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A RADIATION-INDUCED VARIANT OF THE CESTODE, HYMENOLEPIS DIMINUTA, WITH TWO-HOOKED ONCOSPHERES*

Arthur W. Jones
and
James A. Dvorak
Department of Zoology and Entomology
The University of Tennessee

Cestodes, being at all times, even in the adult stage, embryonic, may be expected to give rise to anomalies. The literature of anomaly in cestodes is considerable (Dobrovolny and Dobrovolny, 1935; Wardle and McLeod, 1952), and there would be little point in adding another description of an anomalous worm to that literature unless some light would be shed thereby on the nature of anomalies and their possible importance. The present paper describes a cestode which by inheritance is a member of the common species, *Hymenolepis diminuta*, but by structure is unique, differing in one respect, its 2-hooked oncospheres, from all other members of the species. This unique cestode resulted from an experiment in radiation.

Materials and Methods

Beginning in 1959, infective larvae (cysticercoids) of the rat tapeworm, *Hymenolepis diminuta*, were given heavy doses of radiation (15,000 roentgens at about 500 r per minute, from a cobalt 60 source at the University of Tennessee-Atomic Energy Commission Agricultural Research Laboratories at Oak Ridge, Tennessee) and were then introduced by stomach tube into white rats. The eggs of the tapeworms which developed in the rats were fed to grain beetles (*Tribolium* spp.), and after 15 days the beetles, containing cestode larvae, were irradiated with 15,000 r; the larvae were removed and fed to rats just as before. Eventually eight generations of tapeworms were obtained, the last of which may be said to have received, through its ancestors, 105,000 r of gamma radiation, and, directly, 15,000 r. Eight successive generations had received 15,000 r each.

Adult worms of various generations were fixed in Carnoy's fluid (6 parts absolute alcohol, 3 parts chloroform, 1 part glacial acetic acid) for one hour, and stored in the refrigerator. Then the whole worms were hydrolyzed 12 minutes at 60°C in 1 Normal HCl, rinsed in cold 1 Normal HCl, and transferred to the Feulgen reagent (prepared according to the method given by Gray, 1954) for one hour. The worms were stored in distilled water until needed. Each worm was measured.

For cytological study a portion about 10 mm. long, from the appropriate regions of the strobila, was removed to 45% acetic acid, and small pieces were teased apart on a slide, under a dissecting microscope. Body wall, fragments of muscle, the cirrus pouch, seminal receptacle and ovary we removed, leaving testes, oviduct, and the developi uterus with oocytes in meiosis and embryos in ea cleavage. A cover-glass was applied and gen pressed down, under folded filter paper. Th clear finger-nail polish was applied to the edges the cover, sealing the slide. Such slides could kept without deterioration in the refrigerator se eral weeks for chromosome study and analysis.

A second portion of each worm, 3 to 5 pro lottids, was taken from the posterior region, an these gravid segments were teased in a drop glycerine jelly until they had discharged most their eggs. The fragments of proglottid were moved, and a cover-glass was applied, making nea permanent preparations of the oncospheres wi their membranes.

The remainder of each worm was cut into piec and the pieces straightened upon a slide, while long cover-glass (22 x 60 mm.) was applied a gently pressed down. Then the slide was flood with equal parts of glacial acetic acid and 95 ethanol, a solution which hardened the pieces their straightened, flattened condition. Dehydr tion and clearing were carried out while the wor was under cover-glass pressure; then the cover w removed, mounting resin was allowed to cover t specimen, and the cover-glass was replaced. A dr ing rack in the paraffin oven (58°C) provid rather rapid hardening of the preparations.

Study of the unique cestode with 2-hook oncospheres involved the following techniques. O immersion observation of the chromosome squash with ribbon-filament lamp adjusted to give critic illumination and immersion oil on both slide an condenser (N. A. 1.30) of an American Optical C research microscope, with camera lucida for dra ing, yielded records of chromosome number an morphology. The oncosphere preparation and th whole mounts were studied with a Wild microsco equipped with drawing tube. The entire wor was drawn at low (16 mm.) magnification, and th drawings were used for morphological analysis. Th embryos were drawn under the oil immersion o jective of the Wild microscope. A survey of th entire embryo preparation was carried out to d termine the relative numbers of 2-hooked and oth kinds of embryos in the sample.

The Chromosomes

Analysis of many oocytes and spermatocytes first meiotic metaphase confirmed a chromoson

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Description of Figures 1-10

Figures 1-7 drawn with the camera lucida at the magnification indicated.
Figures 8-10 drawn with the Wild drawing tube at the magnification indicated.

Fig. 1. Early anaphase, second cleavage, showing chromosomes beginning to split at the centromeres, and with two very small supernumerary chromosomes or fragments.

Fig. 2. Metaphase of a cleavage division, showing six larger and six smaller chromosomes.

Fig. 3. Anomalous metaphase of the second cleavage division, or possibly telophase of first cleavage. The chromosomes of the macromere are scattered, but condensed. There are six feulgen-positive spherules in the peripheral cytoplasm.

Fig. 4. A first cleavage division in which only nine chromosomes are present, and there are nine feulgen-positive masses scattered throughout the cell. This condition may actually be a late anaphase of first cleavage in which the chromosomes of the micromere remain scattered, while those of the macromere have already entered a telophase-interphase condition in nine separate masses.

Fig. 5. Metaphase I of meiosis in an oocyte, showing the spiral sperm nucleus and four chromosomal masses, one of which (at right) is an association of four partially homologous chromosomes.

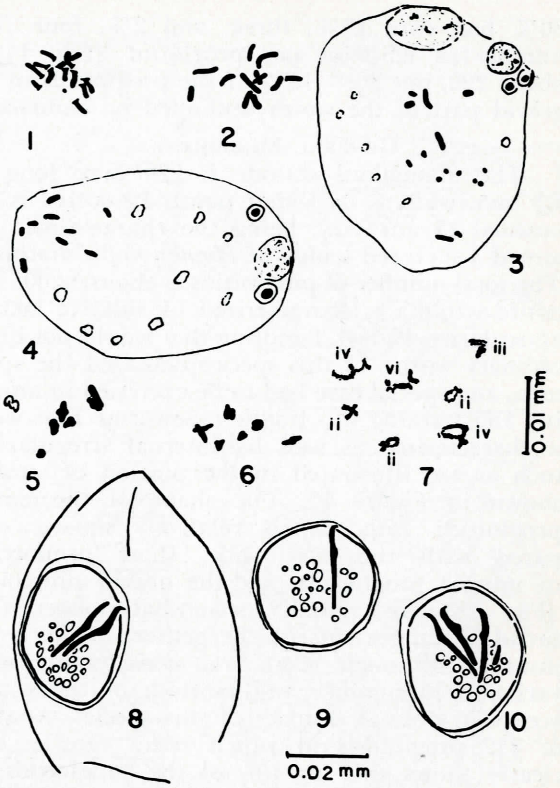
Fig. 6. Six tetrads at early diakinesis of the first meiotic division of an oocyte. The two sizes of chromosomes can be observed.

Fig. 7. Seven pachytene associations drawn from several spermatocytes. The Roman numerals indicate the number of chromosomes thought to be in synapsis in each group.

Fig. 8. Egg from glycerine jelly mount, with shell collapsed and wrinkled, thin embryonic membrane, group of nuclei at base of hooks, and two embryonic hooks typical in size and shape of 80% of the developed embryos.

Fig. 9. Embryo with one hook only. Its shell is not drawn.

Fig. 10. Embryo with three fully developed hooks and a small fragment of a fourth hook.



number of 6 haploid. The chromosomes of this worm exist in two main groups, three pairs being larger than the other three. Thus the morphology is distinctly different from that described by Kisner (1957), who found that normal specimens of *Hymenolepis diminuta* possess one pair of large chromosomes, one pair of small chromosomes, and four pairs of medium chromosomes. Two of the medium-sized chromosomes are somewhat larger and two somewhat smaller than the others in this group. The number, 6 haploid, is the same as that possessed by normal specimens of this species. Metaphases of early cleavage stages confirmed the chromosome number and morphology derived from meiosis. (The latter is more reliable than mitosis, in irradiated material, since it represents the karyotype of the somatic tissues of the worm, while the embryos are the result of fertilization, and their chromosomes have passed through two separate reductions, with opportunity for inversion-fragmentation, non-disjunction, and other meiotic irregularities to occur.) A feature of the cleavage stages studied was the presence of a number of Feulgen-positive masses, somewhat larger than chromosomes, and more diffuse than the latter in staining, scattered throughout the cytoplasm of the cells. Anaphase and telophase of early cleavage stages often seemed incomplete, with the chromosomes of one or both daughter nuclei scattered widely (Figs. 3, 4).

Meiosis in both oocytes and spermatocytes showed evidence that the chromosomes in this cestode have become altered by translocation. Thus in Figure 5 two of the six tetrads are fused into a multivalent association; apparently some portion of one or both of a pair of long chromosomes is homologous to some part of a pair of medium chromosomes, causing the synapsis between these homologous regions to hold together all four chromosomes involved. In spermatocytes, pachytene is often quite clear; Figure 7 shows a number of associations drawn from several spermatocytes, illustrating normal pairs (ii) as well as combinations of more than two (iii, iv, vi) chromosomes. Nearly every spermatocyte at pachytene showed evidence of structural change in the chromosomes.

The Embryos

Departures from the usual six-hooked condition are so rare as to have been seldom noted (Jones, 1962); yet six-hooked embryos could not be found in this worm, and 2 was the commonest number of hooks observed. In a sample of 342 embryos examined, 61 or 18% had no hooks whatsoever, 10 or 5% had one hook only (Fig. 9), 225 or 66% had 2 hooks only (Fig. 8), 32 or 9% had 3 hooks, and 6 or 2% had 4 hooks. Figure 10 shows an embryo with 3 hooks plus a rudimentary 4th. None had 5 or 6 hooks. If the embryos without hooks are not counted, as may be proper since these were in other ways deficient as well, often lacking membranes or cellular structure, then about 6% had one hook;

80% had two; 10%, three; and 2%, four. The number of embryos per proglottid (Fig. 14) is about 200, but 20 – 40% of the proglottids in the gravid part of the worm contained no embryos.

General Morphology

The anomalous cestode is 220 mm. long by 1.3 mm. wide at its widest point. Its scolex is not unusual in any way, being the characteristic unarmed 4-suckered scolex of *Hymenolepis diminuta.* The total number of proglottids is about 1,200. The entire strobila is characterized by relative lack of muscularity. In fact, handling that would not break ordinary worms of this species damaged the specimen, and special care had to be exercised in mounting and staining this fragile tapeworm. This worm is characterized as well by internal irregularities such as are illustrated in the portion of strobila shown in Figure 12. The shape of the mature proglottids (Fig. 13) is relatively square, compared with the evry wide, short proportions of normal proglottids, and the gravid proglottids (Fig. 14) are actually somewhat longer than broad. Arrangements of the genital organs is not unusual, although there was considerably more variation in number and position of testes than occurs in normal cestodes of this species. Analysis of 432 proglottids in which testes can be seen clearly shows that in 1% of the proglottids no testes occur; in 10%, 1 testis only is found; in 25%, 2 testes occur; in 55%, 3 testes (the number characteristic of the genus) occur; in 7%, 4 testes are found; while in 1%, 5 testes occur and in less than 1%, 6 testes can be found. Proglottid reversal (left to right), however, occurs only once in this specimen, although other observers of *H. diminuta* (Kisner, 1961; Job *et. al.,* 1962; Palais, 1933) have recorded this phenomenon as not uncommon. Out of about 600 proglottids observable for the following defect, there are 30 cases of fusion of two genital ducts to form one genital atrium, this frequency of 5% being higher than the frequency (about 0.3%) found by Job (1962) in a population of 25 unirradiated cestodes.

In general, the morphology of the specimen described above differs both qualitatively and quantitatively from that of normal *Hymenolepis diminuta.* As the discussion which follows will emphasize, the specimen, had it been recovered from a wild host, would create a problem for the taxonomist which the word "anomaly" could not solve.

Discussion

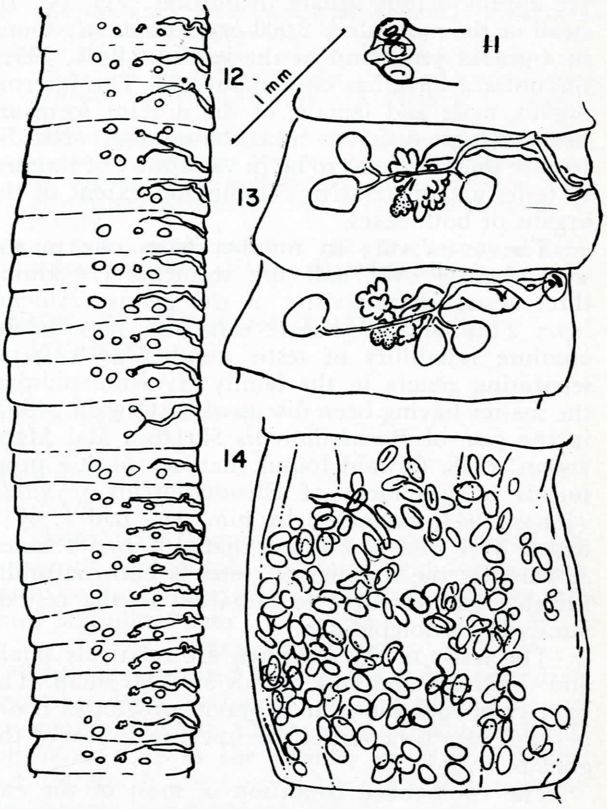
Anomalies in tapeworms have long interested the cestode taxonomists, because they are a possible factor in speciation. Many anomalies should be considered mere induced irregularities in growth. Such are the polyradiate cestodes described and reviewed by Dobrovolny and Dobrovolny (1935), and conditions such as Jones (1946) noted (a 3-suckered scolex) or Goodchild (1958) actually produced by mutilation and transfaunation of tapeworms. Most of the radiation-induced anomalies reported by Schiller (1959) as well as those analysed statisti ally by Palais (1933), Kisner (1961), Kuhlma (1961) and Job *et. al.* (1962) fall into the sam class of induced irregularities. Nothing is know of the genetic basis, if any, for such changes as th reversal of direction of segmentation, displacemen or absence of testes, fusion of genital ducts, or i complete separation of proglottids reported b many of the above workers.

Some anomalies, on the other hand, appear t have been significant in cestode speciation. Ba (1951) discusses the "double" forms of such we known genera as *Oochoristica* (with its doub form, *Pancerina), Andrya* (with *Diandrya) Parano locephala* (with *Cittoteania),* and *Dibothrioceph lus,* the "fish tapeworms" of man and other ma mals, with its double form *Diplogonoporus.* The are examples of tapeworms which resulted from th permanent or genetically determined occurrence an extra set of reproductive organs in each pro lottid. The anatomy of the double worms in a other respects resembles quite closely that of th single worm which, one assumes, represents th ancestral type. (The latter assumption is reaso able, of course, since the great majority of tap worms are single, not double, in their reproducti structure.) It is interesting to note that pol radiate strobilas have been frequently recorded i both *Dipylidium* (which is a double, or "biradiate form) and *Taenia* (which has no double form i nature).

It appears that while most anomalies may b chance occurrences, with no possibility of genet transmission, and hence of no significance as sourc of speciation, some anomalies (in particular th doubling of reproductive organs) have been th source of new genera. Therefore anomalies wi continue to be of interest, especially those whic affect the whole strobila of a tapeworm.

Tapeworm anomalies induced by ionizing r diation were discussed by Schiller (1957), wh suggested that radiation increases the frequency the variations that occur rarely in nature, and th therefore radiation might be useful as a tool t study the differences (among species, genera, etc in number and kinds of anomalies, these differenc having been increased by radiation to measurab frequencies. Schiller, of course, was making th reasonable assumption that most anomalies a non-genetic, and that their occurrence has tax nomic value only as a measure of variability i form or structure. Some anomalies induced by rad ation are conceivably genetic. When chromosom changes occur in combination with extensi morphological differences between the anomalo specimen and its parent stock, the likelihood genetic, hence taxonomic, significance should n be discounted.

The specimen of *Hymenolepis diminuta* d scribed in the present paper shows changes in bo chromosomes and general morphology that distin



DESCRIPTION OF FIGURES 11-14

All drawn with the Wild drawing tube, at the indicated magnification. The width of the portion shown in Fig. 13 is over 2 mm.; this is an exaggeration of the observed width due to flattening of the worm during mounting. The actual width of this region when fixed was 1.3 mm.

Fig. 11. Scolex, showing three of its four suckers and an unarmed rostellum.

Fig. 12. Twenty-one proglottids from the region of developing gonads, showing typical variation as well as two examples of bifurcated or fused female genital canals.

Fig. 13. Two proglottids from the region of sexual maturity, illustrating variation in position of testes as well as in position of genital atrium. The stippled object is the yolk gland, and the lobed structure, the ovary.

Fig. 14. Gravid proglottid with all of its eggs outlined. The remnant of the genital ducts (probably seminal receptacle) appears in upper left corner of the proglottid.

uish it qualitatively from the standard (parent) species, *H. diminuta*.

The latter has 12 chromosomes, occurring as one pair of large, 4 pairs of medium-sized, and 1 pair of small chromosomes (Kisner, 1957). The mutant form has the same number, 12, as the parent species, but its complement of chromosomes lacks the distinctly largest pair, there being only two recognizably different sizes. Moreover, the number 12 is augmented, at least in some of the embryos, by fragments or very small chromosomes.

Aberration in mitosis was observed. As shown in Figures 3 and 4, some cleavage anaphases or telophases seemed irregular, the chromosomes of one daughter nucleus alone being typically aggregated, and the chromosomes of the other nucleus being either scattered, as if the spindle had failed (Fig. 3) or presumably forming separate nuclear fragments, the DNA-containing bodies illustrated in Figure 4. The latter are not yolk masses such as Ogren (1953), Kisner (1961) and others have observed in the early cestode embryo. The above aberrations resemble those observed by Kisner (1961) in cestodes that had been subjected to moderate doses of gamma radiation a few hours before being fixed, and attributed by him to direct disruption of the spindle mechanism, a rather familiar immediate effect of several mitotic poisons such as colchicine. The mitotic aberrations reported by us, however, occurred many cell generations after the radiation had been administered, and must be considered at least partly genetic, rather than toxic, in origin.

In meiosis, the parent species exhibits synaptic regularity, with no evidence of translocated or inverted segments of chromosomes; 6 tetrads regularly form. The mutant specimen, however, rarely shows 6 tetrads in either primary spermatocyte or oocyte; there are usually less than 6 groups, with one or two of the associations involving more than two chromosomes.

The nature of some of the multivalent groups can be seen at pachytene in spermatogenesis (Fig. 7), where chromosomes of different lengths are partially paired, and where three, four and six chromosomes may be in synapsis with parts of each other. Thus it is apparent that the mutant specimen contains genes in different arrangements from that found in normal specimens. It is more than likely, although not demonstrable, that duplications and deletions of genetic material also occur.

The mutant tapeworm does not fit the taxonomically defined *Hymenolepis diminuta* (Rudolphi, 1819) in several respects. Although the scolex (Fig. 11) is typical, the strobila and proglottids are not. Instead of the approximately 2,000 proglottids characteristic of *H. diminuta,* our specimen has only 1,200, although in length it lies within the range, 200-400 mm., generally given for this species (Wardle and McLeod, 1952). It is noteworthy that in twenty-five worms of the standard strain of *H. diminuta* from which the present specimen was derived, Job (1962) found an average number of 1,830 proglottids per worm (range, 1,400-2,200), and an average length of 420 mm. (range 260-540); the parent stock consisted of relatively large worms. The specimen is much more slender (1.3 mm. instead of 3-4 mm.) than the typical form, appearing superficially more like the rodent anoplocephalid, *Oochorcistica ratti,* than like *H. diminuta.* As mentioned under "observations," the strobila of this specimen was remarkably fragile, and lacked normal muscularity. The proglottids show several differences from proglottids typical of the species. The typical mature proglottid is about 6-8 times as wide as long, whereas the mutant proglottid (Fig. 13) is only about twice as wide as long. The gravid proglottids, unlike those of typical *H. diminuta,* are approximately square in outline (Fig. 14). Instead of the more than 2,000 eggs commonly found in a gravid proglottid of the species (Beck, 1951), the mutant form has only about 200. The internal organs, male and female, of the mutant form are not strikingly different from the normal; what difference there is seems to be in variability of number of testes and in relative volume and extent of the organs of both sexes.

The testes vary in number from one to six, with slightly over half the segments containing three testes characteristic of the genus *Hymenolepis.* This variability again calls into question the absolute reliability of testis number as a factor separating genera in the family Hymenolepididae, the matter having been discussed by Oswald (1957) in the case of *Pseudodiorchis* Skrjabin and Matevosian, 1948. Oswald found that out of 465 proglottids of specimens of *Pseudodiorchis reynoldsi* (Jones, 1944) examined by him, 1% had 1, 60% had 2, 37% had 3, and less than 1% had 4 testes. Apparently the number of testes is both naturally variable and sensitive to radiation in the cestode family Hymenolepididae.

The testes of the specimen are relatively small, and the ovary and yolk glands are also small. The paucity of eggs found in a gravid proglottid could perhaps be a function of the inadequate size of the gonads.

The two-hooked condition of most of the embryos is perhaps the most remarkable feature of the mutant specimen. Nearly all cestodes of the subclass Eucestoda have six-hooked embryos. The single known exception is the genus *Anonchotaenia,* the wormlike embroys of which lack hooks. Considerable variation exists among members of the sub-class in respect to embryonic membranes, shell structure, etc., but only the above exception has been noted concerning embryonic hooks. In the sub-class Cestodaria, containing such rare or aberant forms as *Amphilina* and *Gyrocotyle,* the embryo possesses a different number of hooks, ten rather than six, and is called a lycophore instead of an oncosphere. In the present specimen, as noted above, about 80% of the embryos had only two hooks. Thus a unique variation has occurred, one that departs not only from species or family characteristics, but from a basic taxonomic character of the sub-class itself.

If the present specimens had been recovered during a survey or biological exploration, the taxonomic questions raised would be puzzling. But because the mutant specimen has no future, so to speak, it cannot have any sort of taxonomic status. That is the reason why no complete description of it has been given here, other than the above noted differences from its parent stock.

We believe, however, that there is significance in the variations reported here. Schiller (1957), as noted earlier, suggested radiation as a way to increase the frequency of naturally occurring variation. The present study reports the use of radiation to produce a new variant, not found nature. The fact that this variant has characteris that would make it a new species of a new ge perhaps belonging in a new family or even hig category, if the variant were viable and could produce its kind, raises the question, not for first time, of the naming and classification of a ficially produced organisms. Because we are n rearing seven different mutant strains of *Hyme lepis diminuta,* and are studying the biolog characteristics of these strains, we are directly terested in such a taxonomic problem. At prese however, we do not consider it proper or conve ient to place a new name in the literature. Yet t existence of a form such as we have here describ is a warning of difficult taxonomic problems come.

Summary

A tapeworm which differed consistently fro normal worms of the same species was one result treating eight generations of *Hymenolepis dimin* with large doses of gamma radiation.

The mutant form was smaller and more slend than the normal and had relatively longer a fewer proglottids and fewer eggs.

The chromosomes differed from normal chro somes in morphology but not in number, th being evidence of translocation of chromoso segments.

The embryos were 2-hooked instead of 6-hook

The mutant form is considered an example a genetically significant anomaly, potentially a t onomic problem, since experiments in radiati will probably produce morphologically and ph ologically unique organisms which are, unlike t present specimen, able to reproduce.

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OBSERVATIONS OF PRESENT-DAY CARBONATE ENVIRONMENTS IN THE BAHAMA ISLANDS

R. L. Wilson
University of Chattanooga, Chattanooga, Tennessee
R. E. Bergenback
The Pennsylvania State University
State College, Pennsylvania

Introduction

During the fall and winter of 1958 the writers began a detailed study of the limestones of Mississippian age on Lookout Mountain near Chattanooga, Tennessee. Marked lateral and vertical changes were noted in these ancient oolitic limestones. In order to better understand the environments and sedimentary processes responsible, it was believed advantageous to visit an area of recent carbonate formation.

The Bahama Islands, located south and east of Miami, Florida (Fig. 1), are considered to be one of the classic areas for the study of marine carbonate deposition. Since Louis Agassiz's study of 1851, this outdoor laboratory has been of major importance to geolgists.

Acknowledgements

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Sincere appreciation goes to Dr. Leon Johnson, of the Pennsylvania State University, who provided X-ray analyses of carbonate mud samples. The assistance and advice of Dr. Cesare Emiliani of the International Oceanographic Foundation of Miami and Rear Admiral Karo, Director of the U.S. Coast and Geodetic Survey, also is gratefully acknowledged.

Local assistance was provided by Col. Peter Wilson, retired British army officer of Fresh Creek, Andros Town, Bahama Islands. The crew of the yacht, Captain Bob Lackey and Sam Lowe, helped in collecting samples and taking photographs.

Methods of Study

Owing to the limiting time factor, a relatively small number of rock and sediment samples were taken. The unconsolidated sands and muds on beaches, lagoon floors, and river bottoms were spot-sampled for mechanical, X-ray, and spectrographic analyses.

These field samples were supplemented by numerous photographs and brief field descriptions. Airplane flights over the Fresh Creek vicinity provided additional data.

Areas of Study

The areas studied during the 8-day period in December 1959, included: (1) the region around the mouth of Fresh Creek on Andros Island, (2) Big Wood Cay in the vicinity of Middle Bight, and (3) North Cat Cay on the western margin of the Bahama Island group (Fig. 1).

The major portion of this brief investigation centered about Andros Town on Fresh Creek (Fig. 2) because of the relatively large number of closely-spaced environments of carbonate formation and accumulation. These environments include: (1) reef, (2) cay (island), (3) lagoon, (4) beach, and (5) stream. The Middle Bight area displayed characteristics of (1) beach and (2) lagoon. North Cat Cay showed (1) beach and (2) lagoonal environments to advantage.

Fresh Creek Area

The narrow barrier reef off the eastern coast of Andros Island extends a short distance seaward (toward the Tongue of the Ocean) from the exposed cays. It is not continuous and through one of the breaks it is possible to gain access to the Andros Town harbor. The area visited is located off the northernmost island of the Goat Cay group (Fig. 2).

This reef is a living community covered by 7 to 10 feet of water at high tide and only 4 to 5 feet at low tide. The water was very clear and had a temperature of 71° to 75°F. The "rock" consists principally of sand-sized skeletal material held in place by growing coral colonies. Sea fans are the most common. Others include: (1) *Acropora palmata* (Elkhorn coral), (2) *Acropora cervicornis* (Staghorn coral), (3) *Goniolithon strictum*, and (4) several species of *Porites*. Sharp-spined sea urchins were abundant.

Of the many islands in the area, Long Cay was chosen primarily because of its accessibility. It is