mg.; total dose 0.186 gm.	Silvering; increased guanine deposition
Smith, 1949	Coho salmon (*Onchorhynchus kisutch*), fingerlings	Na-thyroxinate by immersion, 1:1,125,000; desiccated thyroid fed	Slenderized shape; increased silvering
Hopper, 1950 (abstract)	See Hopper, 1952		
La Roche, 1950 (abstract)	See La Roche *et al.*, 1950; La Roche, 1951		
La Roche *et al.*, 1950	Atlantic salmon, parr	Na-thyroxinate injected; beef thyroid fed; thyroid extract fed 1:1 with beef liver	Pallor; endophthalmia (illusory—due to thickening of skin); deformation of head; loss of parr marks
Rizzo, 1950 a, b	*Gambusia affinis holbrooki*	No information given	Exophthalmia; hypertrophy of optic nerve; abnormal brain proportions; abnormalities in bones of skull and jaws; hypertrophy of choroid gland of eye
Buser & Bougis, 1951	*Gambusia affinis*, young	Thyroxine by immersion, 5-10 mg./liter	Exophthalmia; elongation of pectoral fins, no elongation of caudal fin
Hoar, 1951	Atlantic salmon, parr	Desiccated thyroid fed	Some increased silvering, but not smoltification
	Brook trout	Same	No silvering in 2 months
	Coho salmon, fingerlings, alevins	Same; also synthetic Na-thyroxinate by immersion, 1:1,125,000	Increased silvering not pronounced
	Coho fry	Na-thyroxinate 1:2,000,000	Pronounced silvering
	Chum salmon (*O. keta*), alevins	Same, 1:1,000,000	Pronounced silvering; thinning of skin; loss of scales; withdrawal of liver fat; slenderization of shape

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Jones *et al.*, 1951	Zebra fish (*Brachydanio rerio*), embryos	Thyroxine; thyroid powder; by immersion, various concentrations	Retardation of morphogenesis and pigment formation
La Roche, 1951	Atlantic salmon, fry, parr, and smolt	Dried beef thyroid fed, 1:1 with diet	Increased dermal thickness
	Brook trout, yearlings	Same	Increased dermal thickness; increased internal organ connective tissue
Gaiser, 1952	Guppy, young	Thyroxine by immersion, 0.0001 per 100	Decreased initial growth rate; earlier onset of sexual differentiation in both sexes; no exophthalmia
Hopper, 1952	Guppy, young	Mammalian thyroid powder by immersion, 0.01%	Increased growth rate, especially in females (females 40-90% larger; males 20-30% larger, than controls); precocious abnormal differentiation of gonopodium in males
Ito, 1952	Goldfish, black & orange (2 fish)	"Thyroid hormone" by injection (no data on dosage)	No effect on pigmentation
La Roche & Leblond, 1952	Atlantic salmon, fry, parr and smolt	Thyroid extract fed 1:1 with liver; dried beef thyroid fed 75% of diet; 200 μg Na-thyroxinate injected intramuscularly 2× weekly	Some retardation of growth (weight, not length); thickening of integument and connective tissue—affecting head shape; pallor due to fewer pigment cells
	Brook trout	Beef thyroid fed 1:1 with liver	Similar
Müller, 1953	Goldfish	Synthetic thyroxine by injection I.P.	Young animals: melanophore expansion. Older animals: melanophore expansion at first, later depigmentation due to loss of melanophores
Smith *et al.*, 1953	Guppy, young, treated with 0.011-0.033% thiourea	Mammalian dried thyroid fed, 1:1 with food or by itself	No protection against retardation of sex differentiation and growth due to thiourea. Thyroid alone: slight delay in male differentiation? No growth effect
Dales & Hoar, 1954	Chum salmon, embryos	Na-thyroxinate by immersion, 1:2.5, 5.0 or 12.5 × 10^6	Speeded hatching; decreased body length; increased growth of body wall and pectoral fins (only); increased guanine deposition; exophthalmia
Gibson, 1954	Zebra fish, embryos	Thyroxine by immersion, 1-100/1,000,000	Same as Jones *et al.*, 1951; also stimulated heart rate
Honma & Murakawa, 1955	Chum salmon, larvae	Thyroxine by immersion, 1:1,000 & 1:1,000,000	Inhibition of weight increase; delay in absorption of yolk; broadening of head, protrusions on head; exophthalmos-like condition; elongated pectoral fins; poor development of body; pallor; silvering (excess guanine)

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Egami, 1957	Medaka (*Oryzias latipes*), adult females, treated with testosterone and thiourea	Thyroxine by immersion, 1/6-1/18 mg./liter	Effects of thiourea mostly overcome by thyroxine treatment (see B. below)
Jones & Huffman, 1957	See Jones *et al.*, 1951; Gibson, 1954		
Langford, 1957	*Fundulus heteroclitus*, adults	Desiccated thyroid, 0.6 mg./day, or triiodothyronine, 0.1, 0.2, 0.5 or 1.0 μg. injected daily	Marked exophthalmia produced
Bjorkland, 1958	Goldfish, young	Thyroxine, triiodothyronine injected I.P. in "small doses" Parrotfish thyroid injected I.P.	Epidermal thickening; pigmentary paling; increased growth in weight and length No morphogenetic or growth effect (immune reaction?)
Matty *et al.*, 1958	*Sparisoma squalidum*, adult female, young *Scarus croicensis*, adults *Bathystoma aurolineatum*, adults (?)	L-thyroxine, 10 or 500 μg injected daily; triiodothyronine, 5 μg daily	Marked exophthalmia, independent of dose
Stolk, 1959	Swordtail (*Xiphophorus helleri*) × platyfish (*X. maculatus*) hybrids, young	Thyroxine fed mixed in food	Increased growth rate; inhibition of melanotic tumor development
Honma, 1960	Chum salmon, larvae	Thyroxine by immersion, 1:1,000,000	Acceleration of metamorphosis, silvering, increased height of skull, elongation of opercular bones, hyperplasia of orbital connective tissue, inhibition of yolk absorption and differentiation of some viscera, retardation of growth rate
Hopper, 1961	Guppy, young	Thyroid powder fed 50 mg./3000 ml. H_2O/diem (no. of fish per tank not specified)	No effect on growth rate or sexual differentiation
B. *Effects of treatment with antithyroid drugs:*			
Goldsmith *et al.*, 1944	Platyfish × Swordtail hybrids, young	0.033% thiourea by immersion	Retardation of growth and failure of sex differentiation
Nigrelli *et al.*, 1946	Guppy, young	0.03% thiourea by immersion	Inhibition of sex differentiation
Zaks & Zamkova, 1947	Loach, embryos	Thiourea 0.033% by immersion	No effect up to 26th day of development

TABLE 12. SURVEY OF EXPERIMENTAL EVIDENCE RELATING THYROID FUNCTION TO GROWTH AND SEXUAL MATURATION IN FISHES[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Frieders, 1949	Platyfish,	0.05% thiourea by immersion;	All died
	Black molly (*Mollienesia latipinna*), Blue Acara (*Aequidens latifrons*), Blue Gourami (*Trichogaster trichopterus*), all young	0.0025% phenyl-thiourea by immersion	Retardation of growth; depigmentation (Blue Gourami: growth decreased at first, but after 5 weeks it *increased* over the controls)
Goldsmith, 1949	Platyfish × Swordtail hybrids, young	0.03% thiourea by immersion	Retardation of growth; inhibition of secondary sexual characters
Iakovlova, 1949 (as summarized by Pickford, 1957)	Sturgeon (Sevriuga), larvae	Thiourea 0.033% by immersion	Inhibition of growth and scute development; retention of larval teeth
Smith, 1949	Chum salmon, alevins Sockeye salmon (*O. nerka*), alevins	0.36% thiourea by immersion	Retardation of growth and yolk usage; deformities observed; no effect on silvering
Hopper, 1950 (abstract)	See Hopper, 1952		
Leloup & Olivereau, 1950	*Dentex vulgaris*	Thiourea 0.5 mg./kg. injected intramuscularly daily	Exophthalmia developed after 2 injections & became accentuated after further injections; fish soon died
Jones *et al.*, 1951	Zebra fish, embryos	Thiouracil by immersion, various concentrations	Enhancement of morphogenesis & pigment formation
Barrington & Matty, 1952	Minnow (*Phoxinus laevis*), adults	0.1% thiourea by immersion	Arrest of spermatogenesis and oocyte growth; inhibition of external sexual characters
Gaiser, 1952	Guppy, young	0.03% thiourea by immersion	Growth and sex differentiation retarded—no females matured in 90 days
Hopper, 1952	Guppy, young	Thiouracil 0.03% by immersion	Females 5-15% smaller than controls; male differentiation failed in most cases—abnormal gonopodial differentiation when seen
Vivien & Gaiser, 1952	Guppy, young	0.03% thiourea by immersion	Retardation of growth and sexual development in both sexes
Warner, 1952	Brown trout, embryos, fry	0.033% thiouracil by immersion	Delay in yolk resorption and slight delay in hatching; impaired viability; no marked growth effect; sluggishness
Chambers, 1953	*Fundulus heteroclitus*, adult males	Thiourea, 0.5 mg./gm. body weight injected 3× weekly, reduced to ½ after 4 weeks	Loss of weight; retardation of growth; regression of testes; enlargement of liver—enlargement and vacuolization of hepatic cells, depletion of liver glycogen and fat; loss of appetite; high death rate
Scott, 1953	Zebra fish, young	0.33% thiourea by immersion	Growth retarded; gonad development retarded in youngest group

Table 12. Survey of Experimental Evidence Relating Thyroid Function to Growth and Sexual Maturation in Fishes[1] (*Continued*)

Author and Date	Species and Stage Treated	Method of Treatment	Observations and Conclusions
Smith *et al.*, 1953	Guppy, young	0.011-0.033% thiourea by immersion	Retarded growth and sex differentiation
Barrington, 1954	Minnow, adults	Thiouracil (no details given)	Completion of spermatogenesis, induced by artificial light, not affected
Dales & Hoar, 1954	Chum salmon, embryos	0.05, 0.1 and 0.2% thiourea by immersion	Reduced growth rate; slight delay in yolk resorption; decreased guanine deposition; no effect on pigmentation
Frieders, 1954	See Frieders, 1949		
Gibson, 1954	Zebra fish, embryos	Thiouracil, 1:500 to 1:100,000	Same as *Jones et al.*, 1951
Sembrat, 1954	Brown trout, embryos	5% methylthiouracil-Na by immersion	Absence of scale development; thinness of skin; increased pigmentation
Fortune, 1955	Guppy, young Minnow, hatchlings	0.05% thiourea by immersion	No effect on growth in either species
Honma & Murakawa, 1955	Chum salmon, larvae	Thiourea 1:3,000 by immersion	No color change; immature character of body form retained
Sembrat, 1956	Brown trout & guppy, young	0.05-0.1% methylthiouracil-Na, by immersion	Inhibition of scale development; thinner skin; inhibition of growth in both species
Egami, 1957	Medaka, adult females, treated with testosterone	Thiourea by immersion, ½-2.0 gm./liter	Pallor; slenderization; marked inhibition of formation of male fin processes in testosterone treatment
Honma & Murakawa, 1957	Goldfish, larvae	Thiourea by immersion, 1:3,000	Inhibition of growth (weight and linear dimensions); inhibition of guanine deposition in scales & of decolorization; bent vertebral column
Grosso & Charipper, 1958	Guppy, embryos	Thiourea by immersion of mother in 0.04% solution	Inhibition of ovarian maturation in adult; decrease in productivity; heterotopic thyroid in newborn young
Pflugfelder, 1959	Guppy	$KClO_4$ by immersion, 0.05%	Inhibition of sexual development
Stolk, 1959	Swordtail × Platyfish hybrids, young	Thiouracil fed, mixed in food	Decreased growth rate; enhancement of melanotic tumor development
Honma, 1960	Chum salmon, larvae, Goldfish, larvae	Thiourea by immersion, 1:3,000	Inhibition of differentiation, decrease in growth rate, abnormal calcium metabolism (reflected by abnormal bone development), increase in carotinoid pigments in goldfish, inhibition of decolorization in salmon
Kajishima, 1960	Goldfish, embryos	Phenylthiourea 0.05% by immersion	Development retarded, body shortened & deepened; exophthalmia; complete inhibition of differentiation of melanin granules in eye & body; xanthophores & guanophores degenerated 2 weeks post-hatching

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[1] This table excludes observations made on metabolic rate, migration, osmotic regulation, regeneration and pituitary cytology.

thyroid initially given (as food), if dissolved throughout the water, would have been approximately 0.0016%; this eventually was doubled but not until the fish had grown considerably larger. This was one-sixth of the concentration used by Hopper (1952) for young guppies (0.01%), and when increased was still only one-third of his dosage. Without chemical assays, it is hard to compare doses of dried thyroid with doses of pure thyroxine, but probably many of the latter were excessive. For example, the 5-10 mg./liter dose of Buser & Bougis (1951) seems to be an extremely high concentration of thyroxine, since it is almost as high as the dried thyroid concentration first cited, and desiccated thyroid certainly is composed largely of materials other than thyroxine itself. That this dosage produced exophthalmia seems not surprising.

In studies on the effects of antithyroid drugs on fish, darkening has been noted: *e.g.*, as a result of the increase in pigmentation (Sembrat, 1954), or a decrease in guanine desposition in the scales, which then revealed the underlying pigment (Dales & Hoar, 1954). Although darkening was noted among the radiothyroidectomized fish studied here, the effect was not analyzed.

3. *Thyroid Activity and Liver Function.*—According to Spellberg (1954), fatty liver in man has been associated with hyperthyroidism, as well as with "anterior pituitary hyperfunction" —among numerous other causes. The first observation differs from experimental results in other mammals. Chaikoff *et al,* (1948) found fatty liver with fibrosis in thyroidectomized dogs after 193 to 799 days, and McClosky *et al.* (1947) noted fatty livers, as well as iron-pigment deposits in the spleens and livers of cats fed thiouracil for 7-8 months. Thirty days of thyroid-feeding reduced fat in the livers of normally fed rats, and protected rats on lipotrophic diets from the development of fatty liver (Leathem & Howell, 1950). These authors also noted that thiouracil, fed for 35 days, caused an increase in liver weight and cell size, without change of fat content. Similar observations were made on rats treated with thiourea up to 60 days by May *et al.* (1946); they found no amelioration of thiourea effects by simultaneous thyroid treatment. Leblond & Hoff (1944) also found that thiouracil-feeding of rats for 21 days led to an increase in liver weight, but that a *decrease* occurred in thyroidectomized animals. No chemical or histological analyses were presented. Sellers & Wen You (1951) found that thyroid hormone influenced the site of fat deposition in the liver of rats. In thyroid deficiency, fat tended to be deposited intracellularly throughout the liver, but in the normal, and especially in the hyperthyroid animal, fat accumulated in the central part of the liver lobule and extracellular fatty cysts were formed. Sellers & Wen You suggested that the protection afforded by propylthiouracil against experimental cirrhosis might be due in part to the reduction of thyroid hormone level, which shifted the pattern of fat deposition from extracellular to intracellular. Finally, Goldberg *et al.* (1950) found no persistent pathological changes in non-cervical tissues, *e.g.*, the liver and kidneys, of radiothyroidectomized rats up to 8 months after treatment.

In fish, only a few observations appear to have been made on the liver in relation to thyroid function, and these are not altogether in agreement. Hatey (1950) reported that thiourea treatment of carp (*Cyprinus carpio*) by immersion led to a decrease in liver size and liver glycogen within 3.5-11.5 days. Chambers (1953) also found a depletion of liver glycogen in *Fundulus heteroclitus,* treated with thiourea by injection for 6 weeks, but this was accompanied by an increase in liver size. Liver cell size was increased and fat content decreased. Pickford (1952) noted a decrease in liver size of hypophysectomized male *Fundulus heteroclitus* treated with thyroxine, but the difference from the controls was not considered significant. The observations of Chambers and Pickford agree with the results obtained for mammals with respect to organ and cell size, but both Chambers and Hatey found glycogen level to be affected in the opposite direction than that seen in rats (Leathem & Howell, 1950; May *et al.,* 1946). On the other hand, Fontaine *et al.* (1953) observed a decrease in liver glycogen after long-term, high dosage thyroxine treatment, at high temperatures, of eels (*Anguilla anguilla*) and rainbow trout (*Salmo gairdneri*), an observation which agrees with the mammalian results. On the basis of these results, these authors hypothesized that the drop in liver glycogen seen in smolts might be at least partly due to thyroid hyperfunction.

Liver size, histology, and fat and glycogen reserves of teleosts differ in the two sexes, and also vary during different stages of the breeding cycle (Olivereau & Leloup, 1950; Immers, 1953; Clavert & Zahnd, 1956a, b; 1957; Zahnd, 1959). In female poeciliids the liver/body size ratio was larger than that of males, but it decreased during the development of the egg yolk, and fat reserves were depleted (Clavert & Zahnd, 1956a). In inactive females and in males, fat reserves were large. Female hormones given to such fish of either sex induced liver changes parallel to those seen in females during vitellogenesis (Clavert & Zahnd, 1957). Tavolga (1949) found that certain female sex hormones when fed, exerted an opposite effect on the livers of platyfish. Liver cells became vacuolated and fatty changes took place—apparently in both sexes. Egami (1955) reviewed the Japanese literature reporting sex and cyclic differences in the liver of fishes. He found in *Oryzias* that the liver is larger in females and has larger cells. Implantation of estrone pellets into sexually active males led to liver changes in the female direction, but in inactive males there was no effect. Implantation of testosterone pellets or ovariectomy of females caused liver changes in a male direction, but more slowly than the changes in treated males. In sticklebacks (*Gasterosteus aculeatus*) and minnows (*Phoxinus laevis*), glycogen storage was greater in females, but it was largely located in the ovaries (Immers, 1953). Its changes were not regarded as responsible for the fluctuations of liver weight occurring during the sexual cycles. Oguro (1956) found the liver of female sticklebacks to be larger than that of males; there was more fat in the male liver, its cells were larger, its cells contained multiple nucleoli, in contrast to single nucleoli in female liver cells, and the mitochondrial morphology differed in the two sexes. Estrogen injected into males caused liver changes in the female direction.

Although the vacuolation of the liver in the radiothyroidectomized platyfish here described could have been due alternatively to accumulation of water, glycogen or fat—all of which could have presented the same picture after the solvent treatments used in preparation of slides —the histological picture was not like any of the illustrations offered by the above authors. Sexual inactivity, although a cause of fat or glycogen deposition in the liver, did not lead, in the reports cited above, to histological appearances even remotely approaching the extreme degree of vacuolization seen in thyroidectomized platyfish. Moreover, normally maturing, incompletely thyroidectomized platyfish developed as severely vacuolated livers as immature, totally thyroidectomized specimens. Therefore it may be concluded that the role of sex hormones in this instance is of negligible importance, in contrast to that of thyroid hormone.

The vacuolated livers in the thyroidectomized and hypothyroid platyfish, if they do represent fatty livers, developed under conditions which accord with the results presented by Chaikoff *et al.*, and the absence of vacuolated liver in thyroid-fed thyroidectomized platyfish is in line with the findings of Leathem & Howell. These mammalian results, and others summarized above, suggested that the presence or absence of thyroid hormone might have a different action on liver physiology than treatment with antithyroid drugs. Only the cats of McClosky *et al.* exhibited fatty liver after chemical thyroidectomy. This experiment was, however, the only one in which either mammals or fish were given antithyroid drugs for more than 60 days. Chambers observed ceroid in the livers of all *Fundulus* that had been thiourea-treated for 6 weeks, and the present author found ceroid in the livers of platyfish thiourea-treated for 10 weeks. Furthermore, vacuolated livers with ceroid were found by the author in platyfish treated with thiourea for 18 weeks. If ceroid is a product related to neutral fat,[8] its over-accumulation might precede and herald the onset of fatty liver. The development of fatty liver might require more prolonged antithyroid treatment than most of these experiments encompassed. The data reported here on thyroidectomized platyfish, as well as on thiourea-treated platyfish, show that vacuolated liver developed gradually over a period of many months (with one exception, vacuolated liver was not found in radiothyroidectomized fish examined prior to 60 days post-treatment). Extensive ceroid deposits found in several specimens were tabulated as equivalent to vacuolated liver.

The observations of Sellers & Wen You may reconcile the above-cited association between fatty liver and hyperthyroidism in man with the experimental results on other mammals if, in the human cases, the excess fat were present extracellularly. In the thyroidectomized and thiourea-treated platyfish the vacuolation was certainly intracellular, so that no conflict appears here. The failure of Goldberg *et al.* to find any pathological changes in long-term-thyroidless rats alone appears unreconcilable with other observations. Only genetic differences come readily to mind in explanation of this inconsistency.

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[8] Ceroid is a clear yellow pigmented material which first was described in experimentally-produced liver cirrhosis of rats (Lillie *et al.*, 1941; Edwards & White, 1941). Its histological appearance and histochemical characteristics were described by Endicott & Lillie (1944); among these are insolubility in alcohol and ordinary fat solvents, and non-stainability by haematoxylin-eosin or Masson's trichrome (used exclusively in the experiments reported here). The identification of the material seen in the livers of the author's radioiodine-treated fish was first suggested by Chambers' (1953) description of ceroid in the livers of *Fundulus* treated with thiourea. Endicott & Lillie suggested that ceroid might either be a derivative of neutral fat or a separate product of abnormal liver metabolism. Ham (1952) described ceroid as an oxidation product of unsaturated fats.

It seems clear, at any rate, that in platyfish the thyroid hormone is deeply involved in liver metabolism, and that lack of it leads to excessive accumulation of ceroid, and probably of neutral fat.[9]

4. *Thyroid Function and Pituitary Development.* —Volumetric studies on the pituitaries of thyroidectomized and normal platyfish suggested that the glandular portion of the hypophysis was sharply reduced in thyroid-fed, athyroid fish, whereas the neural portion developed normally. In solely radiothyroidectomized fish, the entire pituitary appeared to be subnormally developed relative to the size of the animal. This was somewhat surprising, as lack of thyroid hormone might be expected to lead to increased thyrotrophic function on the part of the pituitary, and possibly thereby to an increase in over-all pituitary size. Control platyfish with hypertrophied thyroids were often found to have large ventral basophilic overgrowths of their pituitaries. Enlarged glandular portions of the pituitary also were found in goitrous teleosts by Stolk (1956a,b). Increases in cell number or size among pituitary basophils have been described in antithyroid drug-treated teleosts by Leloup & Olivereau (1950), Scott (1953) and Sokol (1957), and an increase in the number of eosinophils by Vivien & Gaiser (1952). Without specifying cell type, Honma & Murakawa (1955) stated that cells of the transitional lobe of thiourea-treated salmon larvae were enlarged. However, none of the latter authors mentioned a gross increase in the size of the whole pituitary. Atz (1953) and Barrington & Matty (1955) described degranulation of thyrotrophs in the pituitaries of antithyroid drug-treated fish, but did not mention increase in cell number or cell size.

The excessively small pituitaries of the radiothyroidectomized platyfish may have been the result of a general exhaustion of the several pituitary cell types in a futile effort to respond to a variety of physiological deficiencies triggered by long-term athyroidism. Severinghaus (1942), as quoted in a review by Maqsood (1952, page 304), stated that early thyroidectomy in mammals may inactivate the pituitary to the extent that a virtual hypophysectomy is produced. Maqsood cited the findings of several other workers that support this statement. The small size of the glandular portions of the pituitaries of thyroid-fed, athyroid fish, on the other hand, was probably due to inactivity, at least on the part of thyrotrophs, for their function was no longer demanded for normal physiological function of the whole animal, owing to the illusory production of an excess of product by the target organ.

5. *Thyroid Function and Behavior.* An increase in sustained activity after thyroxine treatment and a decrease in activity following thiourea treatment were observed in goldfish and salmonoids by Hoar *et al.* (1952) and Hoar *et al.* (1955). The studies of Woodhead (1959) have shown the importance of thyroid gland activity in the long migrations made by sexually immature, as well as mature, cod (*Gadus callarias*). Many years ago, Marine & Lenhart (1910) noted the sluggish behavior of hypothyroid (goitrous) trout, and they also noted that such trout died more readily than normal fish when taken from the water. Other examples of sluggish behavior after antithyroid drug treatment may be found in Table 12. Although the behavioral observations made on radiothyroidectomized platyfish in this paper were casual, it seems appropriate to note that the activity pattern was in accordance with detailed studies such as those cited, and that greater fragility, as noted by Marine & Lenhart, was found.

6. *Radiation Effects.*—Swelling and hyalinization of blood vessel walls in the thyroid area following moderate and high dosages of radioiodine was noted in adult male rats by Goldberg *et al.* (1950). Their description applies also to the effects seen on the aorta of radioiodine-treated platyfish. Similar in both rats and fish was the fibrous tissue replacement of thyroid tissue, sometimes in whorled form. The process of thyroid destruction in rats and fish followed parallel courses which were characterized by the swelling of the epithelial cells of the follicles, the loss of dense colloid, the disorganization of follicle structure, and the final disappearance of all thyroid tissue, leaving the stromal framework to be infiltrated by fibrous elements.

Most work on the effects of whole-body irradiation on fish has been done with either embryos or mature animals, rather than on young, growing fish equivalent in development to the platyfish studied here. Bonham *et al.* (1948) studied the effects of x-rays on fingerling Chinook salmon (*Onchorhynchus tschawytscha*). After 12 weeks, growth in length was affected only in fish that had received 1,000 r. or more. The cumulative death rate in fish receiving more

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[9] Increased deposition of iron-containing pigment in liver and spleen was among the effects produced in mice that were fed thiouracil on a long-term basis by Dalton *et al.* (1946). This was noted also by McClosky *et al.* (see above) in their thiouracil-fed cats. Although this feature was not systematically studied, it is the author's impression that excessive brown or black pigment deposits were present in the spleens, though not the livers, of thyroidectomized platyfish, and that such deposits also were more frequent in the kidneys, as compared with controls (see Pl. IX, Figs. 4, 5).

than 250 r. was significantly higher than in controls. In fish receiving 500 r. or more, the renal hematopoietic cells were reduced in the first two weeks, with recovery by the 4th or 5th week in survivors of 500-1,250 r. The gonads were not studied. Welander *et al.* (1948) found somewhat greater sensitivity in embryonic and larval Chinook salmon than in the older fish. The lowest dose affecting growth in length was 500 r., and the lowest dose affecting hematopoietic tissue was 250 r. At 500 r., neither mortality nor weight was significantly different from the controls. Pigmentary development was affected only in fish receiving 1,000 r. or more. This also was true of other indices of development, such as eye size and gill growth. Recovery of hematopoietic tissue occurred in fish receiving 1,000 r. or less. Little or no damage to renal tubules and glomeruli was found in the latter groups. Splenic damage paralleled that of the renal hematopoietic tissue in dose effect and recovery. The gonads were retarded by all doses (250 r. and up), but nevertheless, in those receiving 250-1,000 r., they were progressing to definitive sex cell stages, 93 days after irradiation. Other organs examined were not affected by doses of 1,000 r. or less.

In mammals, including man, the hematopoietic tissue is especially sensitive to radiation (Tullis, 1959). At LD_{50} or higher doses (gamma radiation from atomic explosions), hematopoietic tissue was destroyed very rapidly in the lymph nodes and bone marrow, less rapidly in other lymphoid tissue. However, Tullis noted that the reticular (stem) cells are among the most radioresistant in the body; recovery of hematopoietic tissues began within about 4 weeks in survivors. The liver and kidneys were not found to be importantly affected. (Tullis mentioned that "Reversible accumulations of sudanophil fat in the liver have been noted in many animal species," but gave no specific references on this point). Ovaries were noted to be more radioresistant than testes; primordial ova are referred to as being among the most radioresistant of cells. Interstitial cells of the testis also were not noticeably affected.

Metcalf *el al.* (1954) found that in rats given 550 r. whole-body x-radiation, lymphoid degeneration in lymph nodes, spleen and bone marrow occurred, but returned to normal in 17-40 days. Spermatogonia, after destruction, reappeared in 20-40 days. There was no injury to the liver or kidneys.

Lushbaugh (1957), in a review, states that the normal liver is resistant to radiation up to 3,400 r. in mouse, rat and man, and that 20,000 r. did not affect liver regeneration in rats. The pancreas is also radioresistant, requiring more than 2,500 r. for visible effects.

The effects of I^{131} treatment of young and adult platyfish were similar in some respects to those reviewed above, resulting from other forms of irradiation. In particular, the hematopoietic reduction found was characteristic of radiation damage in other animals. However, the persistence of the condition, sometimes acutely, in survivors for 4-8 months after irradiation apparently is anomalous. Moreover, hematopoietic tissue in platyfish similarly treated with P^{32} in equivalent r. dosage (720 r.) was normal 3-4 months after exposure. These observations indicate that athyroidism or extreme hypothyroidism may prevent normal repair of hematopoietic damage resulting from radiation. This is borne out by the improved condition of this tissue in the thyroid-fed, radiothyroidectomized platyfish. The effect of the thyroid here, as in other instances, may not be tissue specific, but merely a reflection of a general positive effect on cell growth.

Although fatty liver apparently may occur transitorily in various species after radiation, this does not seem to be a commonly noted effect. When the P^{32}-treated platyfish, whose livers were quite normal, are considered in relation to the I^{131}-treated fish, the evidence favors the interpretation that the high incidence of severe liver vacuolation found was related entirely to thyroid insufficiency and was independent of radiation. Again, the livers of the thyroid-fed, thyroidless fish were completely normal, which further substantiates this assumption.

Growth retardation in young salmon, although a definite effect of radiation, was not found to be significant at 720 r., the dosage level received by young platyfish treated with I^{131} or P^{32}. There was no effect on the body length attained by P^{32}-treated platyfish, nor were their body proportions noticeably different from those of the controls. Moreover, the thyroid-fed athyroid platyfish were normal in body length and shape; in fact, a slight growth enhancement appeared in these fish, in the form of elongation of the caudal fin. The retardation of growth found in the solely radiothyroidectomized fish therefore may be attributed to the lack of thyroid hormone.

No sign of increased pigmentation, similar to the roentgen - pigmentation of goldfish (Ellinger, 1940), was seen in P^{32}-treated platyfish, nor were any pigmentary differences from controls noted in thyroid-fed I^{131}-treated fish. A generalized darkening was noted in many of the exclusively I^{131}-treated platyfish, but this was of the normal speckled pattern found in all fish of the strain employed and was not especially prominent on the head. This probably was a minor effect of the lack of thyroid hormone (see above).

The effects of treatment with I^{131} or P^{32} on the gonads of young platyfish were dissimilar, although both were adverse. Among solely radioiodine-treated fish, only one developed a gonad of mature appearance. In this male specimen, however, the gonopodium failed to differentiate. A single similar male occurred among I^{131}-treated, KI-treated fish. Apart from these exceptions, the failure of germinal and duct elements alike to develop beyond juvenile stages in thyroidectomized fish of both sexes stands in sharp contrast to the normal sexual differentiation seen in most thyroid-fed, athyroid fish. From these observations, together with those made on P^{32}-treated platyfish (discussed fully below), it may be concluded that the thyroid plays a powerful role in normal sexual maturation and that radiation effects were negligible.

The complete destruction of the germinal cells in the testes of P^{32}-treated males, together with normal gonadal duct development, confirms the observations of Vivien (1953a, b) on P^{32} treatment of immature and adult male guppies (*Lebistes reticulatus*) and swordtails (*Xiphophorus helleri*). That normal male secondary sexual differentiation was found also agrees with Vivien's results, and there seems no question that the sex cells themselves have no endocrine role in the development of these male poeciliids. With respect to females, P^{32} treatment of young platyfish had no readily discernible effect on ovarian development. Fertility was not tested, since the only males made available to these females were their sterile brothers just described. The ineffectiveness of P^{32} treatment of female platyfish is in disagreement with Vivien (1953b), who found an equal amount of germ cell destruction in both sexes. This difference is particularly surprising when doses are compared. The platyfish were treated with 1.7 mc. P^{32} for 48 hours, to give a total dose of 720 er. Vivien treated his fish, maximally, with 200 μc/liter of P^{32}, which was left to decay throughout the treatment of 130 days, fish removed to clean water after 8 days being no less affected. Using formulas given by Glasser *et al.* (1952), we calculate Vivien's procedure to give a total dose of 175 er. (or less, if all the P^{32} had not decayed in 130 days). This is much smaller than the dose given to the platyfish, yet it had a much more profound effect in the females. Two possible, alternative explanations might be, the chronicity of Vivien's treatment, or species differences in the fish. The latter is not likely, since Vivien used fish of two different genera with similar results, and one of his species is congeneric with the platyfish. The first alternative also seems unlikely in view of certain observations made on mammals, which are briefly summarized below.

Long-term gamma irradiation of mice at various dose rates led to observations showing certain similarities, but also profound differences from both Vivien's results and our own. Deringer *et al.* (1954) found that male mice given 1,100 r. at rates of 4.4 r. per 8-or-24-hour day bred normally, while those sterilized by higher total doses (1,760 r.) at the rate of 8.8 r. per 24-hour day recovered their fertility. The fertility of female mice given less total irradiation than males (770 or 880 r.) at the equal rates of 4.4 or 8.8 r. per 24-hour day, or 8.8 r. per 8-hour day, was reduced and some became totally sterile. Unlike males, females sterilized by higher total doses did not recover their fertility. Thus, the reproductive capacity in female mice was reduced or destroyed by doses of radiation which either did not affect male mice, or from which males recovered. Eschenbrenner & Miller (1954) published similar findings on mice and emphasized that inhibition of spermatogenesis was correlated with dose rate, not total dose. They found that interstitial tissue was unaffected up to 5,000 r. total dose. In guinea pigs, the male germ cells were similar in radiosensitivity to those of mice, but the ovaries were highly radioresistant. The latter was also true of rabbits.

The observations on the radioresistance of mammalian testicular interstitial cells agree with all data on P^{32}-treated fish, and also with Vivien's (1952) earlier observations on the effects of x-rays on the testes of swordtails. However, the results with mice are dissimilar to those of the author for platyfish. In the mouse, at a given dose rate, the ovaries of the females were more radiosensitive than the testes of males, but in the platyfish the females were more resistant to radiation damage. Platyfish thus seem to be more similar to the guinea pig or rabbit. In Vivien's experiments, the initial dose rate would have been 8.4 r. per 24-hour day, which was a rate that sterilized female mice, but was a very much lower rate than that given the platyfish (360 r. per 24-hour day). Yet the ovaries of the platyfish suffered no visible degeneration. Thus, the longer term of exposure of Vivien's fish to P^{32} irradiation, although the most likely factor in causing the discrepancy in results on female fish, might be ruled out on the basis of dose-rate considerations.

The observations of Samakhvalova (1935)

and Solberg (1938) on the guppy and the medaka (*Oryzias latipes*), respectively, revealed a high degree of radioresistance in the gonads of both sexes. Doses of 1,500 r. to male guppies and 1,980 r. to medakas of both sexes, in the form of x-rays, caused degenerative changes at first, but recovery followed in 1-4 months, coupled with the return of fertility in all.

Observations such as those reviewed indicate that the effect of P^{32} may not be qualitatively the same as that of radiation in other forms, as far as the gonads are concerned. Vivien (1953b, c) advanced the hypothesis that the action of P^{32} on the gonads commences with the diencephalon, where it interferes with neurohypophyseal control, and that it is not reversible owing to neural destruction. A possible objection to this theory might be the radioresistance of nervous tissue (Tullis, 1959).

SUMMARY

1. Total radiothyroidectomy of platyfish, *Xiphophorus maculatus*, 0.5 to 2.0 months old, by means of immersion in solutions of 5.0 mc./200 ml. of I^{131} for 48 hours was successful. although the death rate after such treatment was high.

2. Gonadal development of completely radiothyroidectomized fish was severely retarded and growth in length also was significantly impeded. Vacuolated (=fatty?) liver appeared in both completely and partially thyroidectomized fish and it increased in severity in a progressive manner as time after treatment lengthened. The hematopoietic tissue in the kidneys was reduced, and the blood vessels in the thyroid area showed radiation damage. Pituitary exhaustion appeared to occur.

3. Feeding of these athyroid fish with mammalian thyroid restored them to a normal condition with respect to death rate, growth, sexual development, liver histology and, in part, hematopoietic development. The radiation damage to blood vessels persisted. The time of maturation of males was slightly advanced, and the gonopodia and testes were normal. One female became gravid. These fish were even more normal than their controls in one respect, *viz.*, their pituitaries showed none of the hypertrophy seen in controls with incipient goiter. Elongation of the caudal fin was noted. No exophthalmia occurred.

4. Treatment of these athyroid fish with potassium iodine improved the mortality rate but did not improve growth or reduce the development of vacuolated liver. Gonadal differentiation was slightly less retarded, but no fish approached maturity at the time that the controls reproduced.

5. Fish treated with equivalent, non-thyroidectomizing, irradiation by means of P^{32} were completely normal in growth, secondary sexual development, body shape and visceral histology. Male gonads were completely lacking germ cells, but female gonads were unaffected. The death rate was as high as that shown by I^{131}-treated fish, but appeared to be differently distributed in time from the I^{131} death rate.

6. The injection of adult platyfish with I^{131} led to severe hypothyroidism, but whether it had any effect on the gonads was doubtful. Vacuolated or ceroid-filled livers and enlarged pituitaries were commonly produced.

7. It is concluded that the thyroid plays a significant role in growth, sexual maturation and liver metabolism of the teleost, *Xiphophorus maculatus*. The thyroid also promotes the recovery of hematopoietic tissue after irradiation, and strengthens resistance to shock and disease.

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EXPLANATION OF THE PLATES

PLATE I

FIG. 1. Radiothyroidectomized platyfish, 6 months old, at bottom left, together with normal broodmates—male above and female below, right. Note the hunched shape and drooping fins of the thyroidectomized fish. × ¾. Photo by Sam Dunton, New York Zoological Society.

FIG. 2. Fish harvested from an experiment in which radioiodine-treated fish were treated subsequently with potassium iodide, or were fed mammalian thyroid. All fish were broodmates and 8 were started in each group. **A**, Radioiodine-treated; **B**, Untreated controls; **C**, Radioiodine-treated, fed thyroid; **D**, Radioiodine-treated, KI-treated. Notice the normal size, longer tails and paler coloring of the thyroid-fed fish, in comparison with the untreated controls. Also note the greater survivorship among the KI-treated in comparison with the fish treated solely with radioiodine. × ⅔

FIG. 3. Fish harvested from an experiment in which radioiodine-treated fish subsequently were treated with KI. All fish were broodmates. The initial groups were: **A**, 5 radioiodine-treated; **B**, 4 radioiodine-treated; KI-treated; **C**, 5 untreated controls. The upper fish in group A is shown alive in Fig. 1. Notice the dumpy body shape in all of the radioiodine-treated fish. × 1

FIG. 4. Fish from three experiments in which young platyfish were treated with P^{32}. Untreated controls appear on the left and the corresponding broodmates treated with P^{32} on the right. The P^{32}-treated fish are normal in appearance, and on the average are as large as the controls. × ¾

PLATE II

FIG. 1. Stripping film I^{131} radioautograph, showing exposure of emulsion grains over food particles in the pharynx of a platyfish. × 100

FIG. 2. Thyroid follicle in the kidney of a radiothyroidectomized platyfish. This follicle is the only example of renal thyroid tissue found in a radioiodine-treated specimen. × 400

FIG. 3. Regenerated thyroid tissue above the bulbus arteriosus in a radioiodine-treated fish. This female specimen was 7 months old and had been treated with I^{131} when 37 days old. × 100

FIG. 4. Regenerated thyroid tissue above and around the ventral aorta in a radioiodine-treated fish 20 months old. This female had been exposed to I^{131} when 50 days old. In this fish, the regenerated thyroid tissue was almost goitrous in proportion, although the follicles retained their distinct identity from one another. × 100

FIG. 5. Fibrous adhesions developed between the aorta and pericardium in an I^{131}-treated fish. The thyroid area above the pericardium and between the gill chambers is filled with loose stromal tissue. × 100

FIG. 6. Aorta surrounded by thick gelatinous fibrous tissue in a young platyfish treated with radioiodine 24 days prior to fixation. × 200

PLATE III

FIG. 1. Kidney of a normal platyfish 10 months old. The dark masses of cells between the tubules are lymphoid tissue; lighter masses consist of nucleated erythrocytes. × 100

FIG. 2. Kidney of a radioiodine-treated broodmate of the normal fish in Fig. 1, at the same age. The kidney tubules are smaller primarily because the fish was much smaller. Chiefly to be noted is the extreme lack of lymphoid cells. × 100

FIGS. 3, 4. Examples of "concretions" (arrows) formed within kidney tubules of radioiodine-treated platyfish. Some concretion-like masses not within tubules may represent degenerate tubules. × 100

FIG. 5. Spleen of a normal 6-month-old platyfish. Pigment-laden macrophages are the source of the black spots scattered within the organ. × 100

FIG. 6. Small, deeply basophilic regenerating kidney tubules in an I^{131}-treated platyfish. × 400

FIG. 7. Extremely shrunken spleen (arrow), which lacks all lymphoid elements, of a 6.5-month-old radioiodine-treated fish. The spleen seems more excessively shrunken than it is because the whole animal was subnormal in size. × 100

PLATE IV

FIG. 1. Liver of a normal platyfish. Capillaries are well delineated by the nucleated erythrocytes within them. × 100

FIG. 2. Extremely vacuolated liver and abdominal tissue in a radiothyroidectomized platyfish. The liver is at the right, pancreatic and abdominal connective tissue to the left, and part of the spleen shows at the upper left. × 100

FIG. 3. Ceroid-filled liver in a radiothyroidectomized platyfish. A rounded mass of ceroid-filled macrophages appears next to the blood vessel at the right of center, and the liver cells themselves are packed with ceroid globules. × 100

FIG. 4. Vacuolated liver in a radiothyroidectomized fish, showing part of the gall bladder at the upper left, with gall ducts entering the liver. × 100.

FIG. 5. Pancreas of a normal platyfish. Two loops of intestine are visible at the upper left and lower right. Vacuoles (fat cells?) and large blood vessels appear among the pancreatic masses. × 100

FIG. 6. Pancreas of a radiothyroidectomized platyfish. The pancreatic tissue is compressed by the great amount of abdominal vacuolation. × 100

PLATE V

FIG. 1. Ovarian development in young radioiodine-treated fish. Left: normal ovary of a 4-month-old fish. Right: ovary of an I^{131}-treated broodmate. These sections through the largest diameter of the ovary show the many fewer ova present in the I^{131}-treated fish. × 67

FIG. 2. Testicular development in young radioiodine-treated fish. Left: normal testes of a 4-month-old fish. Many germinal cysts are present. Right: testes of a radioiodine-treated broodmate. No germinal cysts yet have developed. × 286

FIG. 3. Testes of a normal adult male platyfish, 5.5 months old. Many spermatophores are fully formed and grouped in the ducts (bottom right). × 100.

FIG. 4. Testes of a radiothyroidectomized fish, 6 months old. No gonial cysts have developed in this specimen. × 286

FIG. 5. Ovary of a normal female platyfish, 8 months old. Ova at all stages in development may be seen. × 67

FIG. 6. Single ovum found in a radiothyroidectomized fish, 6.5 months old. No yolk deposition has occurred. A portion of intestine lies to the left of this poorly developed ovary. × 100

PLATE VI

FIG. 1. Ovary of a radiothyroidectomized fish, 8 months old. It contained only the three oocytes shown. These ova are in the reticulated stage that normally precedes the appearance of yolk granules. The oviduct appears at the left. × 67

FIG. 2. Testes of a radiothyroidectomized fish, 6.3 months old. In the left lobe of this testis a few germinal cysts had developed and reached early meiotic stages, but the right lobe showed none. × 100

FIG. 3. Nearly mature testis in a radioiodine-treated male fish, 6 months old. This exceptional specimen had no positively identifiable thyroid tissue, and the anal fin was elongated, but totally undifferentiated (see Pl. VII, Fig. 4). × 100

FIG. 4. Mature testis and heavily vacuolated liver in a second radioiodine-treated male fish, 7 months old. This fish had been additionally treated with KI. Its anal fin was totally undifferentiated (See Pl. VII, Fig. 4), and thyroid tissue could not be positively identified. × 100

FIG. 5. Cross-section through the anterior pituitary of a normal platyfish. Compare with Figs. 6 and 7, below, and with Pl. IX, Fig. 7. × 100

FIG. 6. Cross-section through the anterior pituitary of a radioiodine-injected adult platyfish, 15 months old and 6.5 months post-injection. This fish was almost completely thyroidectomized. The large growth of paler basophiles create a striking difference from the normal pituitary, as seen in Fig. 5. × 100

FIG. 7. Cross-section through the anterior pituitary of an untreated adult platyfish, 24-29 months old. The prominent overgrowth of paler basophiles in this case was accompanied by a quiescent and possibly regressive thyroid. The latter may have been the result of the relatively old age of the fish. × 100

PLATE VII

FIG. 1. Gonopodium of a normal adult male platyfish. Compare with Text-fig. 4, and with Figs. 2-4, below. × 25

FIG. 2. Gonopodium of a radiothyroidectomized fish which also was thyroid-fed. Even though the preservation is poorer than that of the fin in Fig. 1, due to fixation of the fish in Bouin's fluid, it may be seen that all of the terminal hooks and spines are present and normally formed. × 25

FIG. 3. Gonopodium of a P^{32}-treated fish. Normal differentiation of all elements is present. × 25

Fig. 4. Elongated but undifferentiated anal fin of a radioiodine-treated male fish, 10 months old. This fish had no demonstrable thyroid and had infantile testes with no germinal cysts (see Fig. 7, below). × 25

Fig. 5. Testicular ducts of a P^{32}-treated male platyfish, 5.5 months old. No germ cells are present, but the duct system is normally complex. The gonopodium of this fish is shown in Fig. 3, above. × 100

Fig. 6. Testes of a 22-day-old platyfish, born to a female injected with radioiodine 19 days before the birth (arrows). This young fish appeared to have a normal thyroid and no other abnormalities. The testes of this fish are almost as large as those of some radiothyroidectomized fish 6 or more months old. Pancreatic tissue appears to the right of the testes, which are centrally located and suspended from the peritoneum. At this age, the two testes are entirely separated; later, in the course of normal development, they become fused. × 100

Fig. 7. Testes of a radiothyroidectomized fish, 10 months old. No gonial cysts were present. These testes are but little larger than those of the 22-day-old male in Fig. 6. The elongated, but undifferentiated, anal fin of this fish appears in Fig. 4, above. × 100

Plate VIII

Fig. 1. Ovary of a normal pregnant female platyfish. Young oocytes appear at the right, the oviduct at the top, and the neural tube of an early embryo at the arrow. × 100

Fig. 2. Ovary of a radiothyroidectomized, thyroid-fed female. All stages in egg development may be seen in this normally matured ovary. × 100

Fig. 3. Embryo contained in the ovary of a radiothyroidectomized, thyroid-fed platyfish. In this sagittal section, organs such as the eye, brain, kidney, gills and liver may be recognized. × 100

Fig. 4. Section through the pharyngeal area of another embryo contained in the same fish. The arrow points to thyroid follicles. The heart appears at the lower right, and part of the brain at the extreme upper left. × 400

Fig. 5. Ovary of a platyfish treated when young with P^{32}. Oocytes at various stages in development are visible — from the small, darkly basophilic one at the upper left, to the fully yolked egg at the top center. × 100

Fig. 6. Ovary, containing only atretic follicles, of a 17-20 month old platyfish, injected with I^{131} 8.5 months earlier. The oviduct is the darkly stained structure in the central area. ×100.

Fig. 7. Ovary of another 17-20 month old platyfish, injected with I^{131} 8.5 months earlier. In this fish, primary gonial cells appear to be proliferating from the lining of the ovarian cavity, in a manner suggestive of early spermotogenesis (see text). The feminine germinal elements of this ovary appeared to be represented only by atretic follicles. ×100

Fig. 8. Higher power view of the cells proliferating from the ovarian lining in the above specimen. The cells are grouped in balls, as found in the primary gonial cysts of the young testis. ×400

Plate IX

Fig. 1. Pathological nodule found in the abdominal cavity of a male platyfish, 13 months old, which had been injected with I^{131} 3.5 months earlier. This mass appeared to be attached to the testis, of which a portion may be seen at the upper right. ×100

Fig. 2. Tremendous proliferation of epithelial-like cells in the dorsal pericardium and among the transverse pharyngeal musculature of a platyfish injected with I^{131} 41 days earlier. This female was 10 months old when killed. ×100

Figs. 3-7. Pathological effects seen in a female platyfish which appeared to represent the most extreme case of hypothyroidism encountered in this study. The fish was 28-29 months old and had been injected with I^{131} 19.5 months before the time of sacrifice.

Fig. 3. Kidney, showing shrunken condition, edematous subcapsular spaces and lack of lymphoid tissue. ×25

Fig. 4. Kidney, under higher power, showing almost complete absence of lymphoid tissue, shrunken tubules and cysticity. Pigment deposits and whorled "nests" are also in evidence. × 100.

Fig. 5. Spleen, very shrunken, owing to reduction of lymphoid elements, and with large pigment deposit in the center. × 100

Fig. 6. Liver, showing vacuolation, fibrous cysts and "nests." The large, rounded mass at the upper left is a large fibrous cyst within the liver. These changes are similar to those of cirrhosis. ×100

Fig. 7. Pituitary, with an overgrowth of paler basophilic elements of such magnitude that it might well be classed as a tumor. Compare with the normal pituitary shown in Pl. VI, Fig. 5, which is a section taken at the same level. ×100

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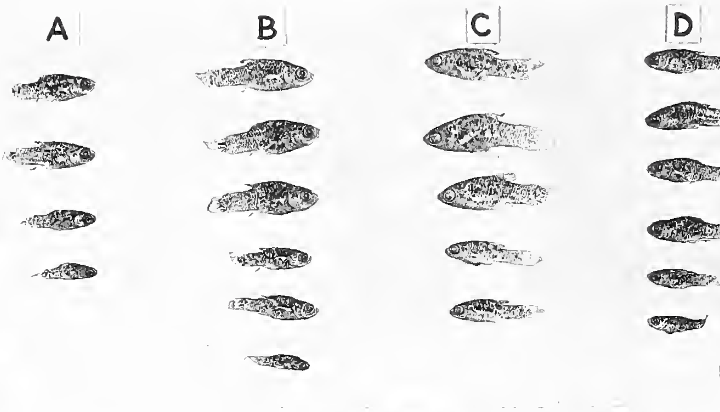

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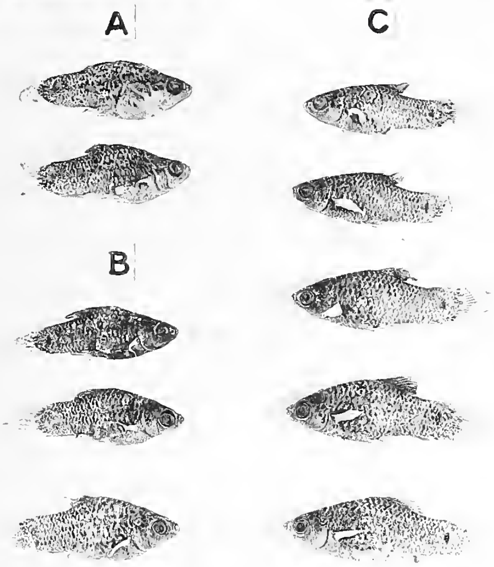

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THE ROLE OF THE THYROID IN THE DEVELOPMENT OF PLATYFISH


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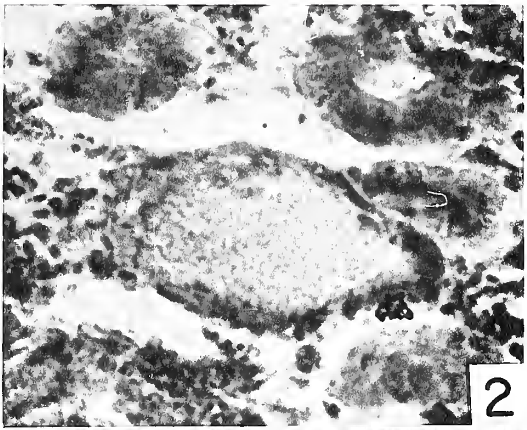
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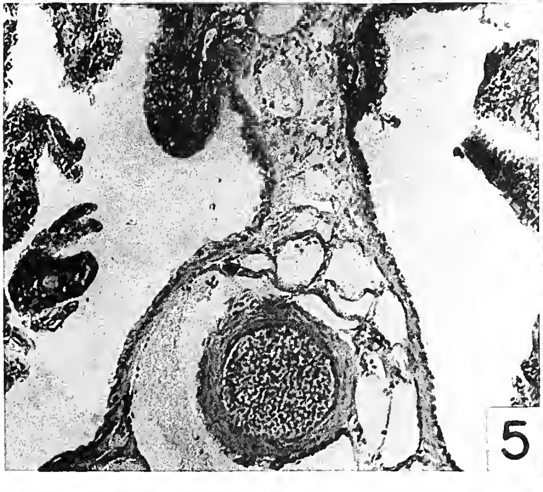
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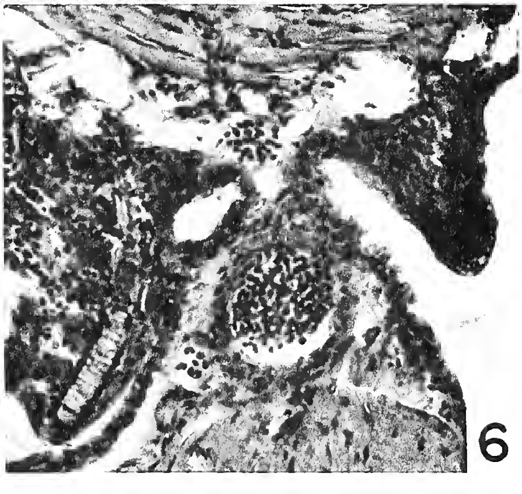
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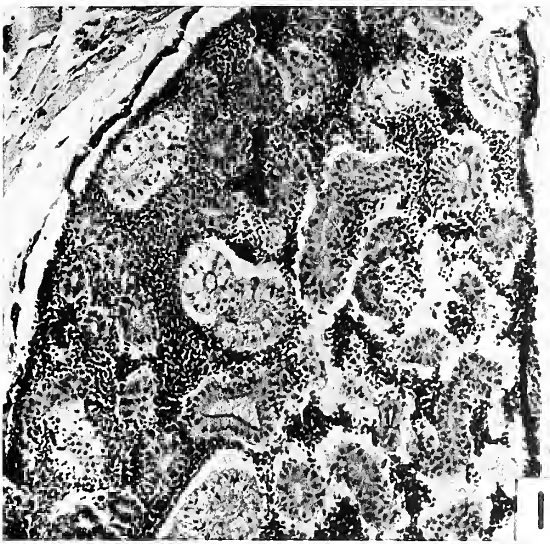
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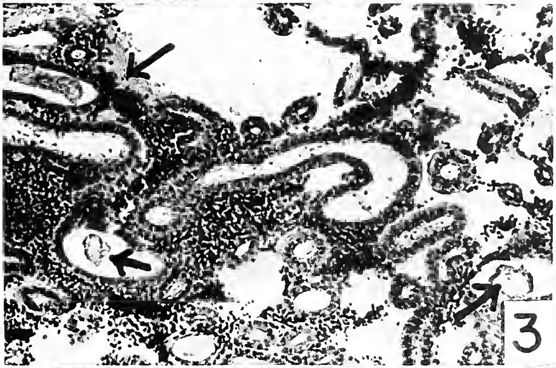
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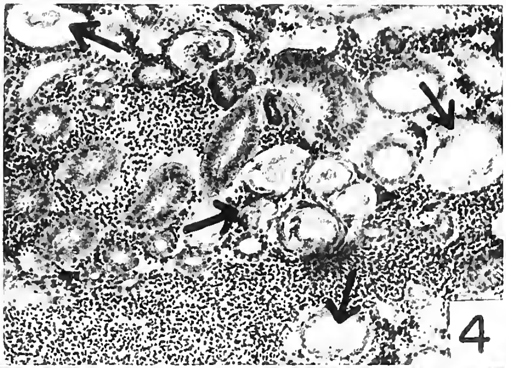
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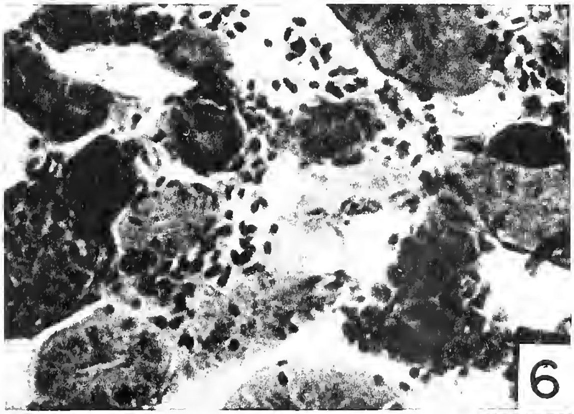
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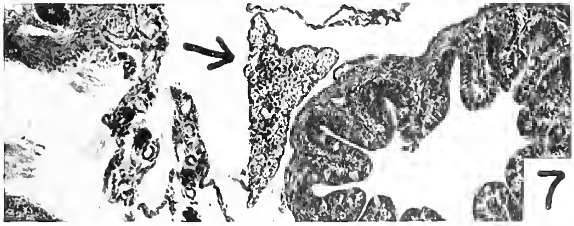
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THE ROLE OF THE THYROID IN THE DEVELOPMENT OF PLATYFISH


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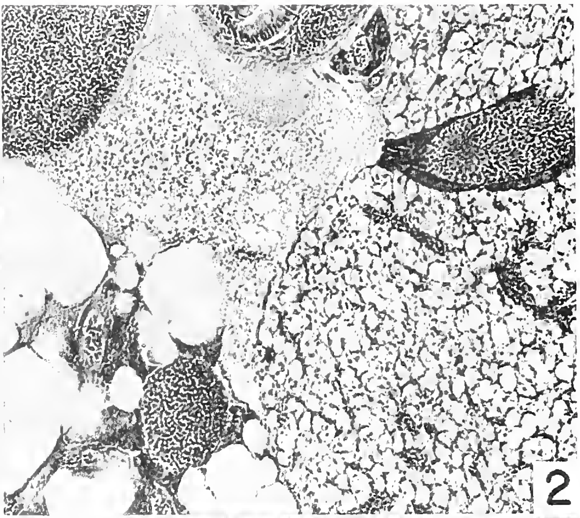
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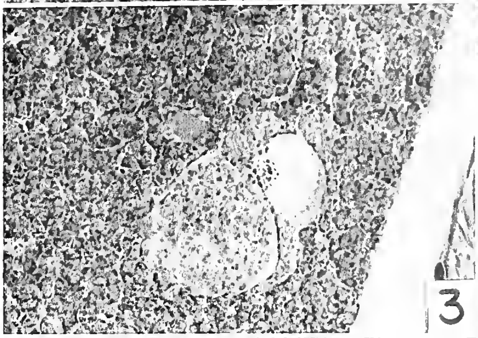
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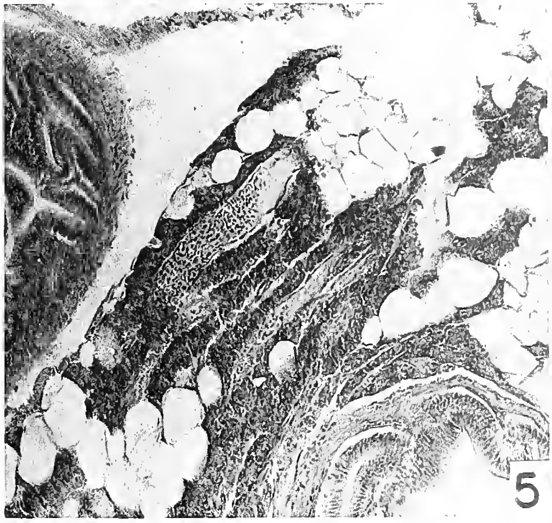
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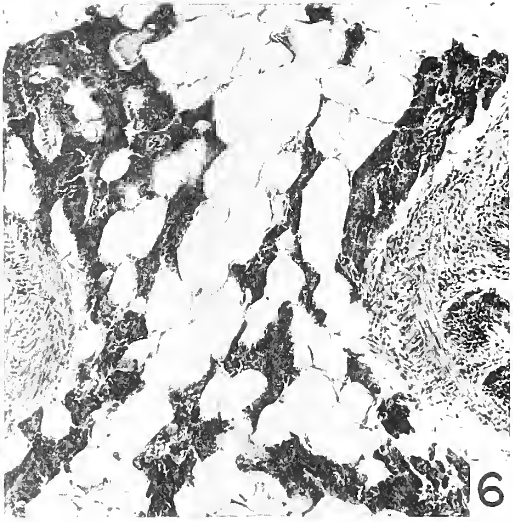
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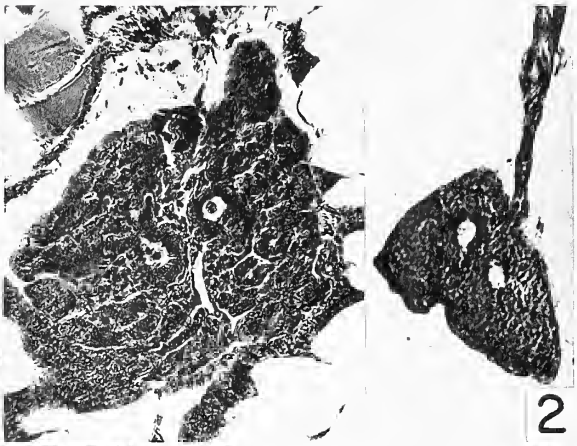

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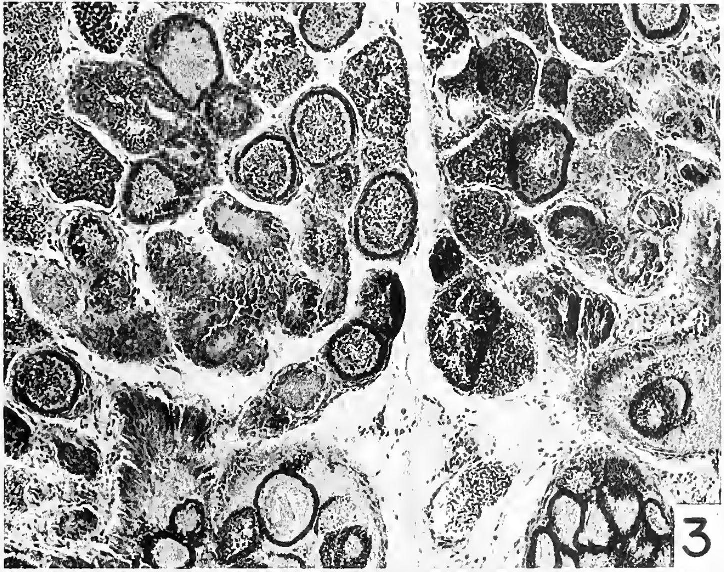

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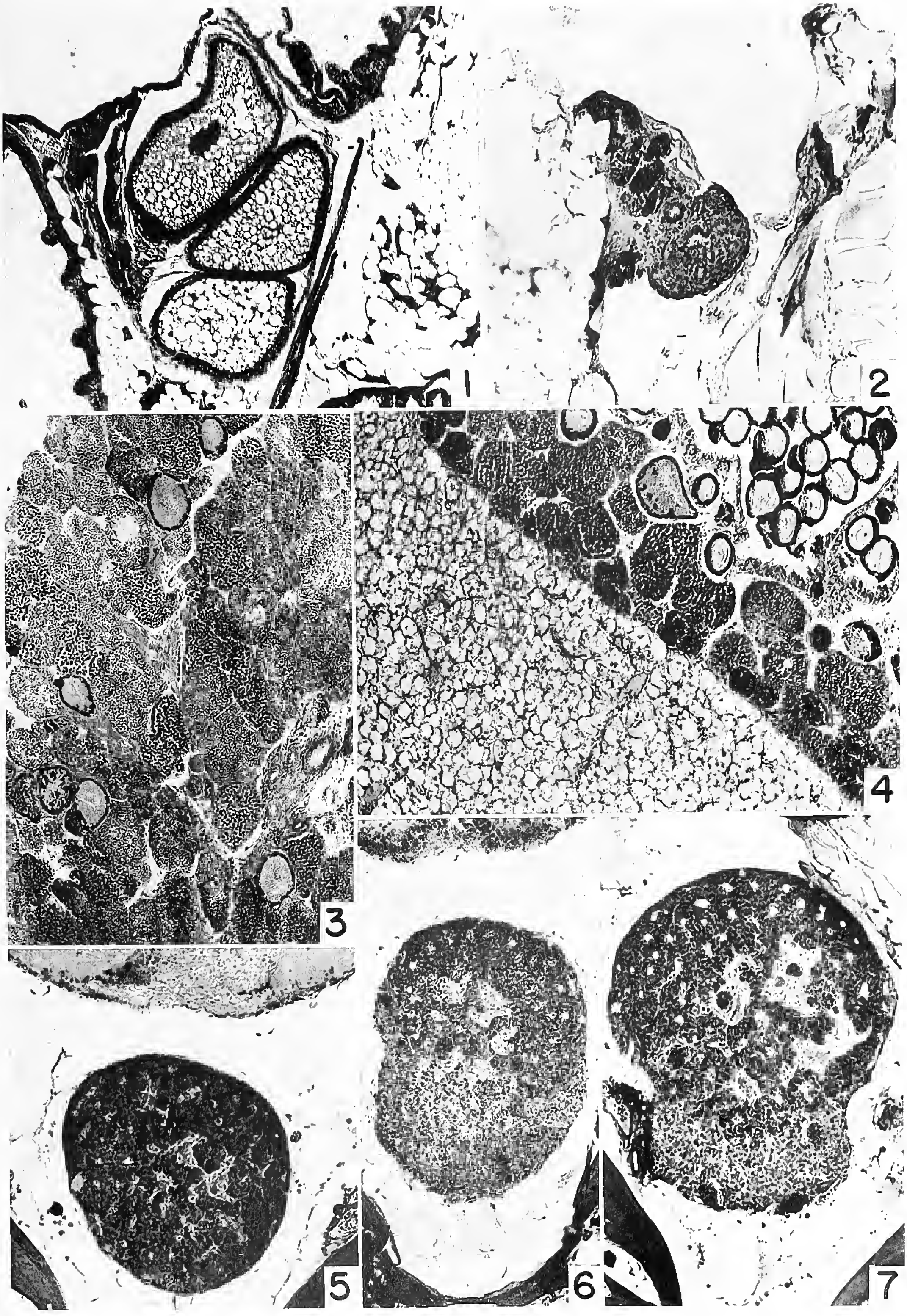
THE ROLE OF THE THYROID IN THE DEVELOPMENT OF PLATYFISH

PLATE VI



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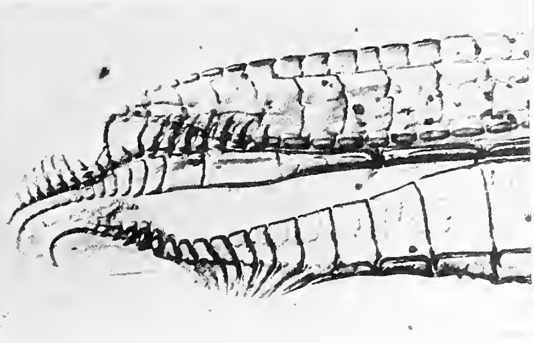

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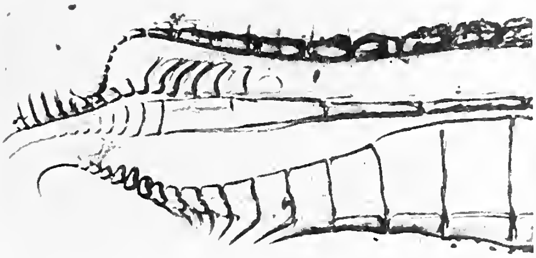

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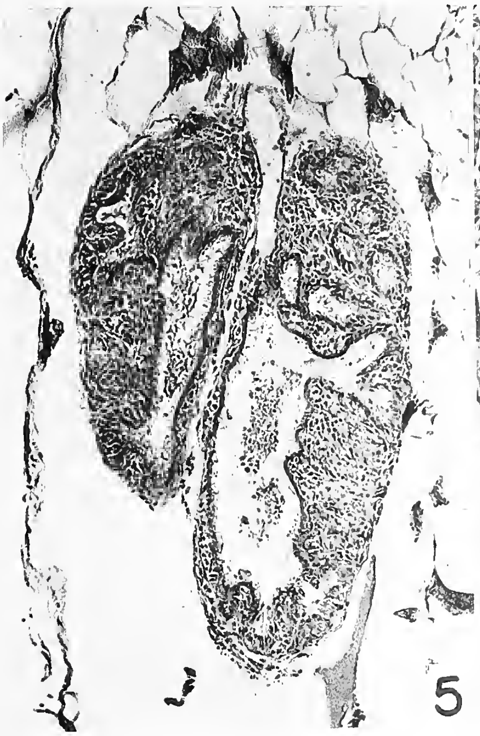

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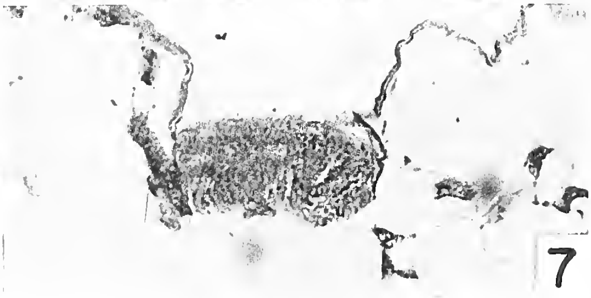

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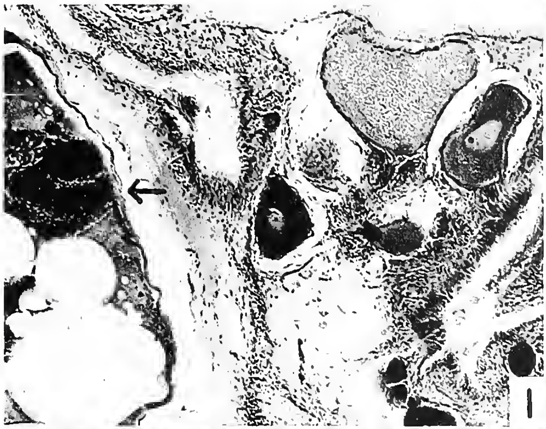
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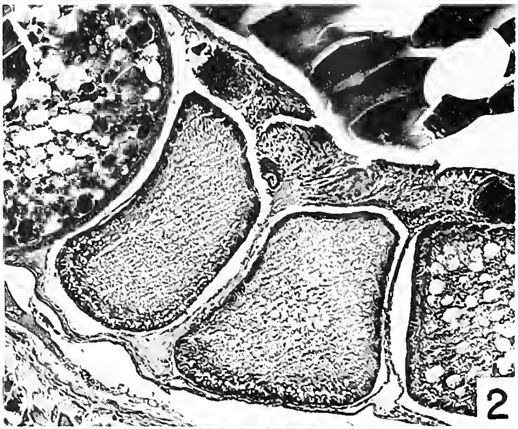
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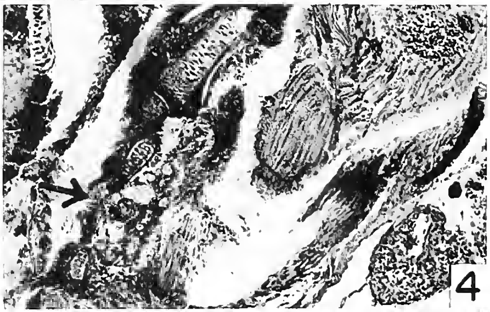
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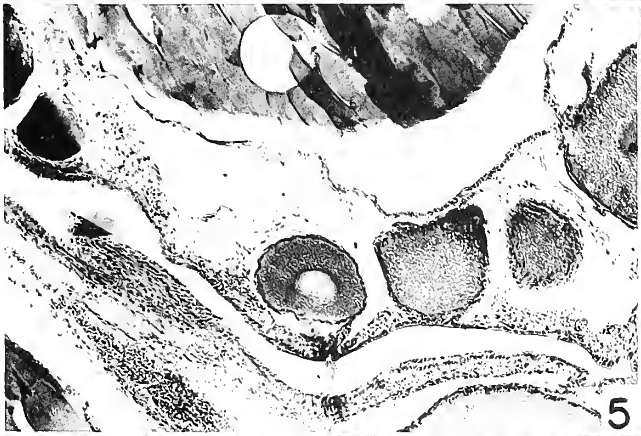
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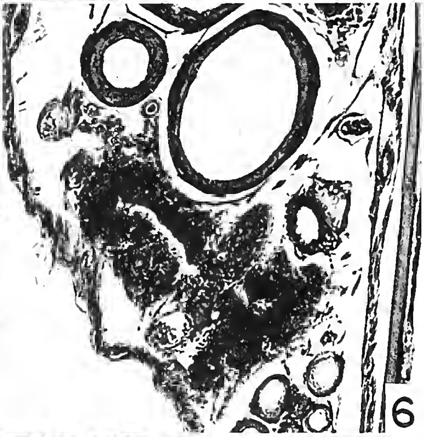
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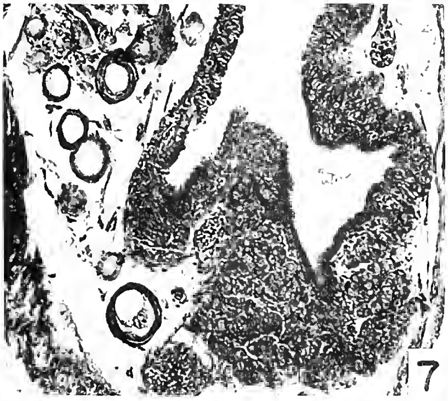
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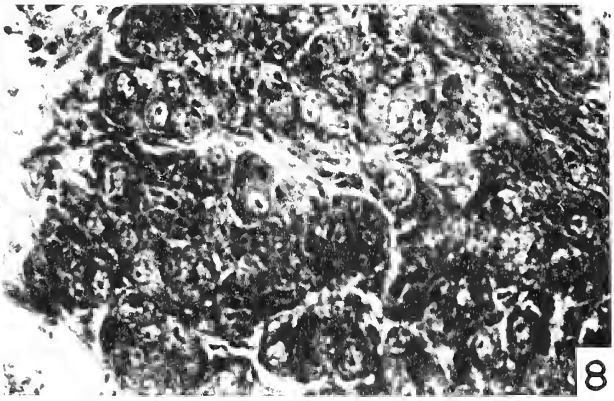
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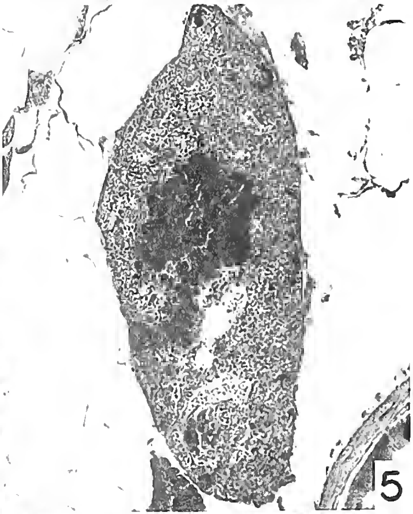
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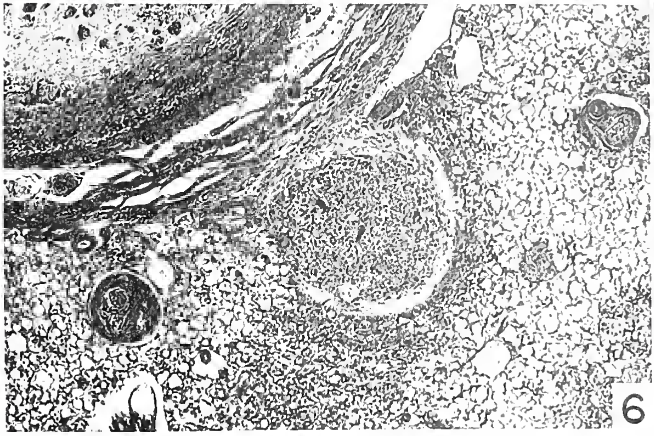
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THE ROLE OF THE THYROID IN THE DEVELOPMENT OF PLATYFISH

INDEX

Names in **bold face** indicate new genera, species or subspecies; numbers in **bold face** indicate illustrations; numbers in parentheses are the series numbers of papers containing the plates listed immediately following.

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