SOME FACTORS AFFECTING RADIOIODIDE METABOLISM IN THE THREESPINE STICKLEBACK *Gasterosteus aculeatus* Linnaeus

by

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We accept this thesis as conforming
to the required standard

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ABSTRACT

Excretion of radioiodide by Gasterosteus aculeatus has been shown to vary directly with increases in salinity and inversely with size of fish. Erroneous excretion values may be produced by an apparent laboratory diuresis which seems to vary with season and salinity. During the initial stages of sexual maturation in sea water temporary changes in excretion occur which result in an increased retention of radioiodide by the fish. These changes show a greater development at lower salinities. The demonstrated dependence of thyroid uptake upon available radioiodide make this parameter an unreliable estimate of thyroid activity. The conversion ratio, except for errors which occur under conditions where the rate of radioiodide excretion is not constant over the period of the measurements, seems to be a valid estimate of thyroid activity.

Technical factors, such as the binding of inorganic radioiodide to precipitated protein, can also produce erroneous conversion ratio values. Differences in the protein binding of inorganic radioiodide suggest that changes in blood proteins occur during sexual maturation. Although a seasonal increase in thyroid activity occurs there is no correlation between this and the increase in radioiodide retention. There is therefore no reason to believe that the thyroid is responsible for the observed changes in retention of radioiodide.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES IN TEXT

LIST OF TABLES IN APPENDIX

I. INTRODUCTION

A considerable body of recent evidence has implicated the thyroid hormone in teleost osmoregulation. Koch and Heuts (1942, 1943) and Heuts (1945) have shown that sticklebacks (Gasterosteus aculeatus), fed desiccated thyroid gland, became less able to maintain osmotic equilibrium. While fish held in fresh water lost chloride, those held in salt water accumulated this ion. In high salinities (above 30‰ NaCl) the condition became lethal. These authors were able to show that the changes in salinity tolerance produced by treatment with thyroid material were similar to those occurring in sexually mature animals. Histological examination indicated that the stickleback had a more active thyroid during the reproductive season. Their explanation suggested that the disturbances in osmotic regulation were produced by the increased thyroid activity. This in turn forced the animal to migrate into fresh water.

An examination of the data presented by Koch and Heuts (1942, 1943) and Heuts (1945) compels one to accept their interpretation of the results obtained at high salinities. The results obtained at low salinities are somewhat contradictory. When animals belonging to a fresh water variety of stickleback (gymnura) were fed desiccated thyroid gland while in fresh water a decline in blood chlorides was observed. Mature fish of this variety showed a similar decline in blood chloride relative to non-breeding animals. Fish of the marine variety (trachura) did not. These animals, when mature, showed a slight

but consistent increase in blood chloride above the non-breeding level. This condition holds true over the entire range of salinities and is exaggerated at very low salinities.

The changes in blood chlorides in Pygosteus pungitius showed essentially the same pattern as observed in marine Gasterosteus (Heuts, 1943, 1945). The effect of thyroxine feeding was not tested on these marine stickleback (G. aculeatus trachura). The probable response can be inferred from the increase in chlorides which occurred in Pygosteus fed on desiccated thyroid material (Heuts, 1943). It seems unusual that the pattern of chloride changes at low salinity (fresh water) should differ in the two varieties of Gasterosteus when it is common to both Pygosteus and the marine form of Gasterosteus.

A further contradiction exists. Although osmoregulatory ability at high salinities was decreased in sexually mature trachura the fish showed a slightly improved ability to osmoregulate at low salinities. Mature gymnura showed a parallel improvement of a lesser degree. It is misleading to state that sexual maturation disturbs or reduces the osmoregulatory ability of the stickleback. This is only partly true. A more correct interpretation is that the region of osmotic stability has shifted to a lower range (Heuts, 1945).

Temperature, also, was shown to have an important effect on osmoregulatory ability (Heuts, 1945). An increase in temperature from 10°C. to 20°C. significantly reduced the range over which stickleback could osmoregulate. Non-breeding fish became less able to tolerate both low and high salinities while mature

stickleback showed a small reduction in their ability to tolerate very low salinities. Although the range of tolerated salinities was reduced, better control over the level of blood chlorides was exhibited (Heuts, 1945). This was especially true at high salinities in mature fish. The increase in temperature also emphasized the greater abilities of mature gymnura and trachura to osmoregulate at low and high salinities respectively. Obviously, thyroxine is not the only factor able to affect the osmoregulatory mechanism.

The work of Baggerman (1957, 1959) supports the idea that thyroxine is involved in osmoregulation. She has shown that treatment of stickleback with thyroxine produced a preference for fresh water. Anti-thyroid drugs induced a salt water preference. Since these results have yet to be verified on hypophysectomized animals their value is considerably reduced. Potential complications arising from thyroxine-thyroid stimulating hormone (TSH) interaction have not been studied. The possibility of detrimental effects of the anti-thyroid drug were largely disregarded in spite of an observed suppression of gonadal maturation.

Hickman (1959) has shown that the apparent increase in thyroid activity of Platichthys stellatus in fresh water was the result of a lower environmental iodide concentration and the resulting deficiency of iodide. Using radioiodide tracer techniques (thyroidal clearance of radioiodine) he presented evidence for increasing thyroid activity with increasing salinity. This was paralleled by increasing oxygen consumption with

increasing salinity, which suggests a metabolic effect. He admits that some of the work with which his own data does not agree cannot as yet be satisfactorily explained (Koch and Heuts, 1942, 1943). It is possible that the discrepancies between the observations of Koch and Heuts (1942, 1943) and Hickman (1959) were the result of the differences in techniques used. The application of the newer radioiodide tracer methods to a study of the stickleback might reveal basic similarities. Since environmental iodide levels may be complicating the problem iodide metabolism as a whole should be considered. The primary objective of the present study has been to study iodide metabolism as a whole.

The metabolism of radioiodide follows certain general pathways. Within a few hours after its injection, a tracer dose of radioiodide (I^{131}) will enter the blood and be distributed throughout the body. From the blood the radioiodide will proceed in one of several ways. Much of it will be removed from the body by the excretory organs. Some will be held in the various organs of the body. The concentration of radioiodide in the thyroid will be especially important because of its concentrating mechanisms. Some of the thyroidal radioiodide will be synthesized into radiothyroxine while the remainder may be either stored as inorganic iodide or released into the circulatory system. Upon reaching the site of utilization, the radiohormone will perform its role in the metabolism of the animal. The disposal of metabolized radiohormone and radioiodide obtained from its deiodination will proceed by a variety of routes.

Many of these possibilities were not considered in this study. The availability of information has resulted in the emphasis of three of the key points in radioiodide metabolism. These are the excretion of radioiodide, the accumulation of radioiodide by the thyroid, and the production of radiohormone. They will be considered with reference to some of the known environmental variables. The available information has also been applied to a critical evaluation of the tracer techniques used.

In summary, iodide metabolism of the stickleback may be involved in a number of different functions such as osmoregulation, salinity tolerance, migration, and sexual maturation. An attempt has been made to evaluate the relation of iodide metabolism to some of these conditions. The use of the three-spine stickleback as the experimental animal has been combined with the newer tracer techniques in an attempt to resolve the differences noted above. Emphasis will be placed on the effects of external salinity and the process of sexual maturation on iodide metabolism.

II. MATERIALS AND METHODS

A. Collection and Care of Fish

The studies being reported were carried out at the University of British Columbia between December, 1959 and July, 1961. The threespine stickleback Gasterosteus aculeatus form trachura was used. Occasional specimens of the forms Gasterosteus aculeatus semi-armata and Gasterosteus aculeatus gymnura were encountered (Heuts, 1945).

1. Source of Fish

All fish were collected in Coal Harbour, Vancouver, B.C., usually within a half mile of the Stanley Park entrance. The usual size ranged from 1 gram to 1.5 grams. Information on size, maturity etc. of fish used in individual experiments is given in Appendix Table I. Seasonally the environmental temperature varied from about 1°C. to 10°C. while the chlorinity probably varied from a high of 15‰ Cl^- to an occasional low of 5‰ Cl^- under exceptional conditions (Dehnel, 1960; Hickman, 1959; personal observations).

2. Holding Conditions

Collections were necessarily made at intervals and the fish transferred to 75 litre tanks at the University of British Columbia. During the immediate post-capture period the chlorinity of the water was maintained at 12-14‰ Cl^- with temperatures held as close as possible to those of the environment. All tanks

were equipped with air breakers and filters to improve holding conditions. After the fish had been allowed to recover from any effects caused by capture and transport they were placed under experimental conditions. A minimum of 2 weeks acclimation was allowed before any observations were made. The work of Koch and Heuts (1942, 1943) and Heuts (1943) indicates that stickleback usually reach osmotic equilibrium within five days after transfer to a hypertonic medium. In one case fish were held for nine months with low mortality. More usually fish held for periods up to four months remained in excellent condition. Holding conditions were sufficiently good to enable specimens to attain sexual maturity.

3. Adjustment to Salinity

Sea water from Vancouver harbour with a chlorinity of 12-14‰ was used as the basic medium. Adjustments in salinity were made by adding dechlorinated water or commercial "sea salt" (kiln dried half ground salt obtained from Vancouver Salt Co.). The "sea salt" was predominantly sodium chloride with traces of calcium and potassium (Dehnel, 1961). The salinity of the medium was determined by use of a modification of the standard Mohr technique using dichlorofluoresein indicator (Hoar, 1960; Strickland and Parsons, 1960). This permitted an estimation of total halide which, for the purposes of this study, is essentially equal to chlorinity. Since halides were of interest in the study this unit was used throughout with a chlorinity of 19‰ accepted as being equivalent to full (100%) sea water. The results were

not expressed as salinity per se although the conversion factor, salinity = (1.805 x chlorinity) + .03 was available.

4. Iodide Restoration

The importance of controlling the concentration of environmental iodide has been established by Hickman (1959). Since full sea water has an iodide concentration of 50 µg/litre (40 µg as KIO_3 and 10 µg as KI; Hickman, 1959) restoration of environmental iodide to this level was carried out. At all salinities fish had access to equal quantities of iodide.

5. Temperature

Temperature was partially controlled during the course of the study. Temperatures ranged from 15°C. in mid September 1961 to a mid winter low of 11°C. and a maximum in July, 1961 of 14°C. All tanks were held within 1°C. to 2°C. of each other and the usual daily fluctuation was less than 1°C.

6. Photoperiod

Since photoperiod has been shown to have important effects upon the maturation of the stickleback (Baggerman, 1957) this factor was controlled. Winter fish were held under eight hours of light while summer fish were held under a 16 hour photoperiod. Changes in duration of light were made at times when new collections were obtained. The intensity of light at the water surface was about 45 ft-c. This figure is of doubtful significance since the type of food used rapidly discolored the water.

7. Feeding

The animals were fed ad lib. every morning, except on the day of injection when they were not fed until several hours after the injection. Fish were never fed on the day that they were killed. Frozen brine shrimp were used throughout the study.

B. Radioiodide Technique

Radioiodide studies were directed toward three key points in the metabolism of radioiodide. The retention and therefore excretion of radioiodide was determined by measuring the radioiodide contained in the body (excluding the thyroid and blood sample). This value was represented as a percentage of the injected dose of radioiodide. The thyroid uptake of radioiodide was also determined by direct measurement and expressed in terms of the injected dose of radioiodide. The rate or efficiency of hormone production was determined by the conversion ratio method (Hickman, 1961). The conversion ratio can be defined as:

$$\frac{\text{hormonal radioiodide of the plasma}}{\text{total radioiodide of the plasma}} \times 100\%$$

The expression of hormonal radioiodide as a fraction of the total blood radioiodide compensates for differences which may result if the concentration of radioiodide in the circulation differs between individuals (Hickman, 1961; Eales, 1961). Since most thyroid hormone is bound to plasma proteins the conversion ratio is usually expressed as:

$$\frac{\text{protein bound radioiodide of plasma}}{\text{protein bound radioiodide + inorganic radioiodide of plasma}} \times 100\%$$

1. Injection Technique

Techniques used were essentially those of Hickman (1959) and Eales (1961). Carrier-free I^{131} was diluted to appropriate concentration with distilled water. A standard dose of 3 µc. in a volume of .01-.03 cc. was injected into each fish using 0.25 cc. tuberculin syringes equipped with a 30 gauge needle. The needle was passed through the epaxial muscle at a point dorsal to the vent. By directing the needle anterioventrally it was possible to inject into the coelom with the muscle acting as a seal to prevent leakage. Generally the injection technique was good. Exceptions occurred in the case of female fish with a maturing ovary. After the gonad reached a size equalling 5% to 10% of the body weight injection became difficult. At this stage the occlusion of the coelom by the ovary resulted in the unavoidable injection of the radioiodide into the ovary (see results and discussion).

2. Standards

Duplicate standards, each consisting of one dose of radioiodide diluted with KI solution were made up in 25 cc. volumetric flasks. One cc. aliquots (4% of a dose) were counted with samples. Standards for the well counter consisted of the 1 cc. aliquot plus 2 cc. distilled water in a counting tube (total volume 3 cc.). Forms of absorbent paper of the same size and shape as the fish or planchets with several layers of paper were used as end probe standards. One cc. aliquots were placed on the paper. The forms were used to evaluate the radioiodide left

in the body and the planchets as standards for determining radioiodide content of gonads. These standards allowed determination of values in terms of initial dose while automatically compensating for radioactive decomposition. Correction for background radiation was made in all cases.

3. Blood Sampling

All sampling was carried out from 10 A.M. to 11 A.M. Pacific Standard Time to avoid possible diurnal variation. The fish were removed from the tanks, gently dried and measured. A clean scalpel cut just posterior to the vent produced maximal blood flow. Blood was collected in heparinized capillary tubes which were then sealed with plasticine. Care was taken to avoid contamination by faeces, urine, and mucus. To avoid dilution of blood by extra-vascular fluid only the initial free flowing blood was collected. Samples were centrifuged under constant conditions in a Servall Angle head type NSE centrifuge - estimated speed was 1800 rpm. Maximal delay between sampling and centrifuging was about 15 minutes.

4. Preparation of Blood Samples

After centrifugation the plasma proteins were precipitated in 1 cc. of 12.5% trichloroacetic acid (TCA.). The sides of the 12 cc. centrifuge tubes were washed with a further 1 cc. of TCA. to remove any adhering plasma. After stirring the sample was centrifuged under constant conditions for approximately 30 minutes, in an I.E.L. Model (S.B.V.) centrifuge. The top speed

of 2500 rpm was reached in a series of stages and held for 15 minutes. The supernatent was decanted and saved. The precipitated protein was washed with 1 cc. portions of 2.5% TCA., carefully stirred, and recentrifuged. A total of three washings, of 1 cc. volume each, were carried out after the initial precipitation. All washes were combined with the initial supernatent. Finally the protein was dissolved with 2 cc. of 3N sodium hydroxide (NaOH) and transferred to a counting tube. An additional 1 cc. NaOH which was used to rinse the centrifuge tube was also transferred to the counting tube. A 3 cc. aliquot of the combined washes was placed in a second counting tube. Components were counted sequentially in a well scintillation counter. Red blood cells were saved and counted, either with the body or separately.

5. Thyroid Sampling and Preparation

The thyroids were dissected out immediately after the fish were weighed. The technique used was standardized. Cuts were made on each side passing through the corner of the mouth and just ventral to the eye. These were extended to the posterior margin of the operculum. A third cut, made along the posterior edge of the operculum, separated the lower jaw region. The vascular gill tissue was carefully trimmed as was the heart if present. The tongue and thyroid area were separated from the operculum cover and placed in 3 cc. of NaOH for counting. The region removed was found to equal 1.96% of the body weight with a standard deviation of $\pm$.70%. Initially the thyroids were

digested in the NaOH before counting. Since under experimental conditions no significant difference was found between digested and undigested thyroids this was discontinued and the thyroids were counted without being digested. The efficiency of the well counter and the very small size of the thyroids probably explain the lack of a significant difference.

6. Determination of Radioiodide Retention

Determination of radioiodide remaining in the body was carried out after removal of the thyroid. Bodies were carefully positioned in a constant manner 5 cm. under the end probe scintillation counter. Standards previously described were counted at the same time. Radioiodide values for both thyroids and bodies were expressed as percent of initial dose after correction for background radiation. Throughout the study the retention of radioiodide has been used as an estimate of excretion. Estimates of excretion were not corrected for the radioiodide in the thyroid. Since the quantity is generally small compared to total excretion (1% - 2%) and fairly constant over the range and period of the experiments concerned the lack of a correction seems justified.

7. Counting Equipment

A Philips High Voltage Supply and Preset Counter were available. These could be connected to either an end probe scintillation counter with a 45 mm. diameter and 38.5 mm. thick NaI(TI) crystal mounted in a lead castle or a scintillation well counter

in appropriate shielding. A Philips Scaler was also available and was used for monitoring glassware and other equipment.

C. Treatment of Data - Statistical Procedures

Standard techniques as described by Snedecor (1956) were used throughout. Usually standard t tests were used for comparison of mean of groups. Where variances were found to be unequal the method of Cochran and Cox was used. In a few cases where the data did not seem normally distributed t rank was calculated. The coefficient of correlation was calculated for excretion and other data. In most cases lines were eye-fitted. Where weight regression lines were calculated standard techniques were used. In the case of retention data, appropriate transformations were carried out.

III. RESULTS

A. Excretion of Radioiodide

1. Pattern of Radioiodide Excretion over a Period of Time After Injection

Excretion as such was not usually measured. Determination of the retention of iodide in the body was simply, directly, and accurately obtained. Changes in excretion were generally based on observed changes in retention. For most purposes 100% dose minus percent dose retained was assumed to equal percent dose excreted. Correction for thyroidal radioiodide was not made (see discussion). In other cases a series of observations over a period of four to six days provide a more accurate estimate of excretion rate. The pattern of excretion is represented by three of these curves (figure 2) obtained over a period of six months (October 1960, February 1961, May 1961) under conditions of high salinity (19‰ Cl^-). The overall picture indicates a logarithmic decline in body iodide. The curves obtained in October and May show a more rapid loss of radioiodide during the first 24 hours than at later times.

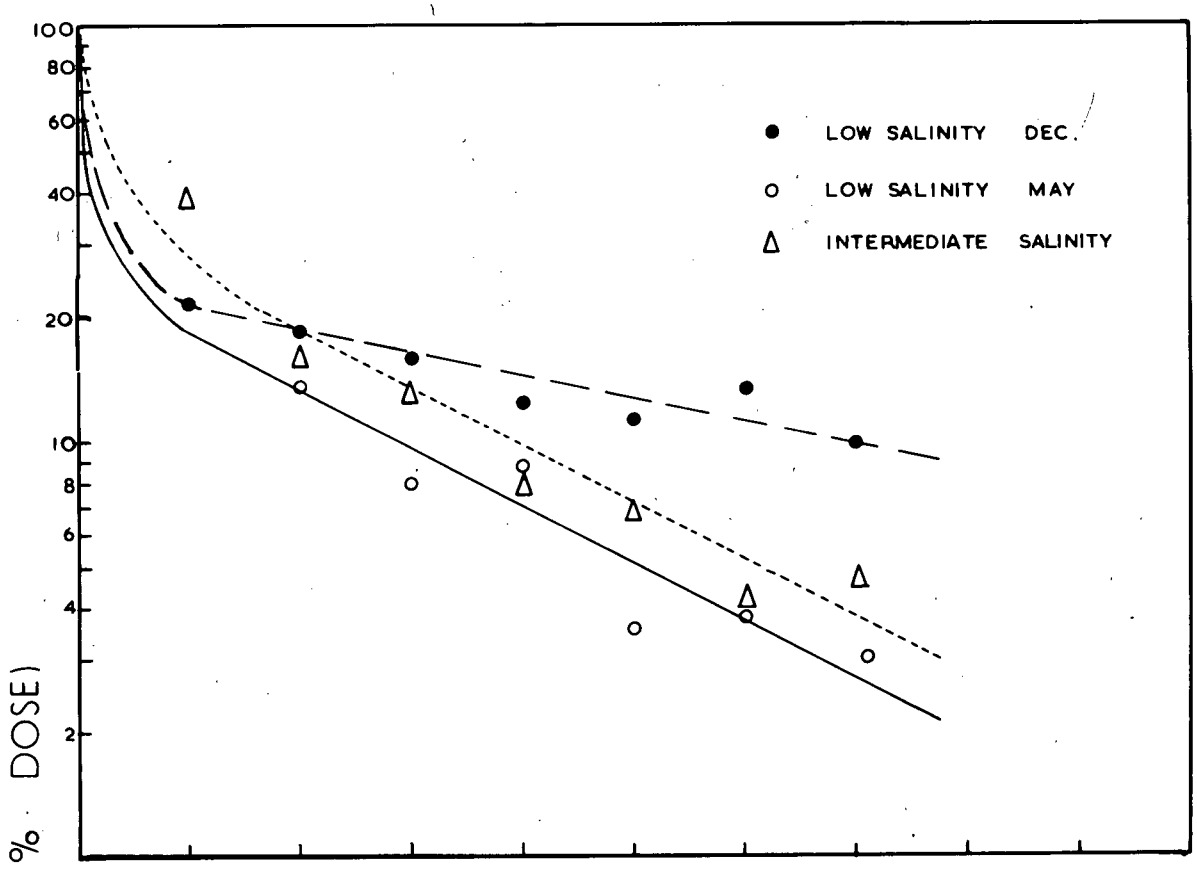
2. Effect of Size of Fish on Radioiodide Excretion

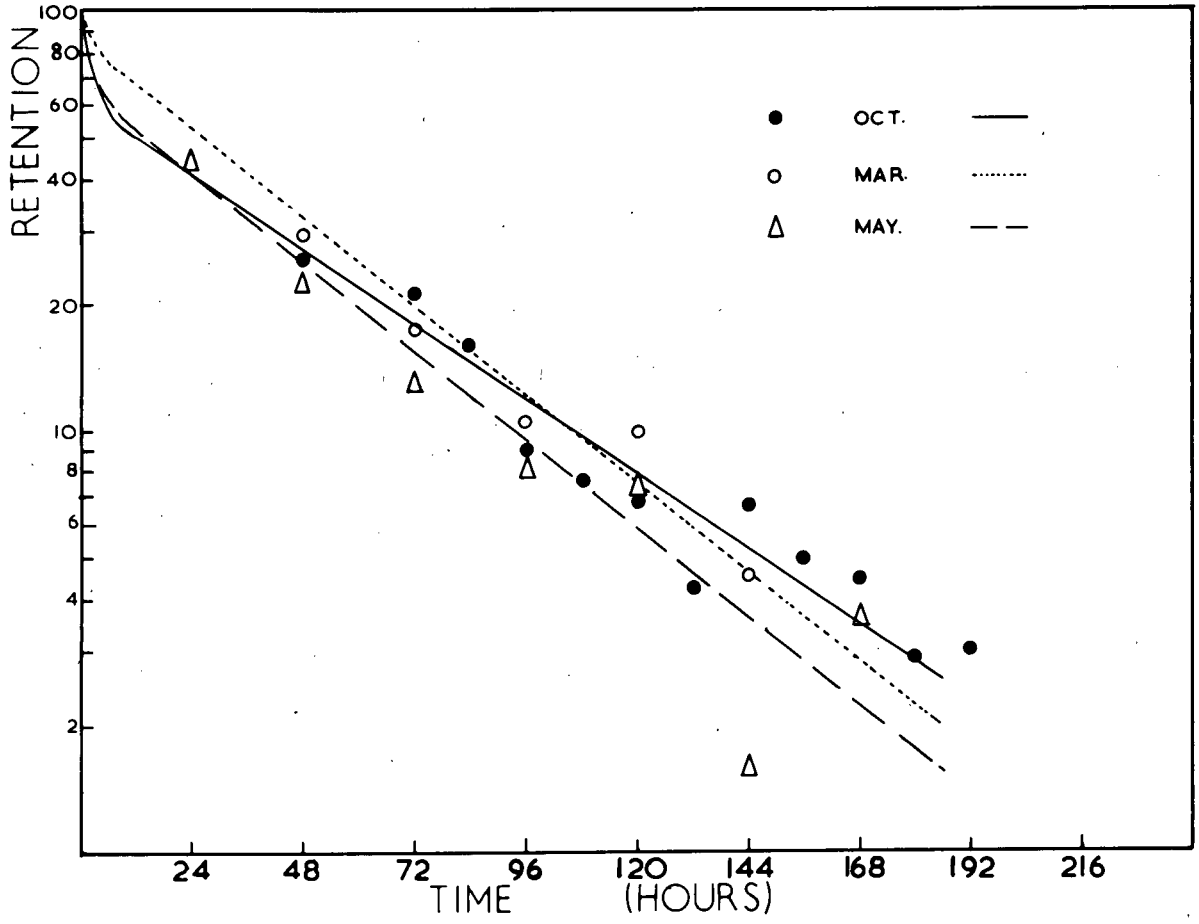
Figure 3 shows a decrease in excretion of radioiodide as fish become larger. The calculation of the coefficient of correlation (r) gives a value of -.565, with 22 degrees of freedom. Since this value is greater in absolute terms than the theoretical 1% level (.515) the null hypothesis (that there

to follow page 15

Figure 1. Changes in iodide retention with time after injection at low and intermediate salinities.

Figure 2. Changes in iodide retention with time after injection at high salinity.

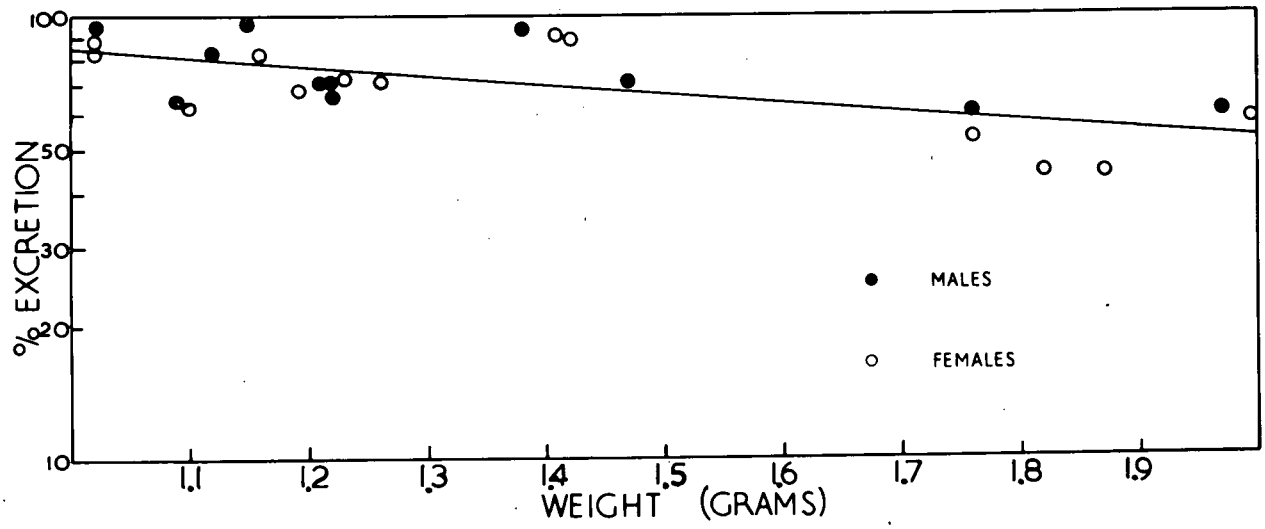

100
80
60
40
20
10
8
6
4
2
(% DOSE)
LOW SALINITY DEC.
LOW SALINITY MAY
INTERMEDIATE SALINITY

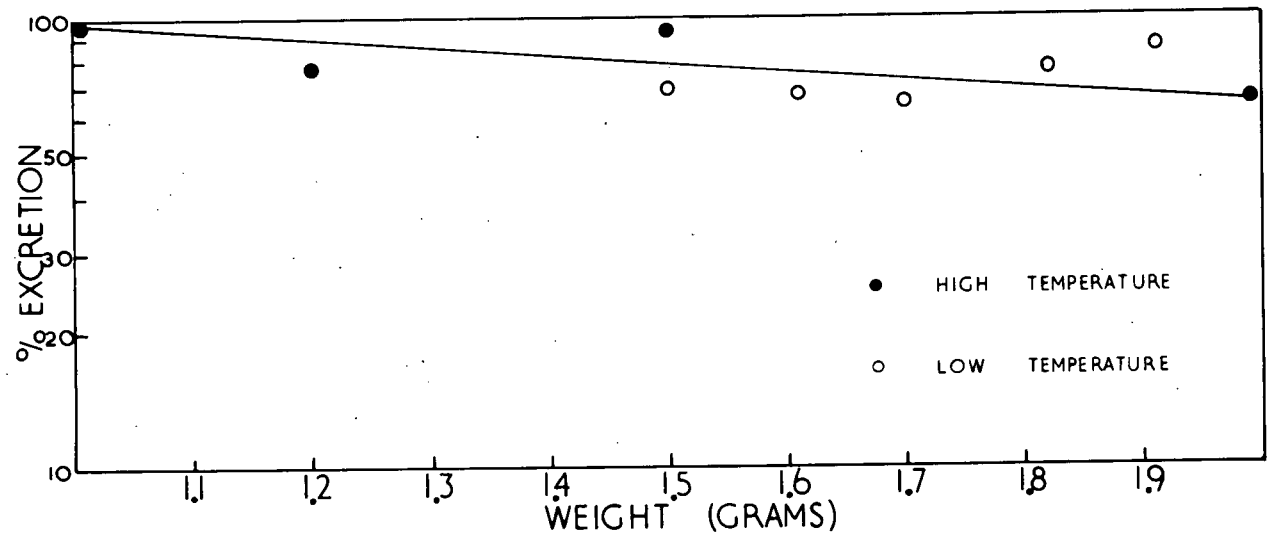

RETENTION
100
80
60
40
20
10
8
6
4
2
OCT.
MAR.
MAY.
24
48
72
96
120
144
168
192
216
TIME (HOURS)

to follow page 15

Figure 3. The relationship between size of fish and excretion of radioiodide.

Figure 4. A comparison of excretion at 10°C. and 17°C.


100
50
30
20
10
% EXCRETION
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
WEIGHT (GRAMS)
MALES
FEMALES


100
50
30
20
10
% EXCRETION
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
WEIGHT (GRAMS)
HIGH TEMPERATURE
LOW TEMPERATURE

is no relation between size and retention) is rejected at this level.

3. Effect of Sex on Radioiodide Excretion

Separation of the above data (figure 3) into sex groups suggests a difference in response of males and females. The females alone showed a significant correlation between size and excretion ($r = -.614$ with 11 degrees of freedom, $r(P.05) = .553$). The correlation of weight and excretion in male fish was not statistically significant ($r = -.455$ with 9 degrees of freedom, $r(P.05) = .602$). An analysis of covariance was used to compare the regression lines of the males and females. The variance ratios (F) calculated for slopes and differences in mean values do not exceed the tabled value at the 5% level. On this basis it was necessary to accept the null hypothesis; that is, there is no significant difference in slopes of regression lines or in the mean value (F slopes = .293 with 20 and 1 degrees of freedom, $F(P.05) = 248.$; F means = .404 with 21 and 1 degrees of freedom, $F(P.05) = 248.$).

4. Effect of Salinity on Radioiodide Excretion

Data on the effect of external salinity on retention of radioiodide are presented in a series of retention curves obtained at external salinities of 5‰ Cl^-, 12‰ Cl^-, and 19‰ Cl^-. The pattern of excretion at high salinity has been presented (figure 2). The curve (figure 1) obtained in June 1961 for intermediate salinity (12‰ Cl^-) is similar to the high salinity curves. The initial rate of excretion (first 48 hours) seems

greater than the rate during the later period. A change on excretion rate was confirmed upon examination of figure 1 which shows the excretion pattern at low salinity (5‰ Cl^-). These curves were obtained in December 1960 and May 1961. The rate of excretion after 48 hours is very slow in contrast to the rate during the first 24-48 hours. There is no doubt that a change in the rate of excretion has occurred. The same condition has been noted at high salinity during October and May. The magnitude of the initial loss is greatest at low salinities and least at high salinities, where it may be entirely absent.

The determination of $t_{\frac{1}{2}}$[1] from the eye-fitted curves provides a more precise estimate of the excretion rate. The $t_{\frac{1}{2}}$ values for high salinity (19‰ Cl^-) in October, February, and May were about 41 hours, 34 hours and 34 hours respectively. The $t_{\frac{1}{2}}$ for intermediate salinity (12‰ Cl^-) in June was 52 hours. The $t_{\frac{1}{2}}$ values for low salinity (5‰ Cl^-) in December and May were 132 hours and 52 hours respectively. There is no consistency between the pattern at different salinities. This will be discussed in relation to possible seasonal changes in excretion.

5. Changes in Radioiodide Excretion with Maturation

Available information on the retention of radioiodide has been plotted for the period November, 1960 to July, 1961 (figure 5). The fish represented from November to February (group 1, figure 5)

1. $t_{\frac{1}{2}}$ is applied to exponential curves and in this case represents the time required for the removal of $\frac{1}{2}$ the radioiodide present at any time (Hickman, 1959).

to follow page 17

Figure 5. Seasonal changes in retention of radioiodide.*

A. Low salinity

B. Intermediate salinity

C. High salinity

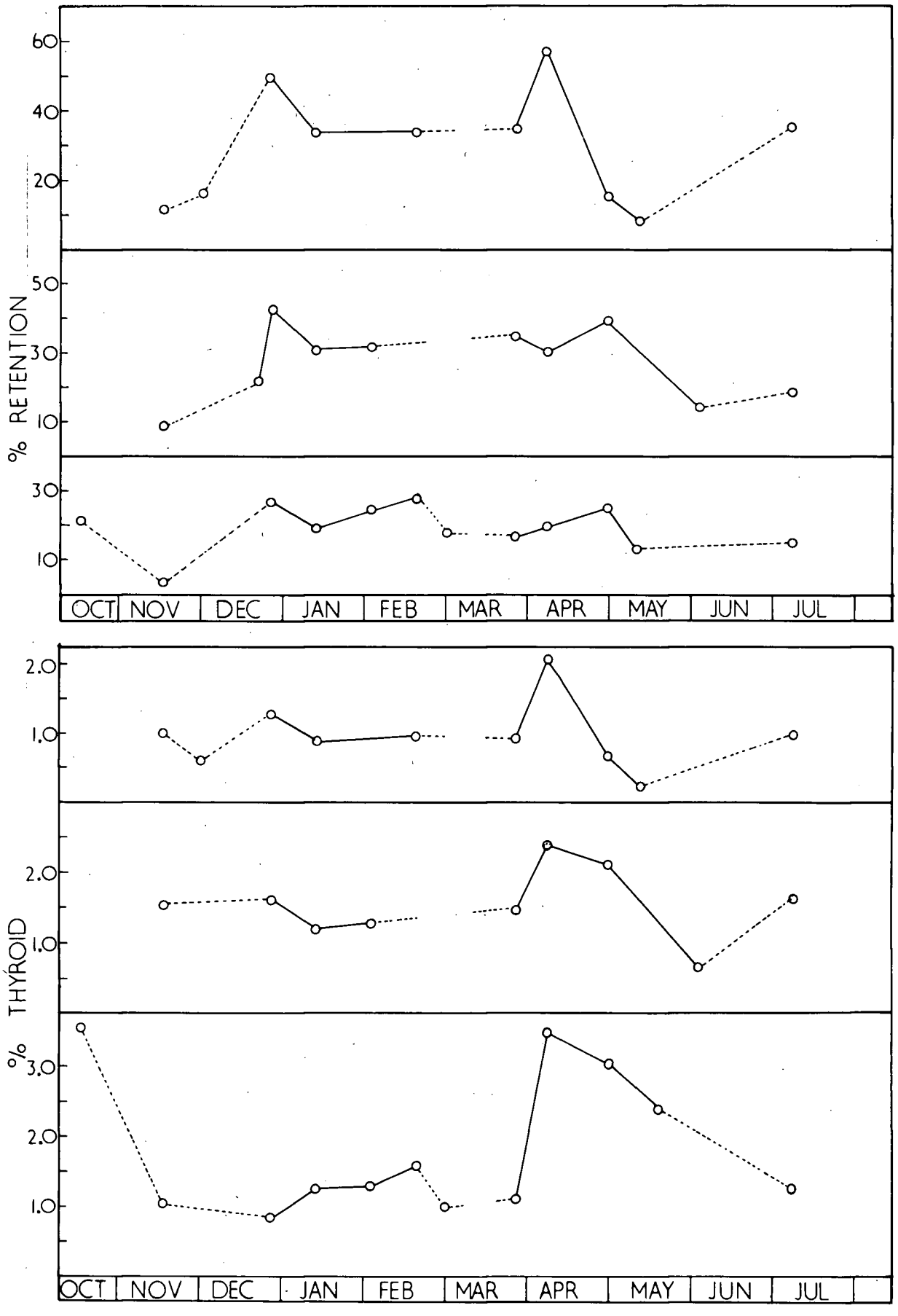
Figure 6. Seasonal changes in thyroid uptake of radioiodide.*

A. Low salinity

B. Intermediate salinity

C. High salinity

*Solid lines are used to connect groups with known equivalent history. Where collection date varied dotted lines are used. The line is interrupted to differentiate between the two major groups studied.


% RETENTION
60
40
20
50
30
10
30
10
% THYROID
2.0
1.0
2.0
1.0
3.0
2.0
1.0
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL

were obtained on November 26 or earlier. As such they were non-breeding animals. Fish from a second major collection on March 10 (group 2) were used from this date until July 10 (figure 5). These fish were probably in a preliminary stage of maturation when obtained.

The results obtained at each of the three salinities have been plotted separately. The low salinity curve shows the greatest change (figure 5). Within one month after capture a marked retention of radioiodide occurred and then declined. The pattern is repeated in the intermediate salinity curve and suggested by high salinity data. The degree to which this retention develops is greatest in low salinity, declining through intermediate salinity to a minimum in high salinity. The data suggest that the onset of the change may be delayed in higher salinities and that the period of increasing retention is prolonged under such conditions. The March collection of fish (figure 5) replicates in principle the picture just described. The change under conditions of intermediate salinity are less pronounced as is the change in the high salinity group.

An analysis of variance (Table I) showed that the changes at each salinity were significantly different, as were the changes occurring with time.

Table I. Results of statistical analysis of seasonal changes in retention.

	Group 1			Group 2		
	F	Degrees of freedom	F(P.01)	F	Degrees of freedom	F(P.01)
Salinity	22.11	2 & 106	4.82	10.37	2 & 87	4.88
Time	83.26	3 & 106	3.98	11.09	4 & 87	3.56

The analyses also indicate that the interaction between salinity and time is significant in the second group of fish (F interaction= 5.9 with 8 and 87 degrees of freedom, F(P.01) = 2.74). This seems to imply that changes are not proceeding at the same rate under all conditions of salinity.

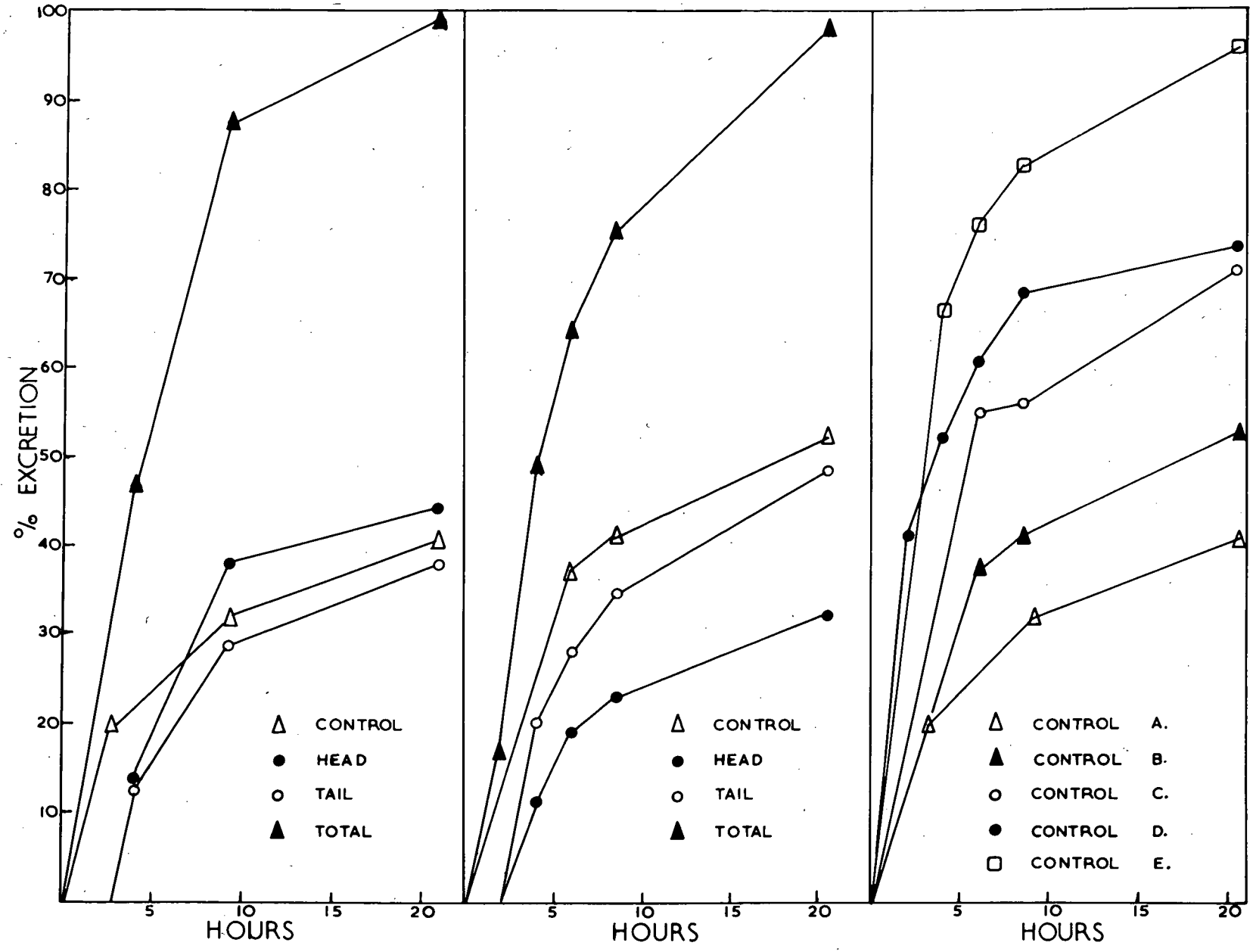
6. Technical Factors Which May Influence Radioiodide Excretion

a. Laboratory Diuresis. The occurrence of laboratory diuresis in marine fish is a common phenomenon (Forster, 1953). Since prolonged holding or handling could have initiated a diuretic condition the effect was of possible importance. The phenomenon itself was not actually studied. Data obtained during preliminary attempts to determine the site of radioiodide excretion are available. Since the data suggest a diuresis they will be presented. The experiment consisted of suspending a fish (previously injected with radioiodide) in a two compartment container. The arrangement was such that the region from the pelvic spines forward (gill area) was in one section while the remainder of the fish was in the other. A rubber partition held the fish in place and prevented mixing of the contents of the chambers. Periodic sampling of each compartment allowed an estimate of radioiodide excretion by both anterior (gill) and posterior (kidney) regions of the fish. Controls consisted of injected fish held in equally small containers without any separation or other attachment. No anaesthetic was used. Considerable agitation was displayed by both experimental fish and controls. On the basis of observations during experiments control "A" was the most reliable indication of normal conditions.

to follow page 19

Figure 7. A comparison of excretion from anterior (gill) and posterior (kidney) regions of the body.

Trial A Trial B Controls


% EXCRETION
100
90
80
70
60
50
40
30
20
10
5
10
15
20
HOURS
CONTROL
HEAD
TAIL
TOTAL
HOURS
CONTROL
HEAD
TAIL
TOTAL
HOURS
CONTROL A.
CONTROL B.
CONTROL C.
CONTROL D.
CONTROL E.

The results are presented in figure 7. It should be noted that the source of excretion during the first two to three hours is unknown and that this excretion is not represented by the anterior and posterior curves. Two points are evident.

1. Excretion of radioiodide by the two experimental fish is much greater than that of the lowest control values (controls A and B).
2. Both anterior and posterior regions of the experimental fish excrete significant amounts of radioiodide.

b. Retention of Injected Radioiodide by the Ovary. Differences in retention of radioiodide by males and females are illustrated in Table II.

Table II. Effect of radioiodide retention by ovary on radioiodide metabolism.

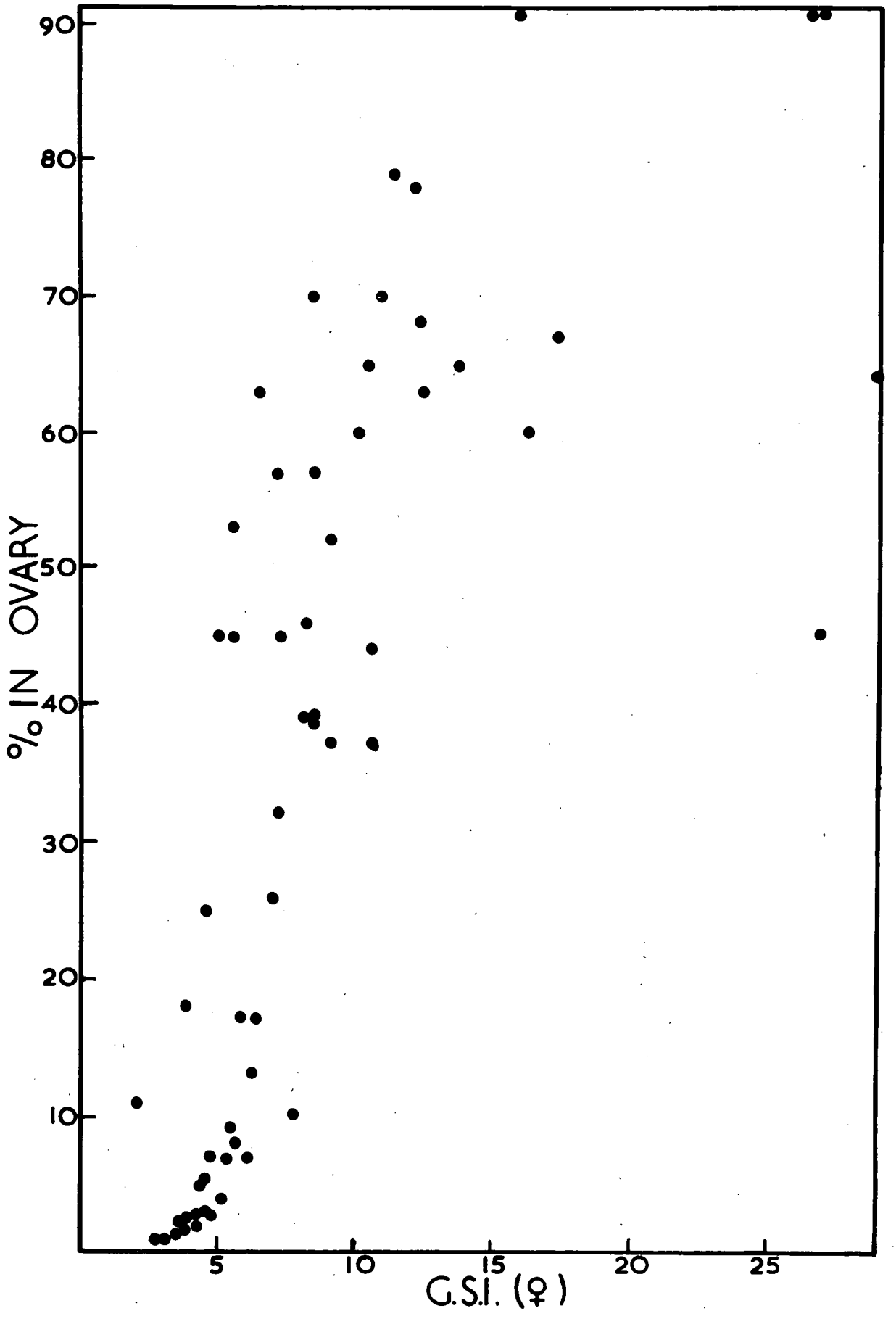
Sex	Size (gm.)	G.S.I.[2]	% I^- retention	% I^- ovary	Uptake % dose	C.R. %	Relative blood conc.
female	1.85	10.54	62.18	40.51	0.40	2.52	0.39
	1.56	12.37	61.00	38.30	0.54	2.97	0.29
	2.14	28.90	75.33	48.22	1.94	2.96	0.75
	1.71	16.27	47.44	28.16	0.31	3.91	0.26
male	1.36	1.44	43.76	0.96	0.95	3.06	1.00
	1.45	2.17	32.27	0.59	0.86	6.37	0.52
	1.60	2.13	48.40	0.70	0.92	5.95	0.80
	1.58	4.14	32.82	1.25	1.37	3.47	1.00

The relationship between gonadal radioiodide content and maturity in females is presented in figure 8. To correct for differences

2. The gonosomatic index (GSI) is assumed to indicate the physical preparedness for reproduction. It consists in the female of $\frac{\text{weight ovary}}{\text{weight body}} \times 100\%$. Since the kidney of the male stickleback functions as an accessory organ it is included with the testes. In fact the kidney showed a greater proportionate change than did the testes. GSI (male) $= \frac{\text{weight kidney + testes}}{\text{body weight}} \times 100\%$.

to follow page 20

Figure 8. Relationship between maturity and radioiodide held by the ovary.


90
80
70
60
50
40
30
20
10
% IN OVARY
5
10
15
20
25
G.S.I. (♀)

in level of excretion the radioiodide content of the ovary has been expressed as a percentage of the total body retention.

7. Factors of Secondary Importance

During attempts to locate sources of variability brief studies were carried out on effects of temperature and of reentry of radioiodide. In the absence of significant effects the studies were discontinued. The results of these pilot studies are presented for completeness even though the investigation was not known to be affected by them.

a. Temperature Effects. Although all tanks were held at the same temperature a general increase occurred from December to July (11°C. to 14°C.). The effect of temperature on iodide metabolism was tested. Fish were acclimated to temperatures of 10°C. (9°-11°) and 17°C. (16°-18°) and equal salinities (14‰ Cl^-) for a period of two months. Although considerable mortality occurred at the higher temperature the deaths occurred at random intervals. The need to take size into account prevented a statistical comparison as insufficient data were available. As an alternative, excretion values were plotted against size (figure 4). Inspection indicates a fairly consistent excretion in spite of the temperature differences.

b. Effect of Reentry of Radioiodide. Reentry of excreted radioiodide into the experimental animal from the environment could alter results. In the stickleback reentry was generally found to be negligible. Examination of uninjected fish held in the same container as a group of injected fish (comparable to

standard experimental conditions) showed only a very slight accumulation (less than 1% of amount held by controls) of radioiodide over two days (see Table III).

Table III. Reentry of radioiodide.

	Body (%)	Thyroid (%)	Blood (%)
No added radioiodide			
Radioiodide content (% dose)	0.237[3]	0.013	0.003
Normal content at equiv. time	24.4	1.03	0.443
Radioiodide added to water			
Radioiodide content (% dose)	0.970	0.058	0.009

Another group placed in a tank to which radioiodide had been added showed a greater accumulation of radioiodide (Table III). The level in the latter case began to reach appreciable amounts but was still too low to have a significant effect on most retention data.

B. Thyroid Uptake of Radioiodide

1. Pattern of Thyroid Uptake Over a Period of Time After Injection

Figure 9 shows the pattern of thyroid uptake over a period of time. Data were obtained from fish held at high salinity (19‰ Cl^-). The accumulation of radioiodide occurred most quickly in the initial 24 hours. The thyroidal content of radioiodide continued to increase until 72 hours after which a gradual decline occurred.

2. Effect of Retention of Radioiodide on Thyroid Uptake

The uptake of radioiodide by the thyroid depends upon the

3. Where % dose of radioiodide exceeds 1% values have been rounded off to the 3rd significant figure.

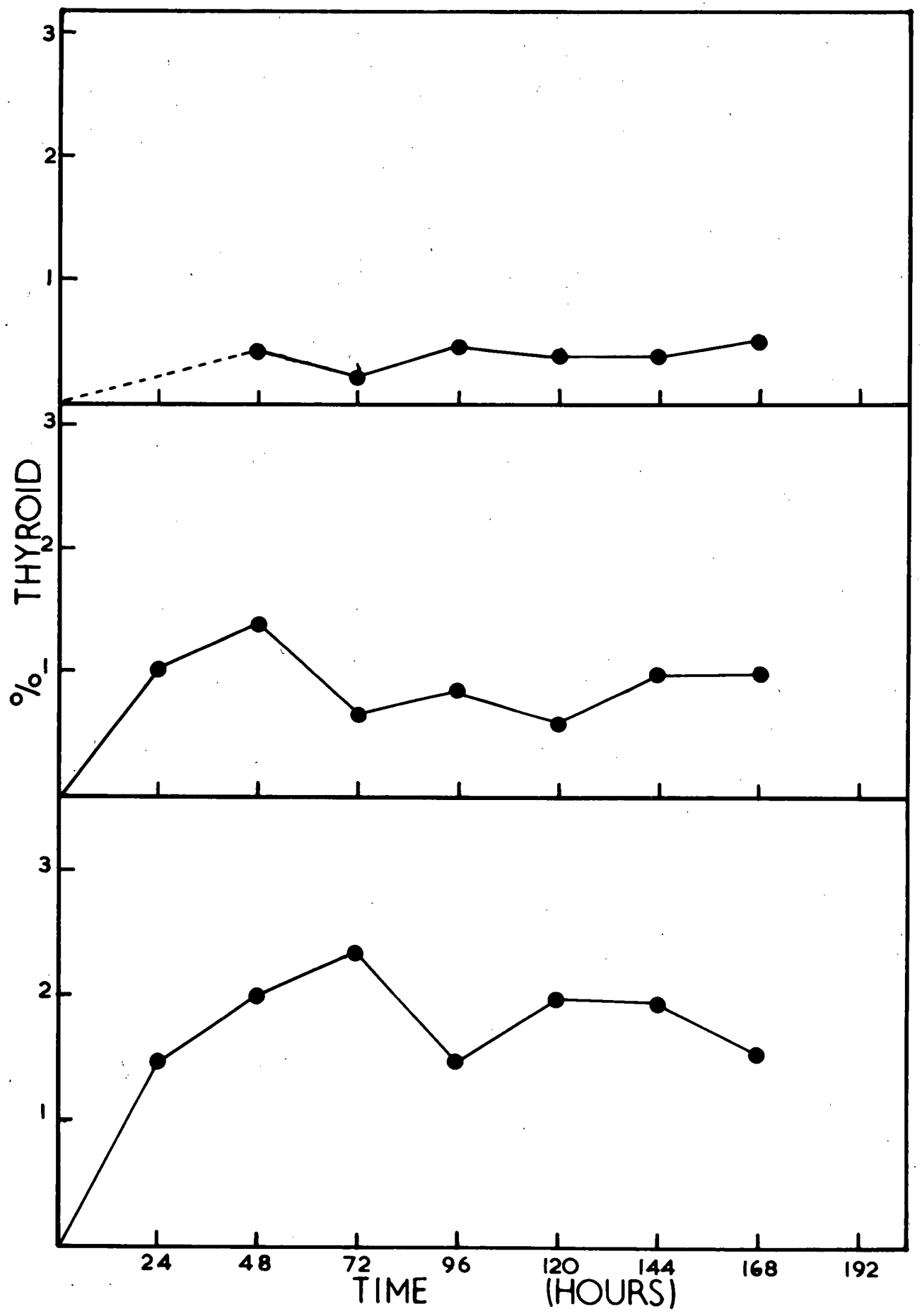
to follow page 22

Figure 9. Changes in thyroid content of radioiodide with time after injection.

A. Low salinity

B. Intermediate salinity

C. High salinity


3
2
1
3
2
1
3
2
1
% THYROID
24
48
72
96
120
144
168
192
TIME (HOURS)

to follow page 22

Figure 10. Relationship between thyroid uptake of radioiodide and body retention.

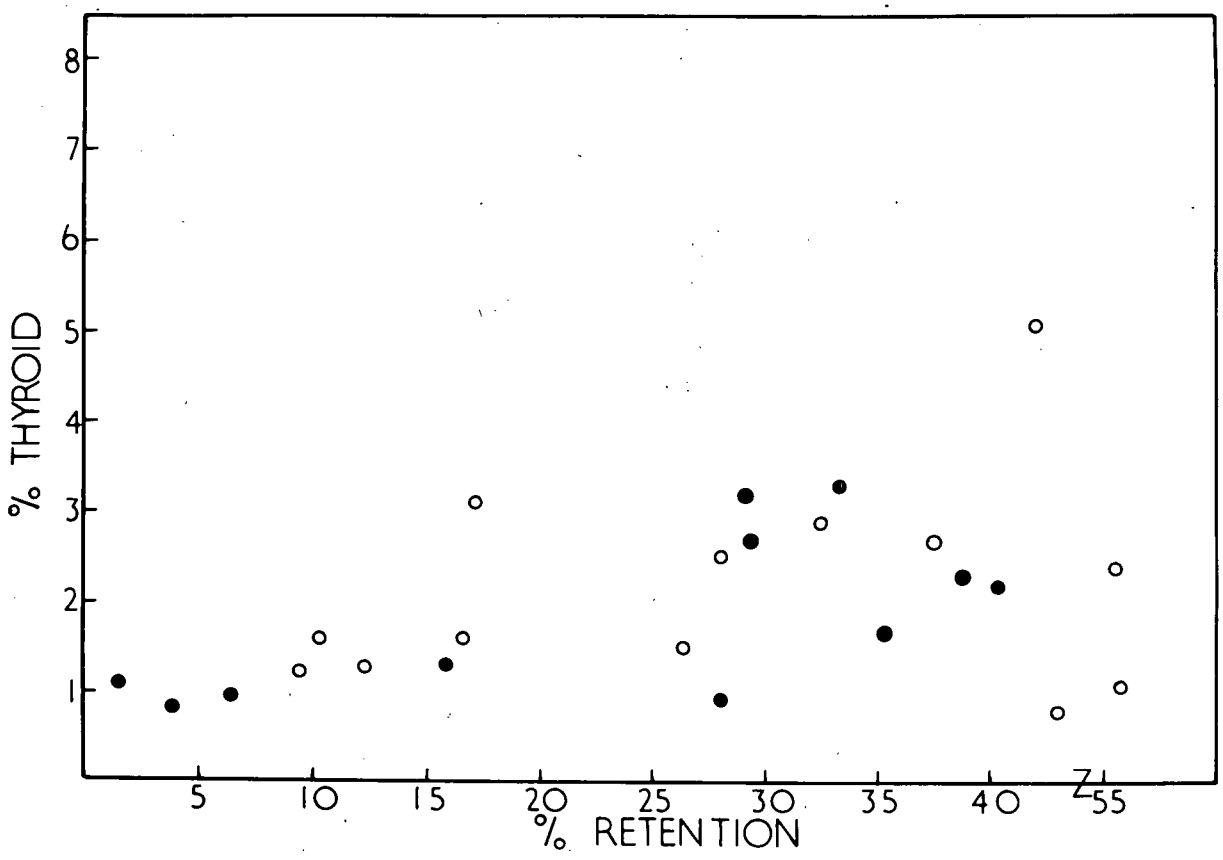
o females

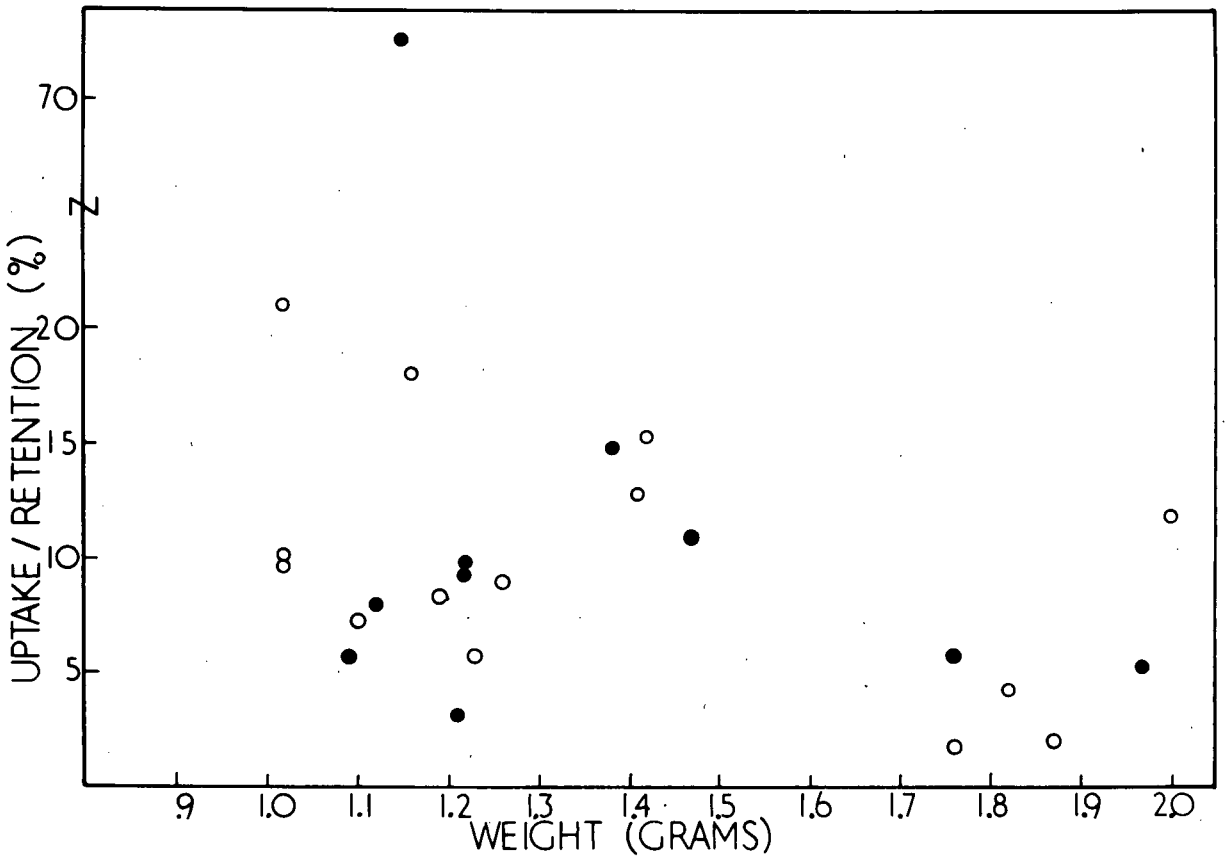
● males

Figure 11. Relationship between uptake/retention and size of fish.

o females

● males


% THYROID
% RETENTION
1
2
3
4
5
6
7
8
5
10
15
20
25
30
35
40
55


UPTAKE / RETENTION (%)
WEIGHT (GRAMS)
5
10
15
20
70
.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0

available radioiodide in the blood and the affinity of the gland for iodide. For this reason a correlation between uptake of radioiodide and retention of radioiodide could be expected under conditions of constant thyroid activity. Figure 9, based on data obtained February 11, 1961 at intermediate salinity, shows that there is such a correlation. Statistically, the correlation is not quite significant at the 5% level ($r = .403$ with 22 degrees of freedom, $r(P.05) = .404$).

3. Effect of Size of Fish on Thyroid Uptake of Radioiodide

Attempts to correlate size and thyroid uptake were unsuccessful. Even when the effect of retention was allowed for (calculation of uptake/retention) no significant trends could be established (figure 11: $r = -.19$ with 22 degrees of freedom, $r(P.05) = .404$).

4. Effect of Sex on Thyroid Uptake of Radioiodide

The data discussed above suggest differences in the uptake/retention relationship may occur between sexes (figure 10). Consideration of the males alone shows a significant correlation between uptake and retention ($r = .694$ with 9 degrees of freedom, $r(P.05) = .602$). The females showed no significant correlation ($r = .190$ with 11 degrees of freedom, $r(P.05) = .553$). Covariant analysis shows a very significant difference between the slopes of the regression lines for males (.04) and females (.01) ($F = 15.04$ with 1 and 21 degrees of freedom, $F(P.005) = 9.94$). Examination of another set of data which was obtained January 2, 1961 at low salinity indicates that in this case retention has not had an

effect on uptake. There were no differences between uptake by the thyroid of males and females under the conditions of the experiment.

5. Effect of Salinity on Thyroid Uptake of Radioiodide

Figure 9a represents the pattern of thyroid uptake at high salinity. Figures 9b and 9c represent uptake at intermediate and low salinities respectively. A general survey indicates a graded series. The percent dose of radioiodide held by the gland is least in low salinity, increasing in intermediate salinity and reaching a maximum in high salinity.

There are also some differences in the pattern of the curves. In low salinity there is a small initial uptake which does not change with time. In high salinity the 96 hour point is the only extreme value. Otherwise a large initial uptake followed by a gradual decline in thyroidal radioiodide is indicated. In intermediate salinity a marked initial uptake (less in absolute terms than the high salinity) is followed first by a fairly rapid loss of iodide, then by what seems to be a slight recovery. A suggestion of the same effect is seen in the low and high salinity uptake curves. Since excretion of radioiodide which has been demonstrated to affect uptake varies with salinity no attempt has been made to compare thyroid uptake values on a statistical basis (see discussion page 41).

The time required for the thyroid uptake to level off is inversely related to the excretion rate in the first 24 hours. The initial rate of excretion increases from high to low salinity

while the time required for the thyroid uptake to inflect decreases from high to low salinity.

6. Changes in Thyroid Uptake of Radioiodide with Maturation

Thyroid uptake at all salinities fluctuates somewhat over the period November to March (figure 6). Although high salinity shows a small temporary increase in January and February the effect is only suggested at other salinities. In April a marked transitory increase in uptake occurs. This effect is greatest in high salinity and more prolonged. As salinity declines so does the magnitude and duration of the effect. Statistical analysis (Appendix Table II) can be summarized in a few words. Under all three conditions of salinity both the increase and decline (in April) are statistically significant. In low and intermediate salinity the increase from May to July is also statistically significant. While the increase in uptake from December 29 to February 21 is statistically significant the decline from February 21 to 28 is not. These changes do not necessarily represent changes in thyroid activity since uptake levels parallel the seasonal changes in radioiodide excretion (retention).

7. Technical Factors Which May Influence Thyroid Uptake of Radioiodide

a. Variation in Dose. Radiation damage resulting from excessive dosage of radioiodide would be expected to alter thyroid function. An experiment was performed to check for possible effects of excessive dosage. Three groups of eight fish (ranging in weight from 1.0 to 1.5 grams) were injected with 1, 3, and 8 μc.

respectively, of carrier-free radioiodide. An analysis of variance was used to compare uptake of radioiodide at different doses. No significant effect was apparent (Table IV) (F for doses = 1.91 with 2 and 15 degrees of freedom, F(P.05) = 3.55).

Table IV. Effect of dose of radioiodide on radioiodide metabolism.

Dose µc./fish	Sex	No. of fish	Wt. mean (range)	Uptake mean (range)	C.R. mean (range)
8	male	2	1.15 (1.09-1.21)	1.30 (0.87-1.73)	2.87 (2.79-2.95)
	female	6	1.39 (1.19-1.82)	2.02 (1.22-2.88)	2.81 (1.12-4.16)
	total	8	1.33	1.85	2.82
3	male	5	1.56 (1.22-1.97)	2.38 (0.90-3.29)	3.28 (1.59-5.14)
	female	3	1.39 (1.02-2.00)	3.26 (1.62-5.06)	4.85/2 fish (2.36-7.35)
	total	8	1.49	2.71	3.73/7 fish
1	male	4	1.13 (1.02-1.22)	1.47 (0.78-2.74)	1.94/2 fish (1.75-2.13)
	female	4	1.44 (1.02-1.87)	2.31 (1.29-3.66)	2.96 (1.27-4.05)
	total	8	1.28	1.89	2.62/6 fish

b. Effect of Radioiodide Retention by the Ovary on Thyroid Uptake. Examination of Table II shows that uptake as well as retention values are affected by this factor. Although exceptions did occur (Table II) females whose ovaries held large amounts of radioiodide usually showed a low thyroid uptake.

8. Factors of Secondary Importance

a. Effect of Temperature. Fish held at 10°C. and 17°C. were examined to see if thyroid uptake differed with temperature (Table V).

Table V. Effect of temperature on radioiodide metabolism.

Salinity	Sex	No. of fish	Wt. mean (range) gm.	Uptake mean (range) %	C.R. mean (range) %
Low	male	5	1.71 (1.61-1.91)	3.19 (1.58-5.90)	4.41 (3.77-5.83)
	female	3	1.74 (1.46-1.93)	0.72 (0.58-1.00)	6.79 (4.13-11.70)
High	male	4	1.42 (1.00-1.99)	2.41 (1.54-3.51)	3.61 (2.62-5.35)
	female	3	1.99 (1.47-2.75)	0.96 (0.48-1.43)	4.18/2 fish (2.76-5.61)

The female data were excluded from consideration because of the large amounts of radioiodide held in the ovary. Although the male uptake values suggest that thyroid uptake is greater at 10°C. the difference is not statistically significant.

b. Effect of Reentry of Radioiodide on Thyroid Uptake. The effect of reentry of radioiodide is shown in Table III.

9. Correction of Thyroid Uptake for Changes in Retention

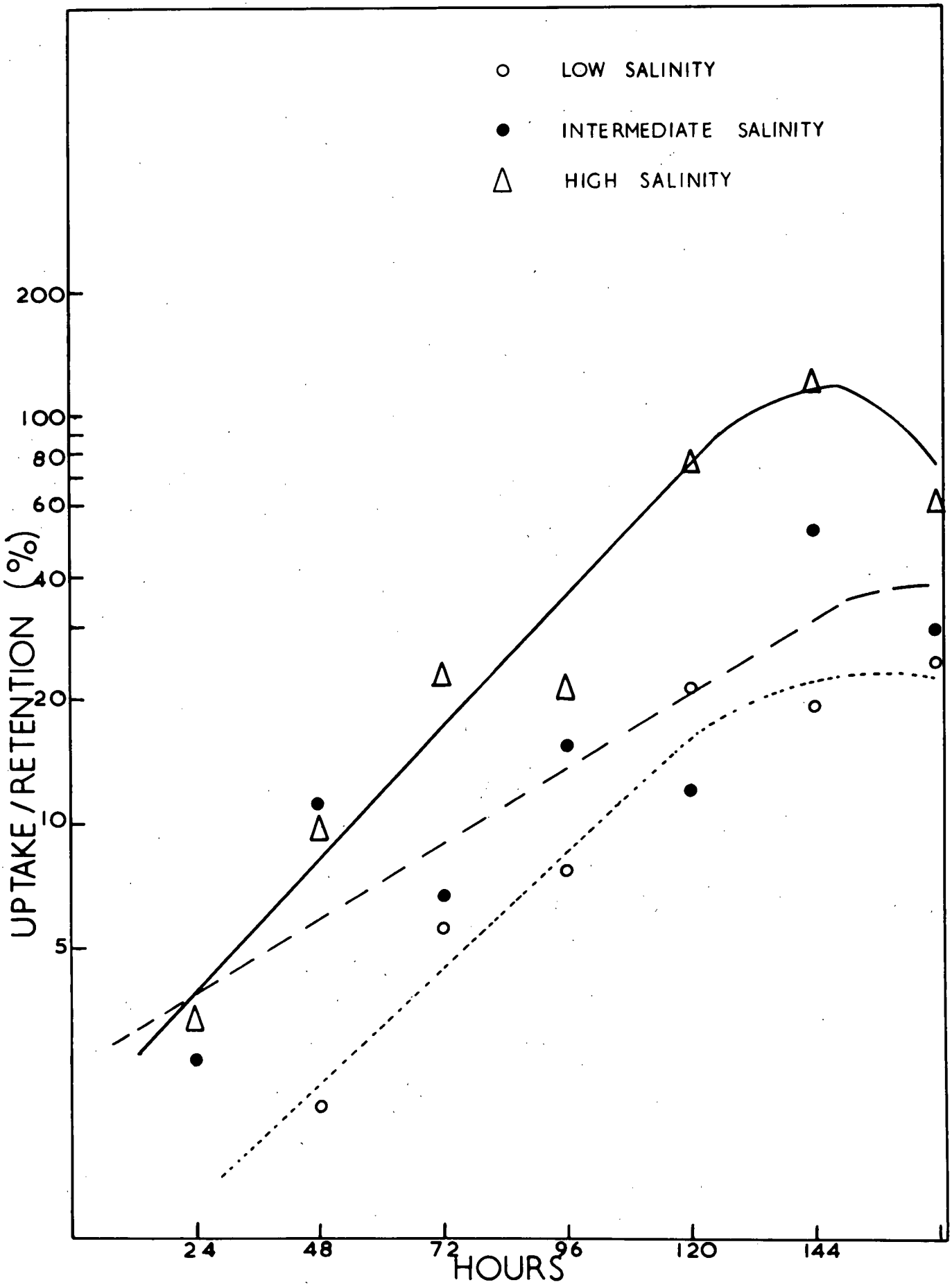
In view of the effect of retention of radioiodide upon thyroid uptake it seems desirable to attempt a correlation by calculating the uptake per unit retention.

The $\frac{\text{uptake of radioiodide}}{\text{retention of radioiodide}} \times 100\%$ has been plotted against time in figure 12. Since thyroid uptake levels off or declines after 72 hours the uptake/retention curve from this time on is a reflection of radioiodide excretion.

a. Effect of Size. Figure 11 shows no correlation between size and uptake/retention.

to follow page 27

Figure 12. Changes in uptake/retention with time after injection at low, intermediate and high salinities.


LOW SALINITY
INTERMEDIATE SALINITY
HIGH SALINITY
200
100
80
60
40
20
10
5
UPTAKE / RETENTION (%)
24
48
72
96
120
144
HOURS

b. Effect of Salinity. Comparison of figure 12a (high salinity) with figures 12b and 12c indicates differences in uptake/retention pattern at different salinities. These curves seem to indicate that the uptake/retention increases from low to intermediate to high salinity. This agrees with the estimate of thyroid activity based on thyroid uptake but not with the conversion ratio data.

c. Changes in Uptake/Retention with Maturation. The pattern of uptake/retention (figure 13) is basically the same as that indicated by percent uptake (figure 6), showing an increase in thyroid activity with salinity and season. Values are generally more consistent.

C. Conversion Ratio

1. Pattern of Conversion Ratio Over a Period of Time After Injection

Changes in the conversion ratio over a period of time after injection are shown for high salinity in figure 15. The proportion of radiohormone to inorganic radioiodide in the blood plasma is seen to increase for the first four days after injection, and then to decline.

2. Relation Between Thyroid Uptake of Radioiodide and Conversion Ratio

The uptake of radioiodide by the thyroid varies with thyroid activity and the availability of inorganic radioiodide. Since the conversion ratio also indicates thyroid activity there should be a correlation between thyroid uptake and conversion ratio. No

to follow page 28

Figure 13. Seasonal changes in uptake/retention.*

A. Low salinity

B. Intermediate salinity

C. High salinity

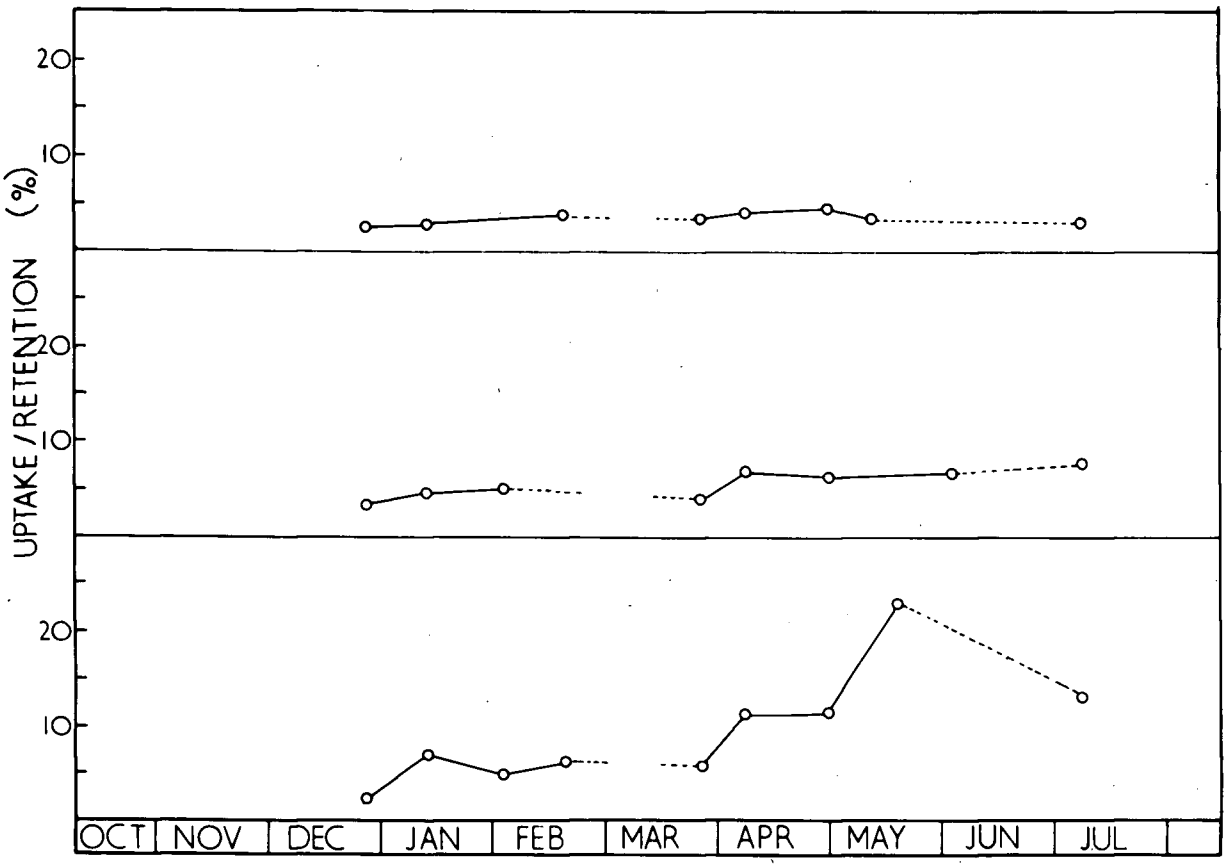
Figure 14. Seasonal changes in conversion ratio.*

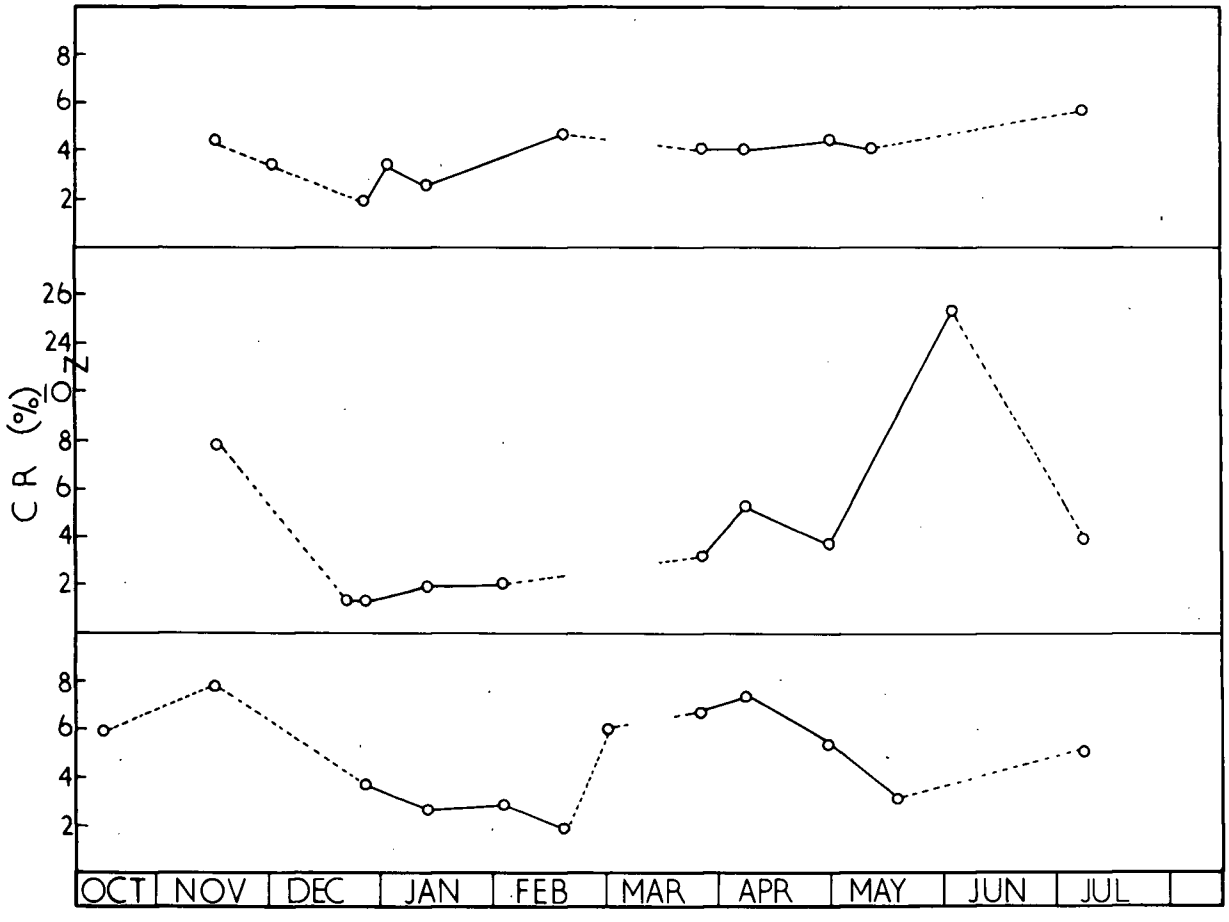
A. Low salinity

B. Intermediate salinity

C. High salinity

*Solid lines are used to connect groups with known equivalent history. Where collection date varied dotted lines are used. The line is interrupted to differentiate between the two major groups studied.


UPTAKE / RETENTION (%)
20
10
20
10
20
10
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL


C R (%)
8
6
4
2
26
24
10
8
6
4
2
8
6
4
2
OCT
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUN
JUL

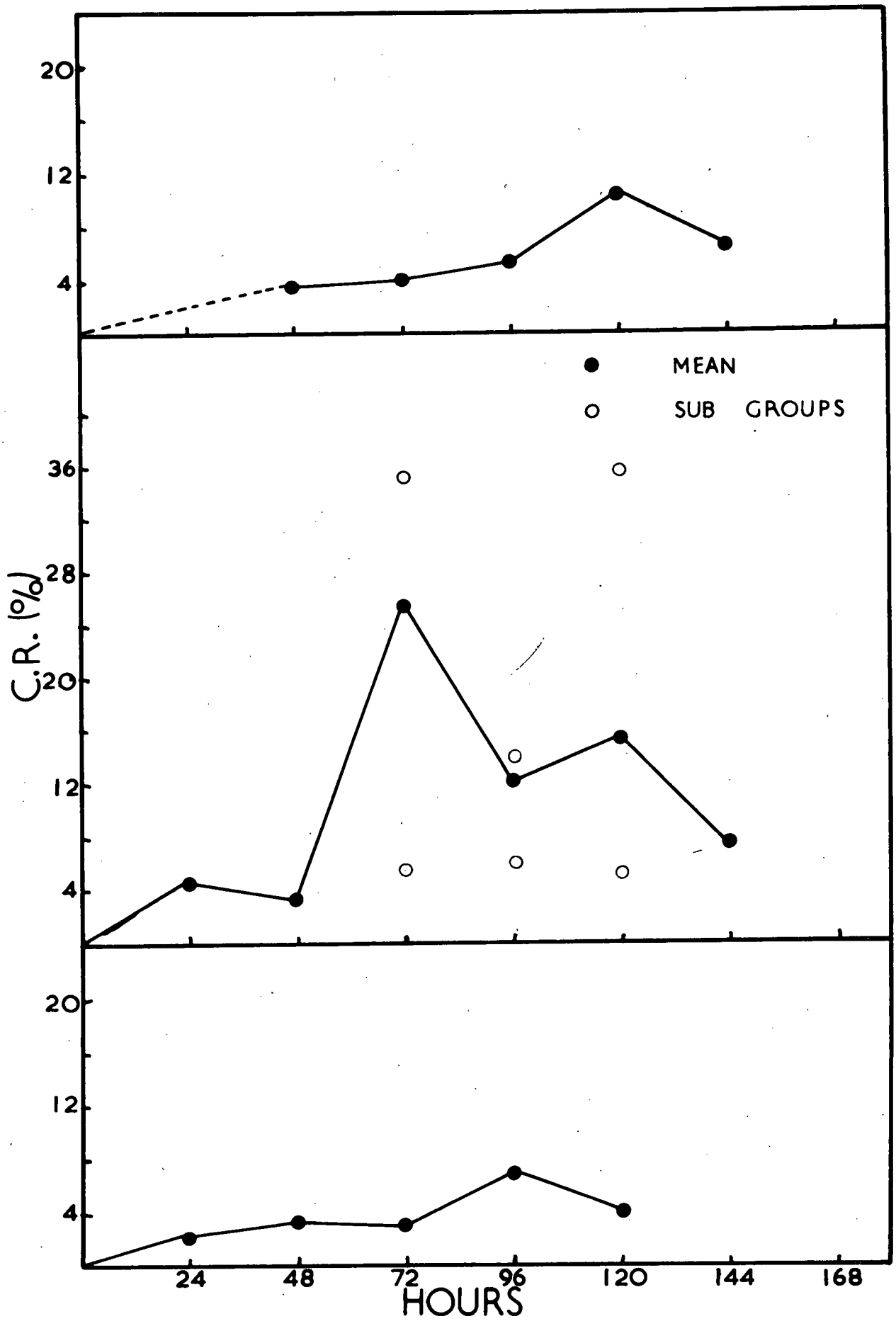
to follow page 28

Figure 15. Changes in conversion ratio with time after injection.

A. Low salinity

B. Intermediate salinity

C. High salinity


20
12
4
MEAN
SUB GROUPS
36
28
C.R. (%)
20
12
4
20
12
4
24
48
72
96
120
144
168
HOURS

correlation could be demonstrated between the two parameters. As the discussion will show this is probably the result of several factors.

3. Effect of Retention of Radioiodide on Conversion Ratio

Under conditions of constant thyroid activity, retention of radioiodide will determine thyroidal uptake of inorganic radioiodide and therefore the amount of radiohormone produced. The advantage of the conversion ratio is that it corrects the estimate of radiohormone production for variations caused by differences in availability of radioiodide. If the conversion ratio fails to make this correction a correlation between radioiodide retention and the conversion ratio could occur. Although the data suggested the possibility of a slight negative correlation the statistical analysis indicated no significant relationship (r for total group = -.093 with 7 degrees of freedom, r(P.05)= .433; r for males alone = -.520 with 10 degrees of freedom, r(P.05) = .666; r for females alone = -.327 with 19 degrees of freedom, r(P.05) = .576).

4. Effect of Size of Fish on Conversion Ratio

Examination of the data showed no correlation between size and thyroid activity as indicated by the conversion ratio.

5. Effect of Sex on Conversion Ratio

Statistical tests were carried out on the available data. The Students t value obtained (.471) with 19 degrees of freedom was within the 50% level (.688). As a result it was concluded

that the differences were not statistically significant.

6. Effect of Salinity on Conversion Ratio

Examination of figures 15c (low salinity) and 15b (intermediate) with figure 15a (high salinity) presented earlier allows one to compare conversion ratio at different salinities. The curves are not quite equivalent since the animals used in later experiments were more mature than those used in the earlier experiments. Changes in maturity are represented in Table VI.

Table VI. Comparison of the degree of maturity of fish represented by the conversion curves (figure 15).

	Low	High	Inter.
Salinity	5‰ Cl^-	19‰ Cl^-	12‰ Cl^-
Date of sample	12/5/61	19/5/61	3/6/61
Male G.S.I.	1.42	1.75	2.44
Female G.S.I.	4.90	8.13	15.18

In figure 15c (low salinity) a slow steady increase in conversion ratio occurred until 120 hours post-injection. After this time the ratio declined. A similar pattern existed in figure 15a (high salinity). The overall pattern of iodide metabolism was apparently accelerated so that the point of maximal conversion ratio was 24 hours earlier than in figure 15c.

Figure 15b (intermediate salinity) showed a different pattern. After a period of fluctuation the conversion ratio climbed to reach a "peak" value at 72 hours. The distinct peak was then followed by an irregular decline in the value of the conversion ratio. Detailed examination of the data of figure 15b indicated two sub groups at 72 hours, 96 hours, and 120 hours. The mean

values of each of the sub groups (subjectively chosen) have been plotted on the same graph. The immense variability in data suggests that the samples were taken at a time when a sudden change in thyroid activity was occurring.

A comparison of the conversion curves (mean conversion) ratios plotted against time after injection) showed several points of difference. Both the maximal conversion ratio and the time at which it is reached are seen to vary. Absolute maximal values are seen to vary in a decreasing order: intermediate> low > high. The time required to reach the maximal value declines in the following order: intermediate> high > low. This, of course, is ignoring the sub group characteristics of intermediate salinity data (see discussion).

7. Changes in Conversion Ratio with Maturation

A study of seasonal changes in conversion ratio indicated no major change of general significance between December and July (figure 14). A slight progressive increase in thyroid activity occurred in low (figure 14a) and intermediate (figure 14b) salinities. In high salinity (figure 14c) higher values were observed in the first samples of the March collection (group two). These values declined through March and April. The decline seems to have been superimposed on a pattern of general increase. The only outstanding feature is the point on the intermediate salinity curve for June 3. Here the single sample was composed of two sub groups. In one the conversion ratio ranged from 20% to 46%; in the other from 3% to 6%. Immense variability existed. Support for the

extremely high values is provided by similar occurrences at other times in the same conversion ratio/time curve from which these data were taken (figure 15). The "seasonal" trends of gradually increasing thyroid activity in low and intermediate salinities continued to be illustrated in spite of, or because of, the use of the new population of experimental animals (group two) for the first time on March 29, 1961.

Statistically, the increase in conversion ratio from January to July are significant in low and intermediate salinities. The surge in intermediate salinity on June 3 is not significantly different from adjacent values because of the extreme variability in the data. In high salinity the decline from the October, 1960 level to the January 13 value is significant as is the decline in value from February 4 to February 22. The increase from February 22 (group one) to April 9 (group two) is significant as is the decline from April 9 to May 19. The increase in conversion ratios between February 22 (group one) to July 8 (group two) is statistically significant. The failure to show statistical differences between the conversion ratios of May 19 and July 8 is directly attributable to the differences in variance. The variance of May 19 data is significantly less than the value obtained on July 8.

8. Effect of Technical Factors on Conversion Ratio

a. Variation in Dose. The effect of the dose of radioiodide on the conversion ratio was tested (Table IV). Doses of 1, 3, and 8 μc./fish were given to groups of eight fish. The average sizes of the groups were 1.28 grams (1.09-1.82), 1.50 (1.02-2.00) and

1.28 grams (1.02-1.87) respectively. An analysis of variance showed no significant differences ($F = 1.00$ with 2 and 15 degrees of freedom, $F(P.05) = 3.68$).

b. Delay in Preparation of Blood Samples. Results obtained during the course of studies indicated that the time taken to separate the protein and non-protein fractions of blood after precipitation of the plasma sample was influencing the conversion ratio. On January 24, 1961 the effect was tested. Blood samples taken from 24 fish representing a homogeneous population were collected at the same time and precipitated in close sequence. The first eight samples were prepared immediately, the second group of eight the following day and the final group 45 hours after killing. The only difference was the time elapsed between the initial precipitation with TCA. and separation of the two fractions.

The mean conversion ratios for the three groups are compared in Table VII.

Table VII. Effect of delay in preparation of blood samples on conversion ratio.

Date	Sub group	Delay (hr.)	C.R. (%)	S.D.
27/1/61	1	2	3.69	1.26
	2	25	8.14	3.20
	3	50	21.39	8.53
9/7/61	1	1.5	4.21	1.54
	2	23.5	40.46	9.17

The t tests carried out indicated highly significant differences between all groups (t, 1 and 2 = 3.618 with 13 degrees of freedom, $t(P.005) = 3.372$; t, 2 and 3 = 4.115 with 14 degrees of freedom, $t(P.005) = 3.326$, $t(P.001) = 4.140$). On July 10, 1961 the test

was repeated as part of another observation. One group (eight fish) was prepared immediately while the second was kept 24 hours before centrifugation was carried out. The results are also presented in Table VII. The differences between the two samples are again highly significant (t, 1 and 2 = 11.0 with 13 degrees of freedom, t(P.001) = 4.221). The 24 hour sample from January was compared with the equivalent July sample. The difference was significant (t = 9.42 with 14 degrees of freedom, t(P.001) = 4.140).

c. Effect of Radioiodide Retention by the Ovary. A statistical comparison of the male and female conversion ratio values (Table II) during the period when the ovary was retaining large amounts of radioiodide shows that the female values are statistically lower than those for males (F = 5.59 with 1 and 70 degrees of freedom, F(P.025) = 5.29).

9. Factors of Secondary Importance

a. Effect of Temperature. The previously outlined temperature study was applied to the conversion ratio. The calculated t value exceeded the tabled t value at the 40% level (t = 1.158 with 11 degrees of freedom, t(P.40) = .876). The higher conversion ratio was found in the fish held at 10°C.

b. Effect of Reentry of Radioiodide on Conversion Ratio. Reference to Table III indicates that only a small amount of radioiodide in the blood (less than 1%) is the result of reentry.

IV. DISCUSSION

The present study is an attempt to re-assess the work of Koch and Heuts (1942, 1943) and Heuts (1943, 1945) on the function of the stickleback thyroid in relation to osmoregulation. It must be assumed that the fish used were equivalent to those studied by Koch and Heuts. This is at present uncertain. The available works (Clemens, Carl, and Lindsey, 1959; Clemens and Wilby, 1961) do not recognise the occurrence of distinct taxonomic varieties in the local populations of stickleback. Observation of intermediate forms indicates the failure of local fish to be clearly defined as to type, suggesting that differences in population might exist. Apparent changes in composition of the local populations with season also introduces the possibility of genetic differences between fish obtained at different times. Differences in results might be the result of differences in the fish populations used.

A. Excretion of Radioiodide

The changes in iodide retention with time indicate that radioiodide is being removed from the body exponentially. This seems to be a general occurrence since it has been reported also by Hickman (1959) for Platichthys stellatus and by Eales (1961) for Oncorhynchus gorbuscha, O. kisutch, O. keta, and O. nerka. The excretion rate ($t_{\frac{1}{2}}$) for the stickleback has been compared with the excretion rates for a number of other species (Table VIII) as reported by Hickman (1959) and Leloup and Fontaine (1960).

The data recalculated from Leloup and Fontaine (1960) is based on the assumption that the two points given represent an exponential excretion pattern.

Table VIII. Comparison of excretion rates of several species of fish.

Species	Author*	Salinity	$t_{\frac{1}{2}}$ (hr.)
Xiphophorous maculatus (Platy)	LF	Fresh + iodide	12
Carassius auratus (Goldfish)	LF	Fresh	16
Gasterosteus aculeatus		19‰	34,34,41
G. aculeatus		12‰ + iodide	52
X. maculatus	LF	Fresh	86
G. aculeatus		5‰ + iodide	52,132
Platichthys stellatus (Starry flounder)	H	Sea water	87
Mugil auratus (Mullet)	LF	Sea water	106
P. stellatus	H	Fresh	111
P. stellatus	H	Fresh + iodide	152
X. montezemae (Swordtail)	LF	Fresh	156
Salmo salar (Parr-Atlantic salmon)	LF	Fresh	170

The excretion of radioiodide by the stickleback in sea water is more rapid than reported for some other species. When excretion by stickleback at low salinity is compared with excretion by fresh water fish the stickleback is seen to occupy an intermediate position. Since the stickleback is in a medium which is essentially isotonic it is not valid to compare its excretion rate with strictly fresh water animals which occupy a hypotonic medium.

*LF - Leloup and Fontaine, 1960
H - Hickman, 1959

The more rapid excretion of radioiodide by smaller fish observed in this study seems to be a general condition since it has been demonstrated in Platichthys stellatus by Hickman (1959) and in Salmo gairdneri by Eales (1962). Although a definite answer to the cause is unknown some possible explanations are available. Hickman (1959) attributed the greater excretion by small fish to their greater metabolic rate. Data presented by the same author showed that during the first few hours after injection of radioiodide both blood concentration and excretion of this material were greater. It may be that the greater concentration of a standard dose in smaller fish has an effect on its excretion. The relatively greater surface area of smaller fish might have an effect also. It is probable that the gill area (and therefore the available excretory surface) is relatively greater in smaller fish (Black, 1957). This might be a part of the cause of the greater rate of excretion. Since these topics are complex and the subject of considerable work it seems unwise to speculate further.

Some definite differences in excretion were observed between males and females. The differences are attributed almost entirely to the retention of large amounts of radioiodide by the ovary. Leloup and Fontaine (1960) have shown that the ovary, among other organs, has the ability to concentrate radioiodide. Reference is made by these authors to the concentration of radioiodide by the ovary of amphibia, birds, and mammals. Although the ovary of the stickleback contains large amounts of radioiodide there is no

evidence to indicate that it was necessarily being accumulated.

A sharp increase in the radioiodide content of the ovary occurred over a range of G.S.I. values from 5% to 10% (figure 8). At this stage of maturation the ovary fills the body cavity and distends the animal. This, plus observations made during the injections of fish, suggest that the initial localization was the result of an unavoidable injection of radioiodide into the ovary. Normally, diffusion would be expected to redistribute the radioiodide in a short time and since the ovary is heavily vascularized the removal of radioiodide should have been a certainty. The ovary, however, retained major amounts even after five days. It seems, therefore, that some mechanism exists which binds and holds the iodide, thus preventing or delaying free diffusion. The data indicated also that radioiodide held by the ovary was not available for excretion, thus making retention values for mature females erroneously high. As a precaution females showing radioiodide retention in the ovary were eliminated from the data.

The failure to demonstrate real differences (if such exist) between sexes can be attributed to the great degree of variability in the retention data. Individual variation, weight related effects, and technical effects resulting from retention by the ovary are obvious sources of variability. The effects of handling, salinity and seasonal changes further complicate the data.

Data presented in figures 1 and 2 show that salinity appears to have two major effects on retention of radioiodide. Both the (stable) rate of radioiodide excretion and the amounts lost during the first 48 hours vary with external salinity. Hickman (1959,

working on Platichthys stellatus) has shown that excretion of radioiodide occurs more rapidly in salt water than in fresh water. In the stickleback the rate at which it was removed from the animals ranged from a $t_{\frac{1}{2}}$ of 34-41 hours in high salinity, to 52 hours in intermediate salinity, and 52-132 hours in low salinity. The trend indicates that excretion in higher salinities is usually more rapid than in lower salinities. The variability is great. Reference to seasonal changes in retention at low salinity (figure 5) shows that the slow rate of excretion based on the retention/time curve obtained in December (figure 1) paralled a period of high retention. The more rapid excretion rate in May occurred at a period of low retention. Although technical factors discussed below may be involved it seems reasonable to attribute the changes in retention to changes in the excretion rate occurring during the maturation of the animals.

An important problem presented by the same data (figure 1) concerns the amount of radioiodide excreted. It is not necessarily greatest under conditions of high salinity as expected. During the first day or two the amount of radioiodide excreted is greatest in lower salinities which have lower stable excretion rates. Reference to figure 1 emphasizes the point. In figure 1 (low salinity) the major portion of the excretion seems to have occurred in the first 24 hours.

A similar but smaller rapid loss of radioiodide was shown in Platichthys stellatus by Hickman (1959) and by Eales (1961) in several species of Oncorhynchus. Hickman was able to show that this initial loss of radioiodide occurred before it had been

distributed through the body and that the greater initial concentration of radioiodide may be the cause. Since the time required for an equilibrium to be established in Platichthys stellatus was greater in salt water than in fresh water (35 hours vs. 15 hours) the greater effect in saline media could be explained. The available data on stickleback are unable to indicate differences in the time required for equilibration of injected radioiodide. The establishment of a constant rate of excretion after 24 hours indicates that in both high and low salinities equilibration has been achieved (Hickman, 1959). Since the effect in the stickleback is greater in lower salinities the differences in time required for the radioiodide to enter the blood and equilibrate fail to provide a complete answer.

It is possible that the loss of radioiodide is the result of laboratory diuresis (Forster, 1953; Forster and Berglund, 1956). Forster has shown that unusually large quantities of chloride are excreted during the diuresis. If iodide metabolism approximately parallels chloride metabolism laboratory diuresis could produce the observed effect.

Information presented below suggests that the metabolism of iodide is similar to that of chloride. Mention has been made of the high concentrations of radioiodide found in the gonad of the stickleback. It may be more than a coincidence that the ovary of the stickleback contains a high concentration of chloride as well as iodide (66.3 mM/Kg.Cl in ovary as compared with 36.6 mM/Kg.Cl in the muscle, Wiggs, 1961). Studies on mammals indicate that a variety of (other) tissues also contain high concentrations of

chloride (Manery, 1954). A comparison with the available data by Leloup and Fontaine (1960) shows that the following tissues have been shown to accumulate both ions: ovary, salivary glands, and gastric mucosa.

Additional information is produced by Manery (1954) who states that the distribution of bromide and chloride between the plasma and tissue (salivary gland) show the same ratio. The ability of blood proteins to bind chloride has been demonstrated (Manery, 1954) while the binding of iodide to protein is well known (Leloup and Fontaine, 1960; Robbins and Rall, 1960). Evidence also exists to show that the notochord of the lamprey concentrates halides in this order: chloride < bromide < iodide (Leloup and Fontaine, 1960). This sequence is related to the increasing size of the halide ion, which has been shown to be of importance in electrolyte studies (Podolsky, 1960). The latter author suggests that this may be related also to some aspects of membrane transport of ions. The evidence seems sufficiently strong to warrant the assumption that the metabolism of halides is very similar. Differences certainly exist but they appear to be quantitative in nature.

Laboratory diuresis is known to occur in marine fish under conditions of stress (Forster, 1953). Stress in the stickleback could be caused by injection of radioiodide. The apparent recovery during the first 24 hours suggests that the diuresis was not severe (Black, 1957). Both Forster (1953) and Pickford and Atz (1957) state that the condition of laboratory diuresis suggests that stress can inhibit an antidiuretic factor.

It is known that marked changes occur in the hypothalamo-

hypophysial system of Salmo gairdneri upon handling and on transfer to sea water (Carlson and Holmes, 1962). These changes are believed to represent changes in levels of oxytocic and antidiuretic substances in the neuro-hypophysis. Carlson's work indicates that the handling effect is not the same as the response to sea water. McBean and Holmes (1962) have shown a reduction in inulin clearance rate of Salmo gairdneri after adaptation to sea water. Carlson feels that this may represent an antidiuresis. Since the stickleback can be expected to have a copious urine flow in fresh water and exhibit a minimal flow under marine conditions (Black, 1957) some antidiuretic mechanism must exist. The speculation of Forster (1953) and Pickford and Atz (1957) as to the inhibition of the antidiuresis therefore seems reasonable.

The graded increase in the diuretic effect from higher salinities to lower salinities presents an additional problem. Why should diuresis occur to a greater extent under conditions of low salinities than at high salinities? The importance of volume receptors in the control of antidiuretic hormone (in mammals) has recently been discussed by Sayers (1959) and Welt (1959). Welt states that

> With respect to the antidiuretic hormone, it is apparent that the effective osmolality of the body fluids is the primary stimulus leading to the elaboration of a concentrated or a dilute urine.

Under strict marine conditions a high osmolality would provide a strong stimulus to maintain antidiuresis. As the osmolality of the body fluids declined (with external salinity) the intensity of the stimulus would decline also. Production of antidiuretic hormone

would be under a weaker control. Stress phenomena could therefore have a greater effect at lower salinities.

The preceding discussion has indicated that a diuretic effect could produce the results and could occur in the manner indicated by the data. Is there any evidence to suggest that diuresis occurs in the stickleback? The classical studies on excretion consider that the teleost kidneys are not important in the excretion of chloride (Smith, H.W., 1930). Hickman (1959) has presented data indicating that the excretion of iodide by the kidneys is relatively small. In both cases extrarenal mechanisms (gill) are considered to be the normal excretory path for the monovalent ions. A similar condition has been demonstrated in Selachians where chloride excretion by the gills has also been shown to occur (Leloup and Fontaine, 1960).

During laboratory diuresis the normal pattern is upset and significant amounts of chloride are excreted by the kidney (Forster, 1953; Forster and Berglund, 1956). Examination of iodide excretion data obtained from divided box studies on the stickleback showed a similar pattern. Approximately equal amounts of iodide were excreted from the posterior (kidney) region as from the anterior (gill) region. These experimental fish were under conditions which would have produced diuresis if anything could (see results, page 19). The assumption that this excretion represents a diuretic condition is reasonable.

The controls which were unavoidably disturbed from time to time show extreme variation in their total excretion patterns. The samples labelled A and B can be considered relatively normal

(compare with high salinity excretion/time curves, figure 2). Comparison of "normal" controls and experimental fish indicates that excretion is approximately doubled during diuresis. Other "control" fish (figure 7) exhibit patterns representing various degrees of diuresis or differences in time at which the onset of diuresis occurred.

On the basis of these data the following assumptions are possible. Laboratory diuresis can occur in the stickleback. The diuresis affects the retention of iodide and is probably the cause of the large initial loss of radioiodide from low salinity fish. The variable degree of diuretic conditions exhibited by the different control animals also provides an explanation of the large variability in retention data.

The reentry of radioiodide is unlikely to have had any appreciable effects. If there was any significant effect it would have occurred only under extreme conditions (low salinity during periods of diuresis). It should be noted that reentry would probably be greater in low salinity than indicated by the data. Since the available evidence agrees with the findings of Hickman (1959) as to the insignificant effects of reentry the topic will not be considered further.

The failure to demonstrate an increase in excretion with increases in temperature as reported by Leloup and Fontaine (1960) may be the result of the experimental circumstances. Because of the variability of the small samples used it is unlikely that anything other than a gross effect could be shown. Seasonal changes

in iodide metabolism such as were demonstrated in figure 5 could have proceeded to different extents under the two sets of conditions. Since the fish had been exposed to the different conditions for two months (April 9 to June 3) there was ample time for such changes which may have masked any temperature effect. It may also explain the unusual pattern of thyroid activity illustrated by both the uptake and the conversion ratio. Although the thyroid of fish usually responds to increasing temperature with an increase in activity (Leloup and Fontaine, 1960) the opposite has been observed (Gorbman, 1959).

The work of Heuts (1945) indicates that the experimental conditions (14‰ Cl^- and 10°C. or 17°C.) would be lethal to the fish used. Harris (1959) has shown that a variety of stresses will inhibit thyroid activity. The mortality in the high temperature tank suggests a condition of stress which might have inhibited the thyroid.

This experiment emphasizes the differences between the present study and the work of Heuts (1945). He has shown that although both combinations of temperature and salinity used in the present study would be lethal, fish at a higher temperature can tolerate a higher salinity. In the present study fish held at 17°C. and 14‰ Cl^- exhibited considerable mortality, while the "more rigorous" conditions of 11°C. and 14‰ Cl^- mortality was negligible. Further work on this topic might prove rewarding.

B. Thyroid Uptake of Radioiodide

The pattern of thyroid uptake in the stickleback indicates

that the major portion of the radioiodide is probably accumulated in the first 24-48 hours and declines after 72-96 hours. The shape of the uptake curve, especially the time required for the attainment of maximal value, has been shown to change with thyroid activity (Leloup and Fontaine, 1960; Eales, 1961). This pattern depends upon the balance existing between the accumulation of inorganic radioiodide and the production and release of radioiodide as hormone. Initially, large amounts of radioiodide are available and relatively large amounts can be accumulated. Later, when less is available the thyroid is unable to accumulate enough inorganic radioiodide to compensate for the radioiodide released as hormone and the thyroidal content of radioiodide levels off and declines.

Figures 1, 2, and 9 show that the maximal level of thyroid uptake is directly related to the amount of available radioiodide. The initial rate of excretion (first 24 hours) is inversely correlated with the time required for the thyroid to attain its maximal level of uptake. In the stickleback it appears that the shape of the uptake curve, as well as the level of thyroid uptake, may be controlled by the availability of radioiodide as well as by thyroid activity.

Usual levels of radioiodide uptake in the stickleback were between 1% and 2% with occasional increases to 3%. Individual values as high as 4% to 5% were observed. These values are similar to the maximal values of 4% or less reported for the mullet (Mugil auratus) and the conger eel (Conger conger) by Leloup and Fontaine (1960) and the starry flounder (Platichthys stellatus) by Hickman (1959). The values are less than those reported for Fundulus

heteroclitus (15%-20%) by Berg (1959).

The variability of the thyroid uptake results is partially caused by technical problems. It was not possible to remove all vascular tissue when preparing thyroids for radioactive counting. As a result, inorganic radioiodide contained in the blood was unavoidably included in the sample. The error from this source will be greatest in the first 24 hours and will decline from that time on. Another complication producing false thyroid uptake values is the fact that thyroidal iodide does not necessarily represent hormonal iodide. For example, large amounts of inorganic iodide are present in the thyroid of the carp, Cyprinus carpio (Leloup and Fontaine, 1960). They indicate that although the effect is most important soon after injection it may persist for a long time.

It has been established that thyroid uptake values and their use as an indicator of thyroid activity will be affected by anything which affects the retention of radioiodide. Although the error produced seems to be extreme in the stickleback, it is not confined to this species since Leloup and Fontaine (1960) have recognized its importance. Unfortunately, not all authors seem to appreciate this fact. The demonstration that correlation between uptake and retention may occur in some sets of data (figure 10) and not in others is probably attributable to the experimental conditions (salinity), technical factors (laboratory diuresis), and thyroid activity itself. In contrast to thyroid uptake measurements the conversion ratio does not appear to be affected by differences in retention.

The failure to demonstrate a relationship between size and thyroid uptake is puzzling since retention has previously been correlated with both uptake and size. Under conditions of constant thyroid activity the thyroid uptake of radioiodide depends upon blood concentration of the ion (Wollman, 1960). Small fish could be expected to have a greater initial blood concentration and therefore a greater initial uptake. The lack of difference in uptake with size at 72 hours may indicate that the more rapid excretion of radioiodide in smaller fish has compensated for the higher initial concentration. The average concentration of radioiodide over the period may have been the same in large and small fish. Although no significant difference is seen in the ability of smaller fish to accumulate radioiodide when its availability is considered (uptake/retention) the data suggest that the thyroids of smaller fish are more efficient. This could be an artifact. It seems reasonable to assume that the level of thyroid uptake depends on the mean blood concentration of radioiodide which may be approximately equal in small and large fish. Since the rate of excretion is considerably greater in small fish the use of a blood concentration figure would create an artificially high uptake/retention or uptake/blood concentration value. Ideally thyroid clearance measurement (rate of uptake/rate of excretion) should be used. This is largely speculation but it does seem worthy of further study.

If small flounder have a higher thyroid activity as a consequence of their metabolic rate as Hickman (1959) has suggested, the same condition could be expected in stickleback. Neither the

thyroid uprake or conversion ratio show such an effect. Errors in the techniques or differences in size ranges used in the two studies could explain the discrepancy. The range of sizes (1 to 110 grams) used by Hickman (1959) was considerably greater than the range (1 to 2 grams) used in this study. Hickman (1959) has emphasized that it is only by using a large size range that some effects can be shown.

The differences in the slope of uptake/retention relationships between males and females seems to indicate that the female thyroid is less efficient. If some of the retained radioiodide in the females was not available for uptake the observed uptake/retention would be less than in a comparable group of males. The females used in this experiment were beginning to retain radioiodide in the ovary. Although the levels held were low (1% to 3% maximum) they might be responsible for the delay in thyroid uptake. Similarly, the only differences between male and female conversion ratio values occur when female gonads retain large amounts of radioiodide. Under such circumstances no conclusion can be reached regarding sexual differences in thyroid activity. Uptake/retention data could represent real differences between sexes but there was no similar occurrence in winter fish.

The changes in uptake at different salinities seems to be the result of variations in radioiodide retention. For this reason no attempt was made to apply statistics to the differences between thyroid uptake values obtained under different conditions of salinity.

Since no significant differences could be demonstrated in the iodide metabolism (uptake or conversion ratio) of fish injected with different doses of radioiodide it seems reasonable to assume that no harmful effects were produced. In terms of μc/gram (approximately 1, 3, and 8 μc/gram) the doses were quite large compared to tracer doses used by Harris (1959), Hickman (1959) and Eales (1961). The failure to observe any effect could be attributed to the rapid excretion of radioiodide and the low level of uptake by the thyroid. The duration of the experiments (three days) would also reduce the risk of radioactive damage to the thyroid.

The most important point demonstrated by this section of the study is the dependency of thyroid uptake on the availability of radioiodide. Changes in the retention of radioiodide have been demonstrated with size, salinity, and sexual maturation. Technical factors (retention of radioiodide by the ovary) have been shown to have an effect while temperature changes probably do even though they were not demonstrated. Unless corrections can be made for these factors thyroid uptake cannot be used as a reliable estimate of thyroid activity. Theoretically the calculation of uptake/retention or uptake/blood concentration should correct for variations in the retention of radioiodide. Unfortunately, such a correction does not seem to be entirely valid under the conditions of this study. Since the same problem affects the conversion ratio it will be considered in relation to the conversion ratio.

The discussion on page 47 has shown that certain conditions might invalidate the use of a terminal uptake/retention or uptake/blood concentration measurement as an index of thyroid activity.

The accumulation of radioiodide by some organ in the body would be expected to invalidate uptake/retention also. The loss of radioiodide from the thyroid before the time at which the observation was made will create falsely low uptake/retention or uptake/blood concentration values. It will become more significant as thyroid activity increases. This point seems to be a major failing of the parameter uptake per unit retention. The contamination of thyroid samples by radioiodide in associated non-thyroidal tissue will create high uptake/retention values. In the present study this error would be greatest under conditions of high salinity where diuresis has the least effect. No detailed study of uptake/retention and uptake/blood concentration was attempted since there was no reason to believe that they are more valid than the conversion ratio.

C. Evaluation of Conversion Ratio

Eales (1961) has demonstrated a good correlation between histological and radioiodide techniques used for measuring thyroid activity. During an extensive evaluation of the conversion ratio he showed that the technique seems to be a sensitive and reliable indicator of thyroid activity. Since the evaluation does not apply to the precise condition of the present study a re-evaluation of the technique was carried out.

The dependency of thyroid uptake on changes in available inorganic iodide has been indicated. A supposedly more accurate method of estimating thyroid activity is the conversion ratio. It attempts to measure efficiency of hormone production while correcting for differences in retention of radioiodide. The conversion

ratio assumes that the amount of radiothyroxine produced depends on the thyroidal uptake of radioiodide. The hormonal radioiodide is expressed as a fraction of total blood radioiodide. This should correct for variations in radiothyroxine production caused by differences in available radioiodide. Even the lapse of time between the uptake of radioiodide and the output of radiothyroxine would have no harmful effects if the rate of excretion was constant since all factors would be proportional. Conversely, if the rate of excretion were not constant the factors would not be in proportion.

When the conversion ratio is calculated a number of assumptions are made. One is that existing blood levels of iodide are representative of (or proportional to) earlier conditions. Examination of figures 1 and 2 indicates that this is not so for conditions of low and intermediate salinities and not always so at high salinity. During the first 24 hours the excretion may be very rapid as a result of diuresis. The stable excretion rate after recovery from diuresis is usually much slower. The cause of the error can be most easily explained by means of a simple calculation based on figures 1 and 2. It must be realized that the complexity of the real situation prevents an evaluation of the effect with available data. Only the principle can be demonstrated.

For simplicity the rate of excretion during the first 24 hours from low salinity (figure 1) and high salinity (figure 2) will be considered equal (the actual difference will increase the error). Thyroid activity will also be held constant and set at an arbitrary level so that the production of radiothyroxine at

48 hours is equal to 10% of the available radioiodide at 12 hours. The effect of observed changes in excretion on the conversion ratio under these conditions is demonstrated in Table IX.

Table IX. Demonstration of error produced by changes in excretion rate.

Salinity	12 Hr. ret. (% dose)	48 Hr. radio-thyroxine (% dose)	48 Hr. ret. (% dose)	48 Hr. C.R. (%)
High	66	6.6	34	20
Low	25	2.5	18	14

Excretion at high salinity (May, 1961; figure 2) shows a relatively exponential pattern. The 48 hour retention is therefore approximately proportional to the radioiodide available during the major period of uptake (figure 9) and hormone production. In this case, the expression of hormonal radioiodide as a percentage of the 48 hour retention figure provided an estimate of thyroid efficiency. Other high salinity data (October, 1960) and February, 1961) showed variations in excretion rate and are subject to the same type of error as discussed below for low salinity data.

Low salinity data violated the basic assumption. By definition the glands were equally active, yet the conversion ratio is 14/20 x 100% = 70% of the true value because of the change in excretion rate. Since conversion ratio is not absolutely reliable unless the rate of excretion is constant its use for the comparison of thyroid activity at different salinities is not justified.

The extent to which radioiodide is accumulated during the diuretic period, the time required for changes in uptake to be

expressed as the release of radiohormone, and the amount of radioiodide accumulated after the diuresis may reduce the error. Factors which can modify the diuresis could also reduce the error in conversion ratio. Such changes occur during the process of maturation. Although unlikely, it is possible that the effect could be constant throughout the study. If so, the conversion ratio will indicate relative levels of thyroid activity.

Another encouraging possibility is that the seasonal changes in retention would modify the bias created by the changes in excretion rate. During periods of increased retention error in the conversion ratio caused by the diuretic effect is likely to be reduced or absent. As a result the conversion ratio will be most reliable during periods of high retention. Even under conditions of constant thyroid activity an apparent increase in conversion ratio would result because of the increased reliability of the conversion ratio. An actual increase in thyroid activity would show an even greater change relative to values obtained during low retention periods. The relative constancy of the conversion ratio within a single salinity condition (figure 14) during periods of high retention may actually indicate a drop in thyroid activity as retention increases. This is, however, speculative.

In view of the moderating effects it is possible that the conversion ratio will indicate relative changes in thyroid activity. These results may not be reliable and when data from different salinities are compared they will be less reliable. It is unlikely that any real evaluation of the relation of thyroid activity and

salinity can be carried out. The estimate of thyroid activity (uptake/retention) would suffer from the same limitations.

The discovery of an unexpected weakness in the conversion ratio suggests that additional evaluation is required. The basic assumption that the protein-bound iodide represents hormonal iodide is probably not entirely justified. It is not completely true for mammalian work because a very small fraction of free thyroxine (T_4) exists in equilibrium with the bound T_4 (Robbins and Rall, 1960). Evidence exists that triiodothyronine (T_3) and T_4 are not completely bound to plasma proteins in fish (Leloup and Fontaine, 1960). The magnitude of error in the conversion ratio caused by this is probably slight since the amount of free hormone is very small (Robbins and Rall, 1960). There is also the suggestion by Barker (1962) that free thyroid hormone may be precipitated by TCA., eliminating the error completely.

On the other hand, monoiodotyrosine (MIT), diiodotyrosine (DIT), and inorganic iodide are bound to blood proteins in fish (Leloup and Fontaine, 1960; Hickman, 1962). In many cases the inorganic iodide is separated from the plasma protein during the preparation of the sample and has no effect (Leloup and Fontaine, 1960). In the stickleback the precipitated blood protein binds inorganic iodide. Since nothing is known of the relation between the precipitated protein and other iodide-containing substances (i.e. T_4, MIT) a need for caution and further study is indicated.

The seasonal change in the binding of radioiodide by precipitated protein further complicates the problem. The obvious explanation is that the blood proteins have changed. Quantitative

and qualitative changes in plasma proteins have been widely demonstrated (Ho and Vanstone, 1961; Vanstone and Ho, 1961; Meisner and Hickman, 1962). These authors have either reported or cited reports showing changes in blood protein as a result of: injection of estrogens and anterior pituitary extracts, migration and temperature changes, smoltification, and sexual maturation of fish. Differences between the sexes were reported for salmon (Vanstone and Ho, 1961). Some similar changes in the thyroid binding plasma proteins have been discussed by Robbins and Rall (1960). Since the changes in plasma protein could affect thyroxine binding and the ratio of free to bound thyroid hormone the reliability of the conversion ratio is not established for the stickleback. Since so many factors have yet to be studied and evaluated the conversion ratio must be used with caution.

D. Conversion Ratio

Examination of figure 15 shows that the conversion ratio curve may vary in two ways. The level of the conversion ratio at any time varies with the activity of the thyroid (Hickman, 1959; Eales, 1961). The time at which the maximum value occurs may also vary (figures 15a, b, and c). Although it seems logical that this time should be related to thyroid activity and therefore to the level of the conversion ratio an examination of figure 15 (a and c) suggests that this may not always be so. The observed differences indicate a need for further study.

Considering the weaknesses in the parameters involved it is not too surprising that percent uptake and conversion ratio are not

correlated. One factor of importance which disrupts the expected correlation is the dependence of thyroid activity upon iodide retention. The time after injection at which samples are taken can also have an effect. After three days (figure 9) the thyroid content of radioiodide has begun to decline. The use of these figures would not be a true indication of thyroid uptake. It is also probable that the production and release of radiohormone in an active gland would cause the level of thyroidal radioiodide to be lower than in a less active gland throughout the whole post injection period. If the conversion ratio (figure 15) indicates the true state of thyroid activity this has happened in thyroid uptake curve figure 9b as compared to 9c.

No consistent relationship occurs between thyroid activity (conversion ratio) and external salinity. Some of the variability may be attributed to technical errors in the conversion ratio. Although they cannot be used in a general comparison of thyroid activity at different salinities conversion ratio values obtained during periods of minimum excretion are more likely to be reliable. An examination of the effect of salinity on thyroid activity has been attempted using this data. Information obtained on December 27 shows that the conversion ratio at high salinity is greater than at low salinity. The thyroid uptake also increases from low to intermediate salinity. The suggestion that thyroid activity increases with salinity is not supported by the high salinity uptake value. Part of the discrepancy is the result of differences in retention of radioiodide while the release of large amounts of hormonal radioiodide could be responsible for the rest of the

depletion. A general trend, except for intermediate salinity value, suggests that thyroid activity increases with salinity. Data obtained in April show a more constant trend. Thyroid uptake (corrected for different levels of retention) and conversion ratio both indicate greater activity at higher salinities. This would confirm Hickman's (1959) observation on the starry flounder (Platichthys stellatus). Unfortunately, the data are too limited to justify any real conclusions, especially when the observed seasonal changes are considered.

Leloup and Fontaine (1960) have shown that the inorganic radioiodide which is loosely bound to the blood protein of some fish is freed by TCA. precipitation. It is not known if the binding of inorganic iodide to blood protein occurs under physiological conditions in the stickleback but it has been demonstrated that the precipitated protein can bind inorganic radioiodide. The occurrence of the effects in at least one other species, (Carassius auratus, Hoar, 1961), demonstrates that much care is needed in using the conversion ratio technique.

The increase in the binding effect from January to July, which may be related to the maturation process, indicates a change in the nature of the blood proteins. If the binding of inorganic iodide to precipitated protein reflects a similar condition in the live animal it might indicate the development of an iodide conserving mechanism. Such a mechanism has been shown to exist in several migratory species of teleost by Leloup and Fontaine (1960) who have shown that an increase in the binding capacity of blood protein for

iodide occurs at migration in the Atlantic salmon (Salmo salar). Although the stickleback can be classed with the rest of the group the goldfish cannot. The observation of a greater effect in mature fish would also be contrary to the results of Leloup and Fontaine (1960) who have shown that inorganic iodide binding declines at sexual maturity. There is also no correlation between the inorganic iodide binding and the observed changes in radioiodide retention.

E. Seasonal Changes in Radioiodide Metabolism

The changes in radioiodide metabolism during the study do not actually represent seasonal changes. It is more correct to interpret them as changes occurring during maturation in two independent groups of fish. The work of Baggerman (1957) indicates that both collections could have reached a physiological state of migration readiness by the time of capture. In this state, exposure to increased temperature and/or longer daily photoperiods would initiate the maturation process or stimulate the process if previously initiated. The transfer to laboratory conditions would probably have been a sufficient stimulus to initiate maturation in both groups since they experienced an increase in temperature. Group two was also exposed to a 16 hour daily photoperiod while group one fish were held under an eight hour photoperiod. It may be important that the first group of fish failed to show any significant sexual maturation. Neither group was completely unresponsive since changes in some aspects of iodide metabolism did occur in both cases. The most apparent change was the gradual

increase in radioiodide retention. It reached a maximum about four weeks after the fish were placed under experimental conditions.

Stickleback (figure 5) about to enter fresh water at the time of peak retention would possess a definite advantage since both the conservation of salts in general and iodide in particular are important in this medium. The conservation of iodide might be of special importance since it, unlike chloride, may not be absorbed from the environment (Black, 1957). Changes of this type have been shown to be related to fresh water migrations (Leloup and Fontaine, 1960).

If the iodide excretion represents the general condition of the osmoregulatory mechanism the data will be more informative. In view of similarities in the distribution of iodide and chloride in body organs as well as apparent similarities between iodide and chloride excretion during laboratory diuresis it is probable that the excretion of iodide does indicate the general pattern of ion excretion.

Baggerman (1957) has shown that stickleback whose maturation was initiated on October 1 developed a preference for fresh water by the second half of October (two to four weeks). Gonadal maturation did not occur until seven weeks had elapsed. In both group one and group two of the present study the peak of iodide retention was at four weeks after exposure to laboratory conditions while gonadal maturation required a somewhat longer period. This suggests that the retention of radioiodide is related to the development of the fresh water preference. A similar conclusion, based on studies of behavior, was reached by Baggerman (1957) who

suggested that the ion retention observed by Koch and Heuts (1942, 1943) might be involved in the control of salt and fresh water preference. It seems reasonable to conclude that the changes in iodide retention observed in the present study are indicative of the type of change reported by Koch and Heuts (1942, 1943).

The increase in radioiodide (salt) retention at the time of fresh water migration supports the theory proposed by Koch and Heuts (1942, 1943) and by Baggerman (1957) which assumes that ion retention is a compelling mechanism. The accumulation of ions affects the physiology of the animal which in turn modifies behavior. The animal must then migrate to fresh water. The seasonal increase in thyroid activity is claimed to be the controlling factor. Increased thyroxine production in the spring is thought to disturb the existing osmoregulatory balance. In order to survive the animal is compelled to migrate into fresh water.

The evidence used by Koch and Heuts (1942, 1943) to support this hypothesis has been discussed in the introduction. Some unusual and somewhat complicated conditions were noted in the changes in blood chloride of the different races of Gasterosteus under different conditions. Houston (1958) demonstrated that both blood and tissue chloride values were related to size in salmonids. The same relationship between size and tissue chloride was demonstrated in the stickleback (Wiggs, 1961). Hickman (1959) has demonstrated that the osmotic pressure of the blood is also dependent on size. Since neither Koch and Heuts (1942, 1943) nor Heuts (1943, 1945) appear to have recognized this variable it may explain some of the complexities in their data. The ability of stickleback

to control the radioiodide retention so that the levels return to normal makes the compulsion theory of Koch and Heuts (1942) improbable. Similar changes involving the development and regression of salinity preference were also demonstrated by Baggerman (1957).

The most important point in the work of Koch and Heuts (1942) was the demonstration that thyroid material fed to stickleback disrupted osmoregulation in low and high salinities. It has been noted that increases in temperature can have equal or greater effects of a similar nature. This suggests that both thyroxine administration and temperature increases are acting through a third and common mediator.

The method used by Koch and Heuts (1942) to administer the thyroid material (feeding desiccated thyroid) is open to criticism (Hickman, 1959). It is known that exogenous thyroxine can alter the production of pituitary hormones other than thyrotropin (Harris, 1948; Olivereau, 1960; Pitt-Rivers, 1960). The treatment used by Koch and Heuts (1942) and Baggerman (1957) may have affected other endocrine organs and these may be the cause of the observed results. Such a mechanism was suggested as a possibility by Koch and Heuts in 1942.

If the retention of salts is caused by thyroxine, parallel changes between retention and thyroid activity should occur. Since iodide metabolism seems to be similar to chloride metabolism iodide retention will be used to follow the changes in the osmoregulatory state.

Examination of changes in thyroid activity during maturation reveals several points. One of the most striking is the failure

of the conversion ratio to show increases paralleling the changes in retention in either December or April. Thyroid activity at such a time may be declining or remaining constant but it is not increasing. The more reliable high salinity conversion ratio declined throughout the period of increasing retention. Some support for a decline in thyroid activity as retention increases comes from thyroid uptake observations in December. The thyroid uptake of radioiodide showed only a slight increase in spite of a major increase in iodide retention. This not only argues against the hypothesis of Koch and Heuts (1942, 1943) but also suggests that something related to osmoregulation may be producing a decline in thyroid activity. This is similar to the decline in thyroid activity suggested under near lethal conditions of temperature and salinity (see page 44). A failure of thyroid uptake to increase paralleling retention could also represent a decline in the efficiency of the trapping mechanism.

In the second group of fish (April) thyroid uptake does increase with retention but the uptake is greater in high salinity where the retention is least. This is virtually the only condition in the present study where thyroid uptake could be considered a reliable index of thyroid activity. The association of high thyroid activity with the lowest retention argues against a salt retaining function for thyroxine. While the thyroid is certainly not disturbing osmoregulation it might be acting in a compensatory role allowing fish to maintain a low retention at high salinity. The changes in thyroid activity might also be the result of some side effect. The trend (illustrated by the April thyroid uptake values)

is supported by the conversion ratios of this group.

A more detailed examination of the relationships between conversion ratio and iodide retention at each salinity (April) shows even less support for the hypothesis of Koch and Heuts (1942, 1943). In the low salinity fish which exhibited the greatest increase in iodide retention the conversion ratio showed no significant change.

Examination of the intermediate salinity data shows that the maximum retention occurs between April 10 and 30. Thyroid activity (conversion ratio and uptake) shows a decline at this time (not statistically significant). It is not until retention is declining at the end of May that the conversion ratio showed an increase. Although statistical significance is lacking the numbers of fish used indicate a real condition. It is possible that the increase represents a normal seasonal development related to sexual maturation. The differences in degree of maturity of the fish used in the different experiments could explain why similar changes in conversion ratio did not occur in low and high salinity studies since the fish in both cases were less mature than those used in the intermediate salinity.

Fish held at high salinity showed only slight changes in iodide retention during April. If a peak occurred it was at the end of the month. Thyroid activity (conversion ratio and uptake) showed a decline throughout most of April and May. In both sets (December and April) of data obtained at high salinity the conversion ratio declined during the period of maturation as retention of iodide increased. The observations made on March 27 and April 8

suggest that the conversion ratio had not been declining since capture but began to decrease as maturation progressed.

The work of Heuts (1945) on Belgian stickleback shows that at the experimental temperature (13°C.) full sea water is near the limit of tolerance for non-breeding fish while mature fish cannot tolerate 2/3 sea water. The high salinity fish were becoming less able to tolerate their medium as they matured. Under such conditions it seems reasonable to suppose that local fish, although tolerant of the medium, were under a condition of stress. The decline in thyroid activity observed during the period could have been caused by stress. Although neither the effects of osmotic stress nor the effect of stress on the fish thyroid have been studied, it is known that stress can inhibit thyroid activity in mammals (Harris, 1959; Pitt-Rivers, 1960).

The observed differences in tolerance between British Columbian and Belgian stickleback may be genetic. It is more likely that the differences are the result of the techniques used. In the present study fish occupied the medium continuously from the time of capture. At capture the animals could tolerate the salinity and acclimation could occur as they matured. The procedure used by Koch and Heuts (1942, 1943) and Heuts (1943, 1945) involved the transfer of fish in stages of increasing salinity to the test salinity. The fish, which were initially acclimated to fresh water (the holding medium) were allowed time (five days, Koch and Heuts, 1943; Heuts, 1943) to attain osmotic stability before being transferred again. Although some osmotic adjustment had been made full acclimation may not have occurred.

When the fish were eventually transferred to a high salinity a lethal accumulation of salts may have occurred before osmoregulatory adjustment could be made. If additional time had been allowed at the intermediate stages acclimation might have permitted the fish to tolerate higher terminal salinities. This would be of great importance if the time required to attain osmotic stability is greater at different salinities or at different times of the year.

The present study has shown no support for the disruptive effect of seasonal increases in thyroxine production on osmoregulation proposed by Koch and Heuts (1942, 1943). As a result some other mechanism must be sought to explain the observed changes in osmoregulation.

V. SUMMARY AND CONCLUSIONS

1. The rate of radioiodide excretion has been shown to vary directly with increasing environmental salinity and inversely with body size.

2. Seasonal changes in radioiodide retention appear to be correlated with the process of sexual maturation. The time at which these changes occur suggests that they may be related to the development of the fresh water preference observed by Baggerman (1957). The increases in radioiodide retention also support Koch and Heuts' (1942, 1943) observations of increases in the ionic constituents of the blood at sexual maturity.

3. Unusual excretion patterns have been demonstrated predominantly in low and intermediate salinities. Evidence is presented which may indicate that the abnormal excretion is the result of a condition of laboratory diuresis.

4. Data are also presented suggesting that extrarenal pathways (gills) are important in iodide excretion.

5. Disturbances in the normal pattern of radioiodide metabolism have been demonstrated in maturing females. The large amounts of radioiodide held by the gonad can invalidate measurements of retention, thyroid uptake, and conversion ratio.

6. Evidence is presented showing that thyroid uptake is an unreliable estimate of thyroid activity where retention of radioiodide varies. This occurs at different salinities and during the process of sexual maturation.

7. Evidence is presented to show that under conditions where

the rate of radioiodide excretion changes during the experimental period the conversion ratio is not a valid measure of thyroid activity.

8. The ability of precipitated plasma proteins to bind inorganic radioiodide has been demonstrated. Where precautions are not taken this can produce erroneously high conversion ratio values. Changes in this effect between January and July suggest that blood proteins change during sexual maturation.

9. No consistent relationship could be demonstrated between environmental salinity and thyroid activity.

10. A significant increase in thyroid activity (conversion ratio) has been demonstrated between mid-winter (December and January) and summer (May, June, July).

11. No correlation was shown between increased thyroid activity (conversion ratio) and the periods of increased retention which occur during maturation. Since the increase in radioiodide retention probably represents an increase in other ions this study provides no support for the theory of Koch and Heuts (1942) that increased thyroid activity is responsible for osmoregulatory disturbances which may occur in mature stickleback.

12. The data suggest that thyroid activity (conversion ratio) may decline under conditions of stress. Such conditions appear to occur when sexually mature fish are exposed to full sea water (19‰ Cl^-) or high salinities (14‰ Cl^-) and high temperatures (17°C.).

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APPENDIX

Table IA. Seasonal changes in iodide metabolism at high salinity.

Date	wt. of fish (range) gm.	G.S.I. male	G.S.I. female	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
17/10/60	0.82 (0.63-1.21)	1.07	1.91	8	21.6 (10.4-30.4)	8	3.55 (1.94)	----	8	5.88 (3.09)
17/11/60	1.21 (0.79-1.81)	1.22	1.53	16	3.1 (0.8-9.7)	9	1.04 (0.44)	----	16	7.32 (8.30)
27/12-60	1.02 (0.79-1.18)	--	--	6	27.0 (21.0-31.4)	5	0.82 (0.28)	2.36	6	3.73 (1.97)
13/1/61	1.31 (0.98-1.61)	1.33	2.34	8	19.2 (6.4-32.6)	8	1.26 (0.73)	7.04	8	2.69 (1.30)
3/2/61	1.29 (1.00-1.80)	1.76	2.87	8	24.6 (16.0-32.8)	8	1.28 (0.53)	4.91	8	2.93 (1.08)
20/2/61	1.51 (1.28-1.72	1.21	2.69	8	28.4 (18.8-44.4)	8	1.59 (0.56)	6.26	8	1.77 (0.79)
1/3/61	1.33 (0.94-1.75)	1.17	3.01	8	17.8 (5.3-27.5)	8	0.99 (0.50)	----	8	6.04 (4.49)
27/3/61	1.22 (0.97-1.49)	1.51	5.11	6	16.6 (4.4-28.6)	8	1.12 (0.44)	5.91	8	6.72 (1.94)
8/4/61	1.39 (0.78-1.70)	1.30	7.26	7	19.6 (5.5-33.4)	5	3.49 (2.43)	11.26	8	7.39 (2.71)
30/4/61	1.15 (1.01-1.27)	1.54	5.78	6	25.0 (12.3-51.6)	8	3.04 (1.86)	11.62	8	5.36 (1.75)
19/5/61	1.61 (1.33-2.03)	1.75	8.13	6	13.1 (7.0-23.9)	5	2.38 (1.06)	23.19	5	3.09 (0.85)
8/7/61	1.86 (1.57-2.22)	3.60	24.78	6	15.2 (3.7-41.7)	7	1.26 (0.66)	13.20	8	5.13 (3.13)

*Uptake/retention values are derived from the averages of individual fish.

Table IB. Seasonal changes in iodide metabolism at intermediate salinity.

Date	Wt. of fish (range) gm.	G.S.I. male	G.S.I. female	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
17/11/60	1.36 (1.02-2.25)	1.28	1.75	16	8.6 (0.5-17.3)	16	1.55 (1.21)	----	16	7.32 (8.30)
22/12/60	1.01 (0.74-1.50)	----	----	8	21.5 (8.3-39.2)	--	----	----	8	1.34 (0.99)
27/12/60	1.21 (0.89-1.97)	----	----	8	42.7 (22.6-69.3)	8	1.61 (1.25)	3.38	8	1.30 (3.03)
13/1/61	1.07 (0.87-1.39)	1.41	2.17	8	31.0 (10.2-49.7)	8	1.19 (0.62)	4.60	8	1.88 (0.71)
3/2/61	1.21 (1.07-1.45)	1.55	2.86	8	31.5 (22.1-37.5)	8	1.28 (0.38)	5.00	8	2.02 (1.00)
27/3/61	1.21 (1.00-1.44)	1.26	3.18	8	34.9 (24.9-53.7)	8	1.46 (0.53)	3.87	8	3.23 (0.31)
8/4/61	1.07 (0.89-1.28)	1.54	3.59	7	33.0 (17.1-42.5)	8	2.38 (0.99)	6.88	8	5.26 (1.74)
30/4/61	1.13 (0.83-1.62)	1.84	5.88	7	39.1 (19.9-52.0)	8	2.10 (0.54)	6.28	8	3.69 (1.84)
3/6/61	1.63 (1.43-1.80)	2.44	15.18	7	14.3 (5.4-24.0)	6	0.66 (0.22)	6.66	6	25.40 (5.66)
8/7/61	2.32 (2.02-2.65)	3.80	14.51	8	18.4 (4.0-50.5)	8	1.63 (0.58)	7.81	16	3.92 (1.13)

*Uptake/retention values are derived from the averages of individual fish.

Table IC. Seasonal changes in iodide metabolism at low salinity.

Date	Wt. of fish (range) gm.	G.S.I. male	G.S.I. female	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
17/11/60	1.29 (0.81-2.04)	1.32	1.60	16	11.4 (1.6-26.4)	16	1.00 (0.41)	----	16	4.08 (4.95)
1/12/60	1.23 (0.78-1.75)	1.17	2.06	8	15.9 (8.0-33.3)	8	0.58 (0.38)	----	8	3.42 (1.57)
27/12/60	0.99 (0.82-1.43)	--	--	8	49.8 (28.0-82.5)	8	1.26 (0.28)	2.45	8	1.86 (1.63)
13/1/61	1.15 (0.92-1.85)	1.15	2.22	8	33.6 (14.4-47.2)	8	0.86 (0.29)	2.73	8	2.48 (1.64)
20/2/61	1.36 (1.15-1.68)	1.20	--	8	33.9 (9.2-80.2)	8	0.95 (0.29)	3.75	8	4.59 (2.08)
27/3/61	1.16 (0.98-1.72)	1.26	3.18	8	34.8 (16.5-60.5)	8	0.91 (0.22)	3.39	8	3.99 (1.05)
8/4/61	1.00 (0.85-1.18)	1.27	3.94	8	57.3 (22.1-84.6)	8	2.02 (1.44)	4.07	8	4.00 (2.17)
30/4/61	1.14 (0.83-2.11)	1.15	4.07	8	15.2 (4.8-44.7)	8	0.66 (0.47)	4.56	8	4.44 (1.16)
12/5/61	1.08 (0.81-1.29)	1.42	4.90	6	8.0 (1.6-19.4)	8	0.21 (0.18)	3.49	8	4.01 (1.51)
8/7/61	1.86 (1.48-2.36)	2.91	14.38	8	35.4 (16.9-83.4)	8	0.98 (0.89)	2.99	8	5.67 (1.73)

*Uptake/retention values are derived from the averages of individual fish.

Table IIA. Summary of statistical comparison of seasonal thyroid uptake observations.

Salinity	Samples compared		No. of fish	Statistic		Tabled value	
Low	Jan. 13	Apr. 8	8+8	t'	2.76	t'(P.05)	2.36
	Apr. 8	May 12	8+6	t'	3.58	t'(P.05)	2.37
	May 12	July 8	6+8	t	6.25	t(P.001)	4.32
Intermed.	Jan. 13	Apr. 8	8+8	t	3.81	t(P.005)	3.33
	Apr. 8	June 3	8+6	t	4.77	t(P.001)	4.32
	June 3	July 8	5+15	t	3.78	t(P.005)	3.15
High	Dec. 27	Feb. 3	6+8	t'	3.34	t'(P.05)	2.42
	Feb. 3	Mar. 27	8+8	t	0.64	t(P.5)	0.69
	Mar. 27	Apr. 8	8+8	t'	2.71	t'(P.05)	2.37
	Apr. 8	July 8	8+8	t'	2.50	t'(P.05)	2.36

t Student's t (Snedecor (1956) p. 45)
t' Modified Student's t for use with unequal variances (Snedecor (1956) p. 98)

Table IIB. Summary of statistical comparison of seasonal conversion ratio observations.

Salinity	Samples compared		No. of fish	Statistic		Tabled value	
Low	Jan. 13	July 8	8+8	t	3.79	t(P.005)	3.33
	Jan. 13	Feb. 20	8+8	t	2.26	t(P.05)	2.15
	Jan. 13	Apr. 30	8+8	T	46	T(P.05)	49
	Feb. 20	July 8	8+8	t	3.58	t(P.005)	3.33
Intermed.	Jan. 13	Mar. 27	8+8	t	4.66	t(P.001)	4.14
	Mar. 27	Apr. 8	8+8	t'	3.20	t'(P.05)	2.37
	Mar. 27	July 8	8+8	t'	0.43	t'(P.05)	2.36
	Apr. 8	Apr. 30	8+8	t	1.76	t(P.1)	1.76
	Apr. 30	June 3	8+6	t'	9.03	t'(P.05)	2.56
	June 3	July 8	6+8	t'	9.14	t'(P.05)	2.57
	Jan. 13	July 8	8+8	t	4.32	t(P.001)	4.14
High	Oct. 17	Jan. 13	8+8	t'	2.70	t'(P.05)	2.37
	Feb. 3	Feb. 20	8+8	t	2.46	t(P.05)	2.15
	Jan. 20	Mar. 27	8+8	t	4.88	t(P.001)	4.14
	Feb. 3	Mar. 27	8+8	t	4.83	t(P.001)	4.14
	Apr. 8	May 19	5+5	t'	3.28	t'(P.05)	2.61
	May 19	July 8	5+7	t'	1.24	t'(P.05)	3.09
	Feb. 20	July 8	8+7	t'	2.69	t'(P.05)	2.43
	Jan. 13	July 8	8+7	t'	1.13	t'(P.05)	3.43

T Sum of Ranks (Snedecor (1956) p. 117)
t Student's t (Snedecor (1956) p. 45)
t' Modified Student's t for use with unequal variances (Snedecor (1956) p. 98)

Table IIIA. Changes in iodide metabolism with time after injection (high salinity) (May 1961).

Time (hours)	Wt. of fish (range) gm.	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
24	1.37 (1.10-1.71)	6	43.9 (35.3-53.5)	6	1.49 (0.43)	3.38	5	2.25 (0.64)
48	1.41 (1.20-1.69)	7	22.7 (11.6-31.4)	7	2.02 (0.77)	9.69	7	3.48 (2.61)
72	1.61 (1.33-2.03)	5	13.1 (7.0-23.9)	5	2.38 (1.06)	23.19	5	3.09 (0.85)
96	1.36 (1.00-1.53)	5	8.5 (2.0-15.5)	5	1.51 (1.07)	21.65	4	6.79 (2.75)
120	1.53 (1.41-1.68)	5	7.2 (0.8-14.9)	5	2.01 (1.18)	79.91	4	4.07 (1.66)
144	1.73 (1.49-2.06)	3	1.6 (1.2-2.2)	3	1.97 (0.66)	121.88	-	----
168	1.41 (1.11-1.70)	5	3.7 (1.7-7.8)	5	1.57 (0.75)	61.92	-	----

*Uptake/retention values are derived from the averages of individual fish.

Table IIIB. Changes in iodide metabolism with time after injection (intermediate salinity) (June 1961).

Time (hours)	Wt. of fish (range) gm.	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
24	1.50 (1.36-1.60)	4	39.3 (32.3-48.4)	4	1.03 (0.24)	2.73	4	4.71 (1.69)
48	1.35 (1.16-1.62)	6	16.1 (4.8-32.0)	6	1.40 (0.56)	11.36	6	3.39 (2.02)
72	1.62 (1.43-1.80)	6	13.1 (5.4-24.0)	6	0.66 (0.22)	6.66	6	25.36 (5.66)
96	1.45 (1.15-1.83)	6	8.2 (1.6-15.5)	6	0.87 (0.31)	15.60	6	12.23 (5.32)
120	1.39 (1.09-1.74)	6	6.9 (1.9-12.8)	6	0.61 (0.34)	12.16	6	15.45 (5.71)
144	1.53 (1.41-1.63)	4	4.2 (0.8-8.9)	4	1.07 (0.46)	53.20	3	7.56 (2.10)
168	1.50 (1.41-1.83)	7	4.7 (2.1-10.9)	7	1.01 (0.69)	29.80	-	----

*Uptake/retention values are derived from the averages of individual fish.

Table IIIC. Changes in iodide metabolism with time after injection (low salinity) (May 1961).

Time (hours)	Wt. of fish (range) gm.	No. of fish	% Ret. (range)	No. of fish	% Uptake (S.D.)	Uptake* / Ret.	No. of fish	% C.R. (S.D.)
48	1.25 (1.10-1.55)	8	13.8 (8.4-20.3)	8	0.43 (0.29)	2.09	8	3.44 (1.63)
72	1.08 (0.82-1.31)	6	8.0 (1.6-19.4)	6	0.21 (0.18)	5.68	8	4.04 (1.51)
96	1.41 (1.05-1.74)	8	8.8 (3.6-14.4)	8	0.49 (0.11)	7.77	8	5.36 (1.45)
120	1.39 (0.98-1.95)	7	3.6 (0.8-8.6)	7	0.39 (0.17)	21.83	8	10.48 (10.25)
144	1.46 (1.24-1.84)	5	3.8 (1.0-8.6)	5	0.40 (0.24)	19.52	8	5.09 (6.46)
171	1.43 (0.82-2.68)	7	3.0 (0.2-11.3)	7	0.50 (0.46)	24.91	-	----

*Uptake/retention values are derived from the averages of individual fish.

Table IVA. Changes in radioiodide retention with time after injection (high salinity, Oct. 1960).

Time (hours)	No. of fish	Mean wt. of fish (gm.)	Mean % Ret.	Range % Ret.
48	8	0.94	26.0	7.6-44.5
72	8	0.82	21.6	10.4-30.4
84	8	0.85	16.3	11.9-29.2
96	8	0.99	9.0	1.1-17.1
108	8	0.85	7.7	2.3-14.8
120	8	0.80	6.8	0.7-14.7
132	8	0.88	4.4	0.9-13.7
144	8	1.07	6.7	1.0-14.0
156	8	0.88	5.0	0.3-13.9
168	8	1.43	4.5	0.6- 8.8
180	8	0.84	3.0	0.4- 5.9
192	8	1.08	3.2	0.7- 6.5

Table IVB. Changes in radioiodide retention with time after injection (high salinity, Mar. 1961).

Time (hours)	No. of fish	Mean wt. of fish (gm.)	Mean % Ret.	Range % Ret.
48	8	1.42	29.8	19.8-43.7
72	8	1.33	17.8	5.3-27.5
96	8	1.35	10.8	4.0-19.7
120	6	1.33	10.2	4.2-14.8
144	5	1.34	4.6	1.6- 8.3

Table IVC. Changes in radioiodide retention with time after injection (low salinity, Dec. 1960).

Time (hours)	No. of fish	Mean wt. of fish (gm.)	Mean % Ret.	Range % Ret.
24	8	1.08	21.5	5.6-33.5
48	8	1.14	18.5	9.7-33.3
72	8	1.23	15.9	4.5-33.3
96	8	1.10	12.4	5.0-25.3
120	7	1.19	11.2	3.4-27.1
144	8	1.48	13.6	6.2-31.2
168	8	132	9.9	5.5-19.2

Table V. Relationships between various parameters of iodide metabolism (intermediate salinity, Feb. 1961).

	Wt. of fish (gm.)	G.S.I.	% Ret.	% Uptake	Uptake / Ret.	% C.R.	% Ret. in ovary
Males	1.09	1.08	35.4	1.73	4.89	2.8	0.44
	1.21	1.17	28.0	0.87	3.11	3.0	0.27
	1.76	1.04	38.9	2.32	5.93	2.7	0.60
	1.47	1.05	29.2	3.20	10.98	5.1	0.48
	1.97	1.34	40.3	2.19	5.42	1.6	0.74
	1.38	1.54	6.5	0.90	14.88	5.1	0.50
	1.22	1.11	33.3	3.29	9.88	1.8	0.53
	1.02	1.08	3.7	0.78	21.10	-	0.04
	1.12	1.47	15.9	1.27	7.99	2.1	0.26
	1.22	1.25	29.4	2.74	9.31	1.8	0.85
	1.15	1.35	1.5	1.09	72.70	-	0.03
Females	1.82	3.73	55.5	2.41	4.34	1.1	3.48
	1.42	3.14	10.6	1.63	15.38	3.2	0.42
	1.19	3.27	32.5	2.88	8.35	4.2	1.54
	1.41	2.84	9.5	1.22	12.85	2.4	0.40
	1.23	1.88	26.7	1.54	5.77	2.3	0.84
	1.26	1.97	28.1	2.54	9.03	3.6	0.91
	1.02	2.51	16.6	1.62	9.77	-	0.46
	2.00	1.18	42.0	5.06	12.04	2.4	2.51
	1.16	3.24	17.2	3.12	18.13	7.4	1.08
	1.76	4.62	43.6	0.78	1.79	2.7	9.70
	1.02	1.86	12.3	1.27	10.33	3.8	0.31
	1.10	2.34	37.5	2.74	7.31	1.3	1.15
	1.87	5.66	55.7	1.09	1.96	4.1	24.76

Table VI. Relationships between various parameters* of iodide metabolism - alternate series (low salinity, Jan. 1961).

Males				Females			
Wt. of fish (gm.)	G.S.I.	% Ret.	% Uptake	Wt. of fish (gm.)	G.S.I.	% Ret.	% Uptake
1.37	1.06	23.75	0.52	0.96	1.59	13.75	0.41
0.82	1.24	23.47	0.59	0.97	2.99	10.70	0.33
0.99	1.07	8.33	0.35	1.07	2.49	11.53	0.71
1.29	1.01	28.06	0.61	1.14	2.75	25.90	1.33
1.37	1.35	13.47	0.37	1.20	2.06	19.86	0.57
1.06	1.04	18.33	0.39	0.91	1.95	12.36	0.49
0.94	1.39	9.65	0.37	0.89	3.42	4.94	0.85
1.01	1.47	21.39	0.50	1.02	2.39	9.58	0.24
1.00	--	19.31	0.59	1.23	1.88	11.95	0.47
1.14	1.04	20.42	0.56	0.93	3.93	16.53	0.32
1.35	1.45	26.67	0.59	0.95	2.55	5.69	0.52
0.90	1.45	6.29	0.16	0.86	2.25	15.42	0.48
0.96	0.94	11.15	0.15	1.06	1.76	20.58	0.54
1.31	1.24	32.01	0.45	1.24	1.60	15.86	0.64
0.87	0.65	5.97	0.20	1.15	1.45	4.59	0.41
1.48	1.16	4.43	0.34	1.17	2.46	3.87	0.18
1.22	1.06	21.15	0.58	1.52	2.08	23.29	0.38
0.95	1.00	12.43	0.62	0.93	2.48	15.00	0.43
0.90	1.25	5.92	0.65	1.62	1.87	28.44	0.35
1.30	1.27	22.15	0.52	1.00	2.15	26.29	0.71
1.38	0.97	13.15	0.39	1.63	2.29	15.86	0.36
1.43	1.12	27.29	0.45	1.22	1.46	22.01	0.30
				1.01	1.96	14.15	0.29
				1.10	1.99	17.58	0.30
				1.02	1.90	17.86	0.43
				0.92	2.62	16.15	0.24

*Conversion ratio data not reliable because of time lapse effect. Negligible radioiodide in all gonads.

Table VIIA. Effect of maturity of fish on retention of radioiodide by the ovary (high salinity, May 1961).

Wt. of fish (gm.)	G.S.I.	Body Ret. (%)	Ovary Ret. (%)	Ovary/body Ret. (%)
1.4	6.4	65.0	11.1	17.1
1.0	7.3	52.5	16.8	32.0
1.1	7.0	42.4	10.9	25.7
1.1	9.1	32.6	16.8	51.5
1.3	8.2	44.7	20.5	45.9
1.1	5.3	12.3	0.9	7.3
1.5	12.2	50.3	39.2	77.9
1.5	8.5	25.8	14.6	56.6
1.9	8.5	50.6	35.5	70.2
2.2	13.8	30.1	19.5	64.8
1.7	5.9	22.5	3.8	16.9
1.5	8.6	19.5	8.6	44.1
1.9	4.6	10.5	2.6	24.8
2.1	7.3	16.6	7.5	45.2
2.0	12.4	32.3	21.8	67.5
1.2	5.6	14.8	6.7	45.3
1.3	6.5	20.8	13.0	62.5
1.1	5.6	9.2	4.9	53.3
1.4	8.3	4.9	1.9	38.8
1.4	11.4	25.2	19.8	78.6

Table VIIB. Effect of maturity of fish on retention of radioiodide by the ovary (intermediate salinity, June 1961).

Wt. of fish (gm.)	G.S.I.	Body Ret. (%)	Ovary Ret. (%)	Ovary/body Ret. (%)
1.8	10.5	62.2	40.5	65.1
1.6	12.4	61.0	38.3	62.8
2.1	28.9	75.3	48.2	64.0
1.7	16.3	47.4	28.2	59.5
2.1	19.1	42.2	44.5	105.5
2.3	26.8	66.3	29.9	45.1
1.5	4.5	21.5	1.0	4.7
1.6	11.0	40.0	27.9	69.8
1.4	26.9	49.1	44.7	91.0
1.7	16.0	55.7	51.2	91.9
1.7	10.1	40.3	24.3	60.3
1.4	8.3	3.3	1.3	39.4
1.7	26.8	36.2	32.8	90.6
1.5	8.1	11.3	4.4	38.9
1.6	7.9	16.4	1.5	9.1
1.6	7.2	18.4	10.4	56.5
2.0	17.3	40.4	27.2	67.3

Table VIIC. Effect of maturity of fish on retention of radioiodide by the ovary (low salinity, May 1961).

Wt. of fish (gm.)	G.S.I.	Body Ret. (%)	Ovary Ret. (%)	Ovary/body Ret. (%)
1.2	4.5	20.3	0.6	3.0
1.1	4.5	11.8	0.3	2.5
1.3	4.7	8.4	0.6	7.1
1.2	4.4	17.7	0.8	4.5
1.1	5.6	24.9	2.2	8.8
1.2	3.9	9.9	0.2	2.0
0.8	3.9	4.4	0.1	2.3
1.1	3.7	19.4	0.4	2.1
1.3	5.7	31.7	2.6	8.2
1.3	4.3	11.0	0.2	1.8
1.3	5.1	5.1	2.3	45.0
1.1	3.1	10.1	0.1	1.0
1.6	3.0	14.4	0.2	1.4
1.5	3.9	8.0	1.4	17.5
1.4	4.4	6.2	0.2	3.2
1.8	4.0	8.6	0.2	2.3
1.5	6.2	12.1	1.6	13.2
1.0	2.1	0.9	0.1	11.1
1.2	6.0	7.0	0.5	7.1
2.5	9.2	26.8	9.9	36.9
2.2	10.6	9.1	3.4	37.3
2.2	5.1	29.4	1.3	4.4

Table VIII. Comparison of excretion from anterior and posterior regions of the body.

		Time after injection (hr.)				
Salinity	Excretion (% dose)	2.8	4.0	9.2	20.6	27.9
Low	from "gill"	-	14.1	38.4	44.8	47.0
	from "kidney"	-	12.5	29.0	38.5	34.8
	total	20.2	46.8	87.6	103.5	102.0
	control A	19.6	--	32.0	41.4	47.8

		Time after injection (hr.)				
Salinity	Excretion (% dose)	2.0	4.0	6.0	8.5	20.5
Intermed.	from "gill"	-	11.7	19.3	23.4	32.6
	from "kidney"	-	20.3	28.4	35.1	48.8
	total	17.1	49.1	64.7	75.6	98.5
	control B	-	--	37.4	41.7	52.6
	control C	-	--	55.3	56.1	70.9
	control D	41.5	52.4	61.0	68.7	73.7
	control E	-	66.7	76.3	83.1	96.0