A COMPARATIVE STUDY OF IODINE METABOLISM IN JUVENILE ONCORHYNCHUS

by

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Members of the Department

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April, 1961

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ABSTRACT

Comparative histological and radiochemical studies of iodine metabolism in juvenile Oncorhynchus revealed good agreement between thyroid epithelial height and ability to convert I^{131} into protein-bound I^{131}(PBI^{131}). The ratio of I^{131} to PBI^{131} in plasma samples (Conversion Ratio) was considered superior to other thyroid assays reviewed.

Peaks in thyroid activity and loss of I^{131} from the body occurred in sockeye and coho at the time of downstream migration, but in chum and pink only when postmigrants were retained in fresh water. In coho and sockeye these changes were transitory, in chum irreversible and in pink prolonged. On the above basis, thyroxine was assigned no specific role but a theory of smolt evolution was proposed and related to a phylogeny within the genus Oncorhynchus.

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INTRODUCTION

Life cycles of anadromous salmonids follow a generally consistent pattern. Spawning and juvenile development occur in fresh water, while the usually longer phase of adult growth takes place in the sea. Such a life history involves precise timing of both upstream and downstream migration. At the critical period in juvenile life when the downstream migration commences, the parr may undergo a marked metamorphosis termed smoltification, which is also accompanied by behavioural and physiological changes.

Morphological changes include a subepidermal guanine deposition and melanophore disintegration (Robertson, 1948), a thickening of the epidermis (Pickford & Atz, 1957, p. 172), an increase in adrenocortical volume (Olivereau, 1960), while chloride secreting cells appear in the gills (Nishida, 1953). These changes are associated both with increased salinity tolerance (Fontaine and Baraduc, 1954) and with a marked preference for salt as opposed to fresh water (Baggerman, 1960). At the metabolic level, the smolting parr has been observed to have increased oxygen consumption, a fall in hepatic glycogen (Fontaine & Hatey, 1950) and also changes in lipid metabolism (Lovern, 1934). Of equal significance are certain behavioural changes which favour downstream displacement (Fontaine, 1954; Hoar, 1958).

Closely correlated in time with all these changes is a marked thyroid hyperfunction as determined by the histological state of the follicles (Pickford & Atz, 1957, p. 133; Hoar, 1939, 1950). Certain experiments involving TSH, thyroxine or iodinated casein administration seem to indicate thyroid hyperfunction as causal to many features of smoltification

(summarized by Pickford & Atz, 1957). However, the parr induced to smolt by such means does not show exactly the same complex of physiological change found in nature.

There are two alternative explanations. Either the thyroid has a compensatory function allowing a fish preadapted to salt water to continue to live in fresh water, i.e. alleviates osmotic stress or, histological picture is an artifact and is merely the result of continued existence in the iodine deficient fresh water habitat (Hoar, 1950, 1952).

Hoar and Bell (1950) refer especially to the confusing picture in Pacific salmon. Histologically they show low thyroid activity at the time of migration, only becoming hyperactive if detained in fresh water. This is in marked contrast to Atlantic salmon (Hoar, 1939; Leloup & Fontaine, 1960) where, by various methods, heightened thyroid activity has been associated with smoltification and downstream migration. However, even in this species, it has been claimed that the thyroid of the smolt has a reduced ability to fix iodine and that the ratio of thyroxine iodine to total iodine in the plasma is reduced (Pickford & Atz, 1957, p. 133).

It is evident from the above that confusion exists concerning not only the role of the thyroid, but also concerning its state of activity. This stems partly from lack of a precise definition of thyroid activity - namely, total rate of hormone output, as opposed to either an increase in efficiency of any iodine trapping mechanism or to the level of hormone in the blood, which can itself be influenced by peripheral utilization. Different methods of determining thyroid activity only take certain of these aspects into account and are not necessarily equivalent. There is also an obvious need for a study of radioiodine metabolism in juvenile Oncorhynchus.

On this basis, a study of iodine metabolism has been made on four species of the genus Oncorhynchus in an attempt to follow seasonal changes

in thyroid activity. An improved radioiodine technique (Conversion Ratio method) has been employed and fully described. In the course of the investigation, data obtained by this method has been compared to histological and other radioiodine techniques in order to evaluate the various methods of thyroid assay. This was necessary to assess earlier work and to facilitate later study.

II. SURVEY OF METHODS FOR DETERMINING THYROID ACTIVITY

Methods for determining thyroid activity fall into three main categories, all of which have been applied to salmonids. They are (i) CHEMICAL (ii) RADIOIODINE and (iii) HISTOLOGICAL. In the following account emphasis has been laid on histological and radioiodine techniques. No observations were made using chemical techniques and only a brief summary has been given.

A. CHEMICAL METHODS

By such methods, it is possible to determine the total I^{127} in the thyroid or blood plasma and also to discriminate between free iodide (I^-) and that bound to protein (hormonal). Reference to application of such methods to salmonids has been made by Leloup & Fontaine (1960).

It is important to remember in evaluating such data that the blood concentration of thyroxine is not necessarily indicative of the gland's output. One possible way to increase hormone output owuld be to increase peripheral utilization. Under such conditions, the blood thyroxine level would temporarily drop and acting via the feed-back mechanism to the hypothalamus-pituitary axis (Brown-Grant, 1957) could stimulate increased hormone output so that the blood level would eventually be reinstated. Thus although there might be little change in blood thyroxine level, the hormone output could be greatly increased. Determination of total thyroxine level does not take into account the dynamic aspects of hormonal production, and since its value is always prone to changes in peripheral utilization, it should not necessarily be considered a reliable indication of thyroid activity.

B. RADIOIODINE METHODS

Several methods have been used to determine thyroid activity in fish. They can be considered in two categories. There are those determinations

in which the affinity of the gland for iodine is considered indicative of the thyroid state while others implicate the rate of hormone output.

Since under the action of certain thyroid inhibitors, the thyroid may accumulate vast quantities of I^{131} and convert none to thyroxine^{131}, it is evident that the two process are independent to some extent and not necessarily comparable measures. The most fundamental measure of thyroid activity would be to calculate the rate of output of thyroxine from the gland and any method which is not concerned with this aspect of thyroid activity is therefore suspect.

In the former category, the most common method is to measure the percentage of an injected dose of I^{131} taken up by the gland over a fixed time period (% thyroid uptake). Setting aside the above criticism, the method has the following disadvantages.

(i) It assumes a constant loss of radioiodine from the body between successive determinations. Thyroid uptake is dependent on the reservoir of available I^{131}. Where loss of I^{131} is great, there is reduced chance of any molecule being taken up and thyroid uptake would appear less even though the activity of the gland was unaltered. Such depletion of the I^{131} pool could be brought about either by excretion or by the affinity of extra-thyroidal tissues for I^{131}. Where excretion is concerned, marked variation has been shown in the Atlantic salmon (Leloup & Fontaine, 1960). Also significant is the affinity in some fish of certain tissues such as notochord, ovary and muscle for I^{131} (Leloup & Fontaine, 1960). Variation in the rate of I^{131} uptake by the blood from the coelom is another source of variation capable of effecting the I^{131} blood level and hence thyroid uptake.

(ii) Hickman (1959) has shown that the I^{131} accumulating potential of the thyroid is significantly decreased by the addition of iodine to natural fresh water. This is in accordance with a homoiostatic mechanism

in which the gland's affinity for iodine is altered relative to the I^{127} concentration in the water. It is generally considered to be greater in the lower concentration.

(iii) Mere thyroid uptake measurement after a fixed time period gives no idea of the loss of I^{131} from the gland as thyroxine^{131} or protein - bound I^{131} (= PBI^{131}). In some cases this loss is appreciable and occurs quite early. Working from this principle, some workers (Fromm & Reineke, 1956; Leloup & Fontaine, 1960) have measured the biological half-life for the loss of I^{131} (presumably as PBI^{131}) from the thyroid. This has been claimed as a measure of the loss of hormone.

(iv) Finally, the method involves accurate dissection of the thyroid gland which in teleosts is notoriously diffuse and in certain fish such as goldfish is partially located in the head kidney region (Chavin, 1956).

However careful the dissection, an inevitable source of error arises from the blood trapped in the thyroid region itself. In counts taken soon after injection this source of error will be high and then exponentially decrease. In this respect, all counts on thyroid uptake will be falsely high and the source of error is always likely to be greatest after injection.

The main criticism of thyroid uptake concerns lack of appreciation of the rate of extrathyroidal I^{131} clearance. This has been taken into account by the thyroid clearance method employed on fish by Hickman (1959) and Baggerman (1960) where the method has been fully explained. Essentially, this is a ratio between two rates of I^{131} clearance, (a) the general clearance from the blood and (b) the thyroid uptake which indicates what per cent of the loss has gone to the thyroid, i.e.

$$\text{Thyroid clearance} = \frac{\text{rate of } I^{131} \text{ uptake during 't' minutes}}{\text{mean blood concentration of } I^{131} \text{ during 't' minutes}}$$

$$= \frac{\%\ \text{thyroid uptake } I^{131} \text{ in time 't'}}{\text{mean blood concentration } I^{131} \text{ in time 't'}}$$

This method is still susceptible to certain hazards of estimating I^{131} concentrating in fish by thyroid uptake. As employed by Baggerman (1960), the method involves measurement of I^{131} level at 5 - 10 hours after injection. At such a time it is considered that essentially only I^{131} will be in the blood, but Baggerman herself has emphasized the need for investigation on this point. Secondly, such a choice of time means that I^{131} loss is being measured at its most rapid and variable phase (Hickman, 1959).

A far superior method would not involve direct measurement of the per cent of the dose taken up by the thyroid and also take into account the rate of secretion of PBI^{131} into the blood stream. An approach to such an ideal is provided by the C. R. (Conversion Ratio) method. This has been mathematically derived by Riggs (1952) and adapted to the present microtechnique by Hickman (personal communication).

After a fixed interval of time the following ratio is determined in a given sample of plasma.

$$\frac{PBI^{131}}{I^{131} \not / PBI^{131}} \times 100 = \frac{\text{hormonal } I^{131}}{\text{total } I^{131}} \times 100$$

Superficially it is a percentage representation of the ability of the thyroid to remove iodine from the blood, convert it to thyroxine and secrete it to the blood where it is found as the protein-bound form. It is thus representative of iodine uptake, thyroxine synthesis and hormone output, the three main phases of thyroid function. The validity of the method has been more fully criticised in the following study of iodine metabolism in the

juvenile Oncorhynchus. The account is based partly on personal data, partly on results from the literature and partly on speculation.

C. HISTOLOGICAL METHODS

Many workers are of the opinion that the histological appearance of the teleost thyroid is indicative of the gland's activity (Pickford & Atz, 1957, p. 129). However, Swift (1955) and Fontaine (1953) have disagreed with this generalization. Swift (1955) refers to the following quotation from Carter (1933) "neither the size nor the histological appearance of a gland is necessarily correlated with the amount of secretion which is pouring into the circulation". However, in a later paper he does demonstrate a good general agreement between radioiodine and histological technique (Swift, 1958). Despite such contradictions almost all aspects of thyroid histology have been related to the activity of the gland. In the following treatment a general survey has been made of the reliance of the criteria used.

Histological changes may be considered as follows:

1. Follicular and extra-follicular changes
2. Changes in the colloid
3. Cytological changes.

1. Follicular and Extra-follicular Changes

Total follicular mass is claimed to increase with sustained thyroid hyperfunction in both Atlantic and Pacific salmon (Hoar, 1939, 1952). To estimate thyroid activity in this manner, detailed examination of the entire thyroid must be made. In theory all follicles should be accounted for. In the very variable fish thyroid, this presents a definite problem as it is difficult to make allowance for duplicate counting or omission of some follicles. In view of its dependence on prolonged growth processes, total follicular area is probably one of the slowest changing features of the gland,

in addition to being perhaps the most tedious to measure.

According to Hoar & Bell (1950) the quiescent thyroid follicle is of spherical shape in Oncorhynchus. In hyperthyroid chum salmon they become tufted and irregular in shape. This is probably a direct result of the increased mass of cells per follicle which causes mechanical buckling and irregular extrusion into extra-follicular spaces, while frequent budding of the active thyroid would also contribute to irregularity. Gaylord & Marsh (1912) and Hoar (1952) mention in addition a "pathological condition" diagnosed by the disorganised appearance of clumps of thyroid epithelial cells.

Such a character is difficult and tedious to quantify, but various workers have based conclusions on observations partly dependent on follicle diameter. Hoar & Bell (1950) were of the hesitant opinion that more active thyroid follicles had a larger diameter, but pointed out that the trend was not inconsistent with the growth of the fish. On the other hand, Stolk (1951) has claimed that in Lebistes the follicle diameter is smaller in the hyperthyroid gland. This could be due to the fact that in an active thyroid more smaller follicles have just budded and is indicative of the lack of reliance that might be placed on follicle diameter as a measure of the gland's activity.

The principle extra-follicular change associated with hyperthyroidism is a general increase in vascularity. The erythrocytes may even come to lie in the follicle (Gudernatsch, 1911). The erythrocytes are capable of intruding between the follicle cells (Rasquin, 1949) and may eventually cause rupture of the follicle. According to Bargmann (1939) this is not an uncommon means of follicle discharge. However, vascularization would be difficult to quantify. A possible method might be to estimate the percentage of follicles containing erythrocytes but this might only provide estimates

above the level of activity at which the phenomenon occurs.

2. Changes in the Colloid

Peripheral vacuolation is evident in histological preparations as secretion occurs. This is an artifact but is still indicative of hyperactivity (De Robertis, 1949). Vacuolation or amount of colloid present is difficult to quantify but a method has been employed by Fortune (1955). By projection of outlines on to paper, then cutting out and weighing these outlines, the ratio of colloid mass to epithelial mass has been determined. This ratio though not precisely definable in physiological terms, takes into account both the change in colloid content and the increase in epithelial mass (hyperplasia and hypertrophy). It has many advantages and no major criticism apart from its lengthiness.

A comparable mathematical derivation has been used by Lever (1949) and Stolk (1951) relating the ratio of follicular internal diameter to the number of cells in the same follicle. It appears in this instance that internal diameter represents the amount of colloid present, but fails to take into account the appreciable effect of vacuolation. Other disadvantages include restriction of observation to spherical follicles, which in some instances may be rare and may even be spherical as a result of reduced activity (Hoar & Bell, 1950).

Staining has also been shown to vary (Pickford & Atz, 1957, p. 129; Hoar, 1952). In the inactive gland the colloid is very viscous, acidophilic and homogeneous. The change in staining reaction to acid dyes (eosin) is brought about by a change in pH in the colloid as the result of enzymatic hydrolysis (De Robertis, 1949). This is associated with hormone release and is stimulated by TSH. As such it is difficult to quantify. According to Pickford (Pickford & Atz, 1957, p. 129), the staining reaction in Fundulus

may not always be in agreement with other histological criteria.

3. Cytological Changes

Since primary tyrosine iodination and also hormonal release are currently considered simultaneous properties of each and every epithelial cell, and since both phases of thyroxine metabolism are indicative of gland activity, it is not surprising that many workers have concentrated on measuring thyroid function in terms of epithelial changes. Hoar & Bell (1950) and Vivien (1958) have described various cytological states appropriate to different phases of activity. Changes occur in nuclear position, vacuolation of cytoplasm, staining reaction of cytoplasm and presence of colloid in the cell. Most noticeable, however, is a change in cell height (Pickford & Atz, 1957, p. 129; Hoar, 1939, 1950, 1952). These have been the most widely used of all histological criteria.

However, as pointed out earlier, its applicability is controversial. The aim of current observations in sockeye smolts was therefore twofold. In the first place evaluation of cell height against other easily quantified histological criteria was made. Cell height was then assessed as an index of thyroid level against more modern radioiodine techniques.

III. MATERIALS AND METHODS

A. LIVING MATERIALS

PINK SALMON (Oncorhynchus gorbuscha) and CHUM SALMON (Oncorhynchus keta)

These were obtained on May 7th as recently hatched fry from Cultus Lake Hatchery. The pinks were kept indoors throughout and a few fish survived until December. They ranged in size from an average of 0.2 gram in June to a maximum of 10 grams in September. Initially the chum were also kept indoors but early in July were transferred to a large outdoor concrete pool where they survived well until October when fungus killed most of them. They ranged in size from an average of 0.3 gram in June to 17 grams in November.

COHO SALMON (Oncorhynchus kisutch)

These were obtained by seining from Salmon River. Hauls were made in November and January (1959) and August (1960). The size range was 2 to 6 grams depending on time of capture.

SOCKEYE SALMON (Oncorhynchus nerka)

All sockeye came from Cultus Lake and were trapped as yearling downstream migrants (4 - 6 grams in June). During June and early July, heavy mortality was suffered partly from fungus, partly from wearing away of the snout on the rough concrete surface of their tank and partly from unknown causes. Some survived to November of the same year and attained a weight of 30 grams. Sockeye smolts were also available which had been retained in fresh water under hatchery conditions (University of British Columbia) three and four years, and by November of their fourth year weighed up to 150 grams. It is interesting to note that none of these sockeye ever developed red or pink flesh and that some of the males showed partly developed gonads.

All fish were kept (unless otherwise stated) under hatchery

conditions (University of British Columbia). They were held in freely running dechlorinated water which ranged in temperature from 4 to 15° C, reaching its peak value in September. No sudden fluctuations in temperature occurred. Food consisted of Clark's Commercial Trout Food which was administered in one of four grades appropriate to the size of the fish. Most fish were fed twice a day and fry at more frequent intervals.

B. RADIOIODINE TECHNIQUE

1. Injection

Radioiodine in the form of carrier-free iodine, diluted with distilled water, was injected intraperitoneally using a 30-gauge needle and 0.25-ml tuberculin syringe. The injected dose was usually of the order of one microcurie per gram of fish , and was injected in a volume appropriate to the size of fish (0.005 to 0.05 ml). To prevent leakage, the method advocated by Hickman (1959) was employed, in which the dose was injected through dorsal musculature which acted as a seal to the wound. Aliquots of each dose were reserved as standards (for thyroid uptake and excretion estimates) and were diluted with potassium iodide.

2. Blood Sampling and Conversion Ratio Technique

At fixed periods after injection, the fish was killed, weighed, measured and a sample of blood taken by cleanly cutting the tail in the region of the caudal peduncle and drawing up the blood into a fine heparinised capillary tube as it welled from the haemal artery. Care was taken to withdraw as pure a sample as possible, avoiding contamination either from body slime or especially from the cerebrospinal fluid.

One end of the capillary tube was plugged with 'plasticine' and the sample centrifuged. When separation of corpuscles and plasma was complete,

the tube was broken at their junction and the length of the plasma in the capillary tube measured. This was later used as a measure of the amount of the amount of plasma. Direct weighings were also taken in many cases. The plasma was then blown into 12.5% trichloracetic acid (TCA) in a thick-walled 12-ml centrifuge tube where precipitation of proteins immediately occurred. The precipitate was centrifuged and the supernatant decanted off. The precipitate was then washed two or three times with 2.5% TCA to ensure that all I^{131} was washed free from the protein-bound I^{131} (PBI^{131}). The PBI^{131} was then dissolved by adding 1N NaOH. Aliquots of the PBI^{131} solution and I^{131} solution were then counted in a well scintillation counter. The volumes of reagents used varied with the size of the blood sample. In large fish 4 ml of 12.5% TCA were used and with smaller fish 2 ml. Similar amounts of 2.5% TCA were used for washing the precipitate.

3. Thyroid Sampling

The standard method of thyroid dissection was to remove the basi-branchial region of the first three gill arches, trimming away all hyoidal musculature, gill filaments and most of the gill arches. In sockeye smolts it was found that the dissected thyroid constituted 0.23% of the body weight with a standard deviation for ninety-six fish of 0.045. A similar relationship held for large as well as small fish. The trimming technique was therefore considered consistent. The thyroid was then dropped into a "clearsite" counting tube containing Bouin's fixative (many thyroids were histologically examined after counting) and then counted against a standard of comparable volume (4 ml) in a well scintillation counter. Thyroid uptake was expressed as a percentage in terms of the injected dose.

4. Body Sampling

The bodies, lacking throat thyroid, were counted against a standard

to determine the percentage of the dose still retained. The standard consisted of piles of filter paper cut to the shape and thickness of the fish and evenly permeated by a known proportion of the dose diluted with potassium iodide. Both bodies and their standards were counted under an end-probe scintillation counter with a 45 mm diameter and 38.5 mm thick NaI (Tl) crystal shielded in a lead castle.

C. HISTOLOGICAL TECHNIQUE

Thyroids were dissected from the basibranchial region in accordance with the distribution of thyroid tissue described by Hoar & Bell (1950). Tissues were fixed in Bouin's formol-picric-acetic acid fixative and prepared for histological study by routine methods. Tissues from the region of the second basibranchial region were serially sectioned (10μ). Harris' haematoxylin and eosin stains were used throughout.

IV. RESULTS

A. RADIOIODINE

1. Data on General I^{131} Metabolism

Studies of I^{131} metabolism were made on underyearling coho in early September with low thyroid activity(Table VI; Fig. 6), in underyearling chum in July (Table VIII; Fig. 8) and August (Table VII; Fig. 7) when the thyroid activity was low and high respectively, and in three year sockeye smolts with an extremely active thyroid (Table IX; Fig. 9).

In order to follow the fate of I^{131} in these small fish, up to 100 individuals were injected and then killed in groups of 3 to 12 at intervals from 8 to 24 hours. Means were calculated for each group and considered representative of the I^{131} metabolic state in the population as a whole. Where possible the following were measured at each time interval:

(i) I^{131} Biological Coefficient[1] (Fig. 1)

(ii) % Retention of dose in body (Fig. 2)

(iii) % Uptake of dose by thyroid (Fig. 3)

(iv) PBI^{131} Biological Coefficient (Fig. 4)

(v) Conversion Ratio (Fig. 5)

The data have also been presented collectively for each separate conversion curve in an attempt to show how the Conversion Ratio for each species can be interpreted in terms of the measurements made (Table VI - IX; Fig. 6 - 9).

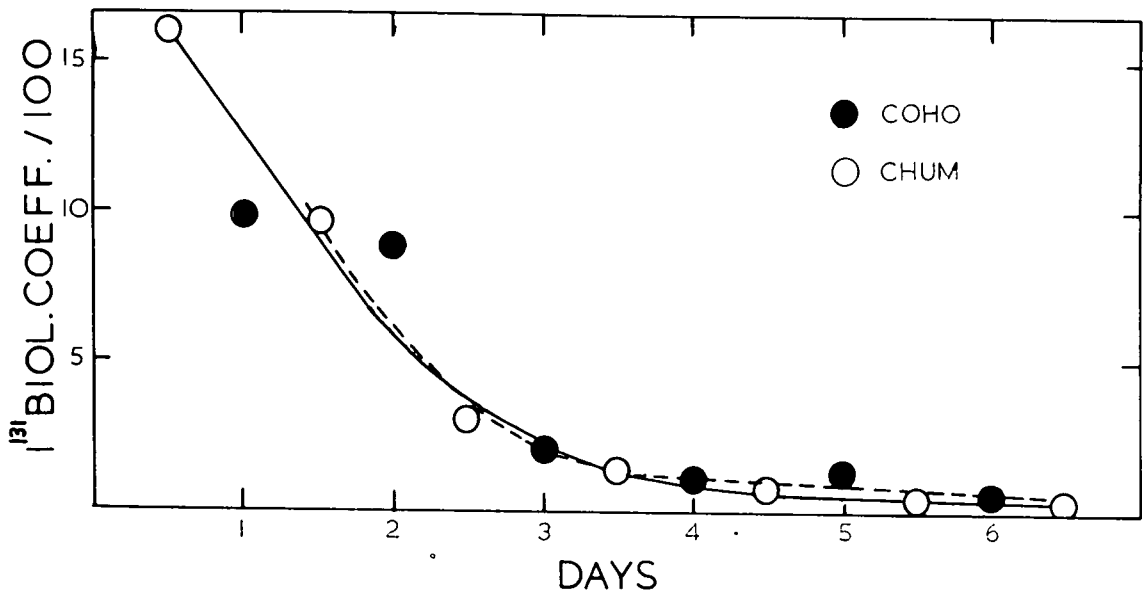
1 The "I^{131} Biological Coefficient"

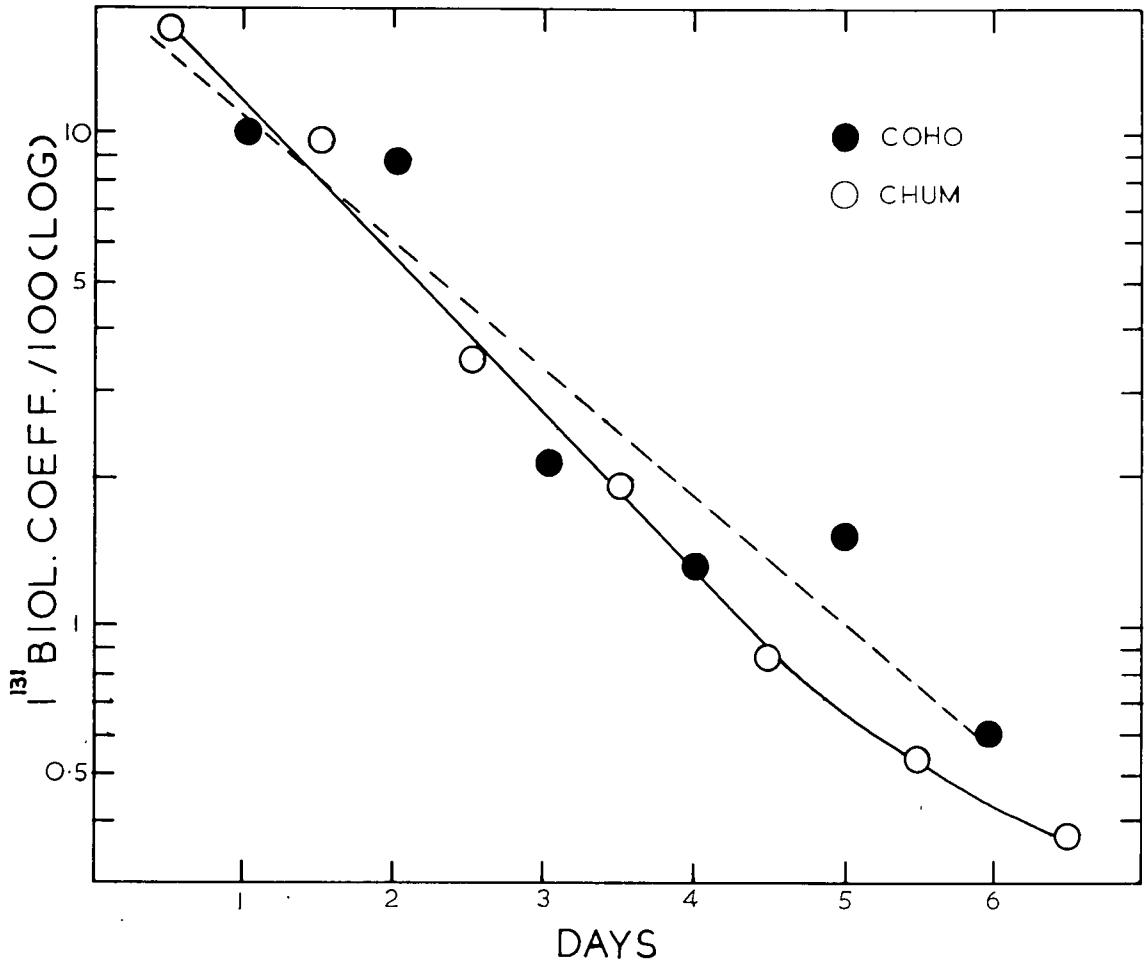
$$= \frac{\%\text{ of the dose represented as } I^{131} \text{ in the sample}}{\text{mass of the sample}} \times \text{body weight}$$

This particular measurement was chosen as it makes allowance for variations in specimen size. If the same dose is injected into different sized fish, then the absolute concentration is inversely related to its body weight. Thus allowance for size is merely made by multiplying "concentration" by "body weight" (Comar, 1955).

To follow page 16.

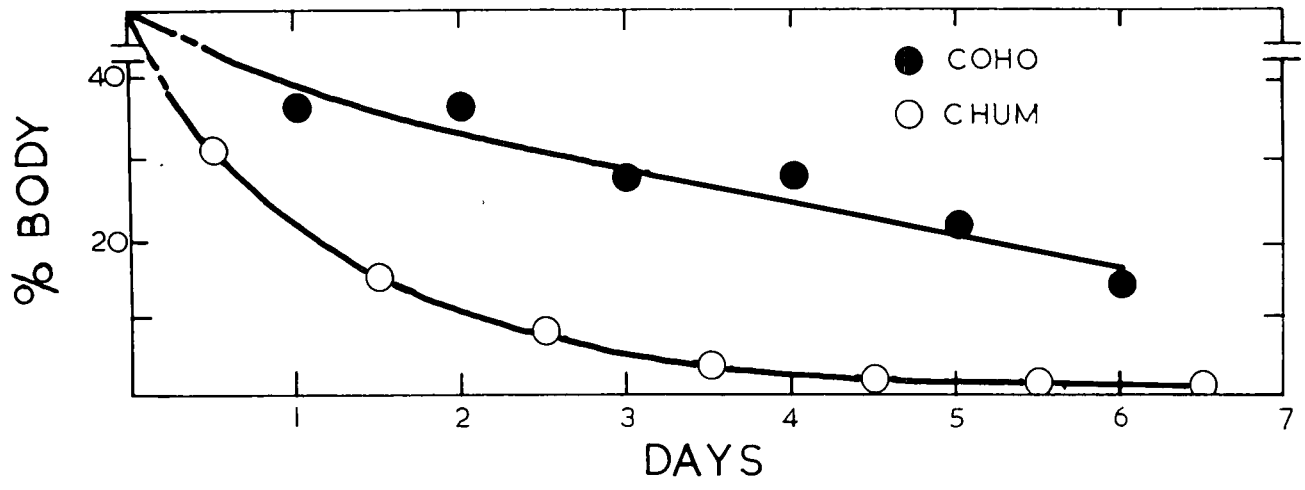
Fig. 1. Loss of injected I^{131} from blood plasma in underyearling coho and chum salmon.


15
10
5
^{131}I BIOL. COEFF. / 100
COHO
CHUM
1
2
3
4
5
6
DAYS


10
5
1
0·5
^{131}I BIOL. COEFF. / 100 (LOG)
COHO
CHUM
1
2
3
4
5
6
DAYS

To follow page 16.

Fig. 2. Loss of injected I^{131} from body (less thyroid) in underyearling coho and chum salmon.


% BODY
40
20
COHO
CHUM
1
2
3
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5
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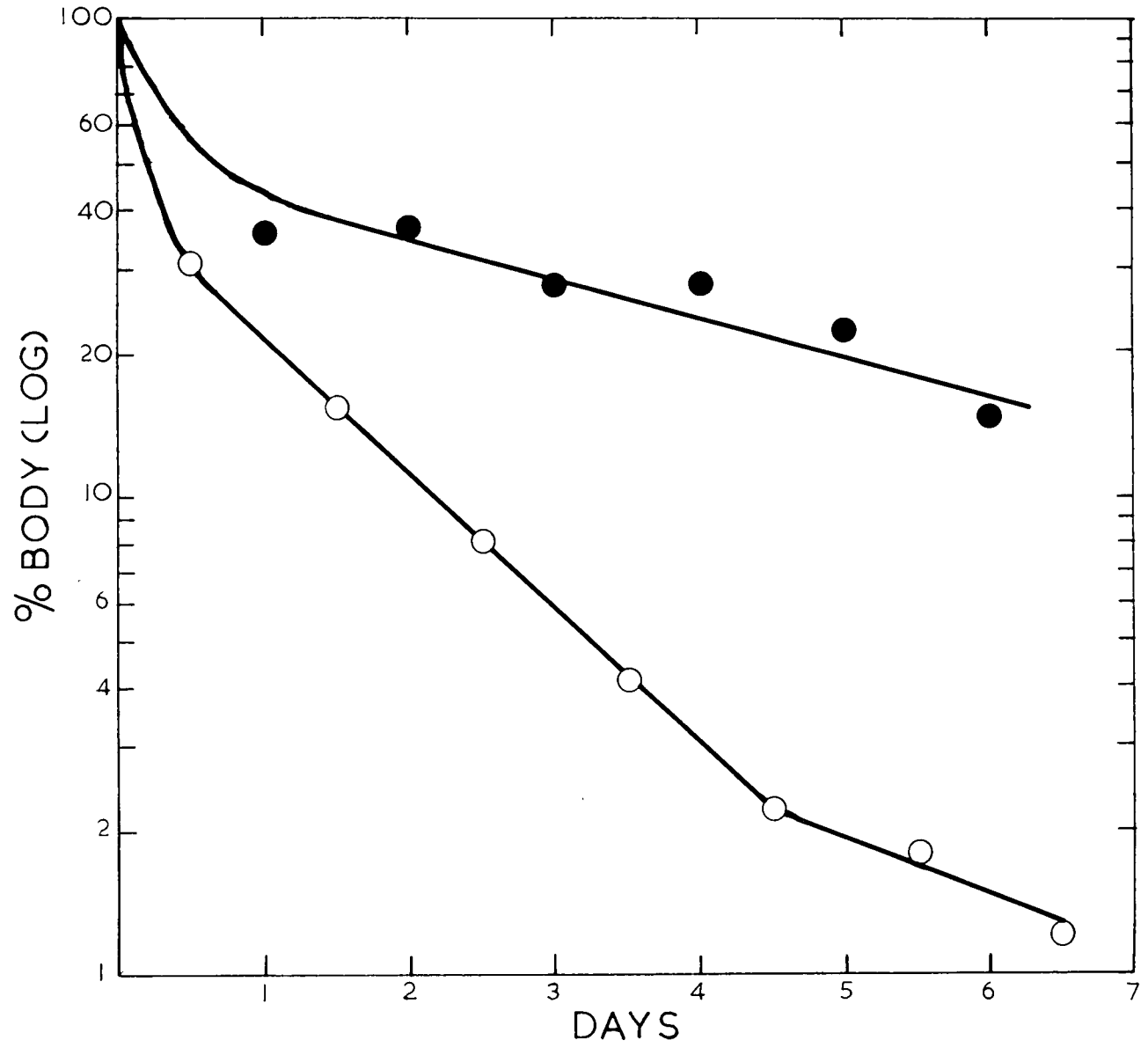
7
DAYS


% BODY (LOG)
100
60
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20
10
6
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1
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DAYS

To follow page 16.

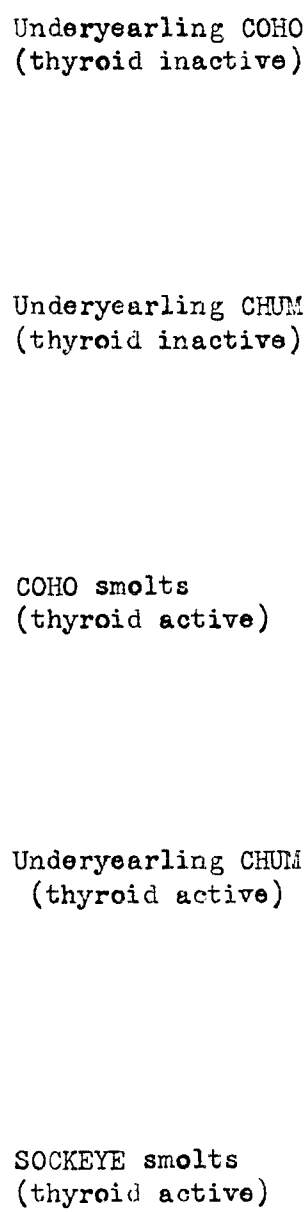
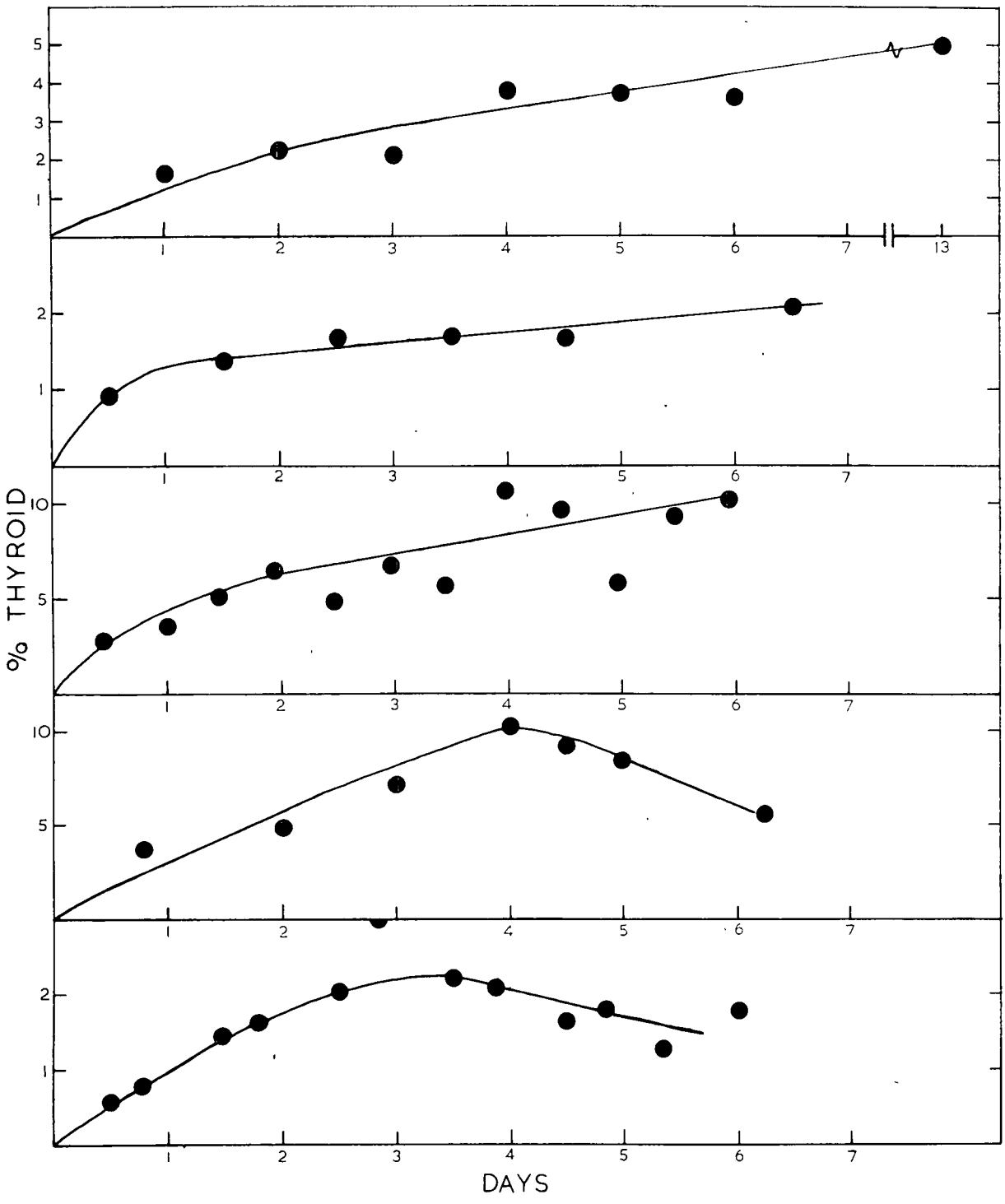


Fig. 3. Thyroid uptake of injected I^{131} in juvenile Oncorhynchus.


% THYROID
DAYS

2. Comparative Data

Conversion Ratio (C. R.) determinations were made on juvenile Oncorhynchus maintained in fresh water (Table IV; Fig. 11). These tests were checked over certain phases of the season by histological observations and in certain cases by determinations of per cent uptake by the thyroid (Tables XII - XV; Fig. 19 - 21).

CHUM SALMON had a high C. R. when first examined in July. This value declined in August and then rose to a steady value of 25 - 30% which was maintained until November. In addition, histological measurement revealed that the first peak in thyroid activity as measured by the C. R. method had only recently been attained (Fig. 20). This implies that at the time of migration, their thyroid activity was probably lower.

COHO and SOCKEYE SALMON showed definite seasonal trends in their first year. There was low thyroid activity in winter followed by a marked peak value in spring coinciding with the period of downstream migration. A very similar pattern seemed evident in underyearling coho, except that thyroid decline appeared slower and even in August showed relatively high activity (Fig. 11).

PINK SALMON showed a decline in thyroid activity from July to October. However, survival was poor and it is possible that data were obtained from a highly select group of fish.(Fig. 11).

That the state of thyroid activity in these hatchery maintained fish was representative of changes in nature was indicated by histological observations made on coho downstream migrants from the Capilano River in early June. Four migrants examined had mean cell heights of 4.58μ (individual values were 4.71, 5.00, 3.99, 4.59). This showed very good agreement with the laboratory-reared fish (fig. 19).

The retention of I^{131} in the body (less thyroid) after 108 hours was

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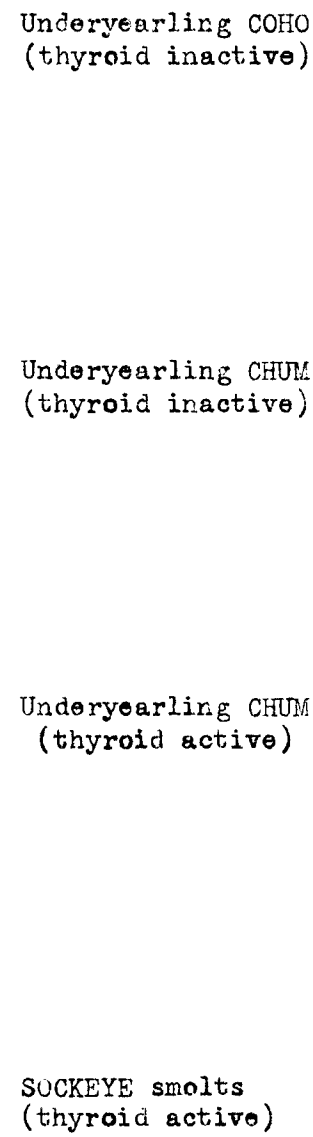
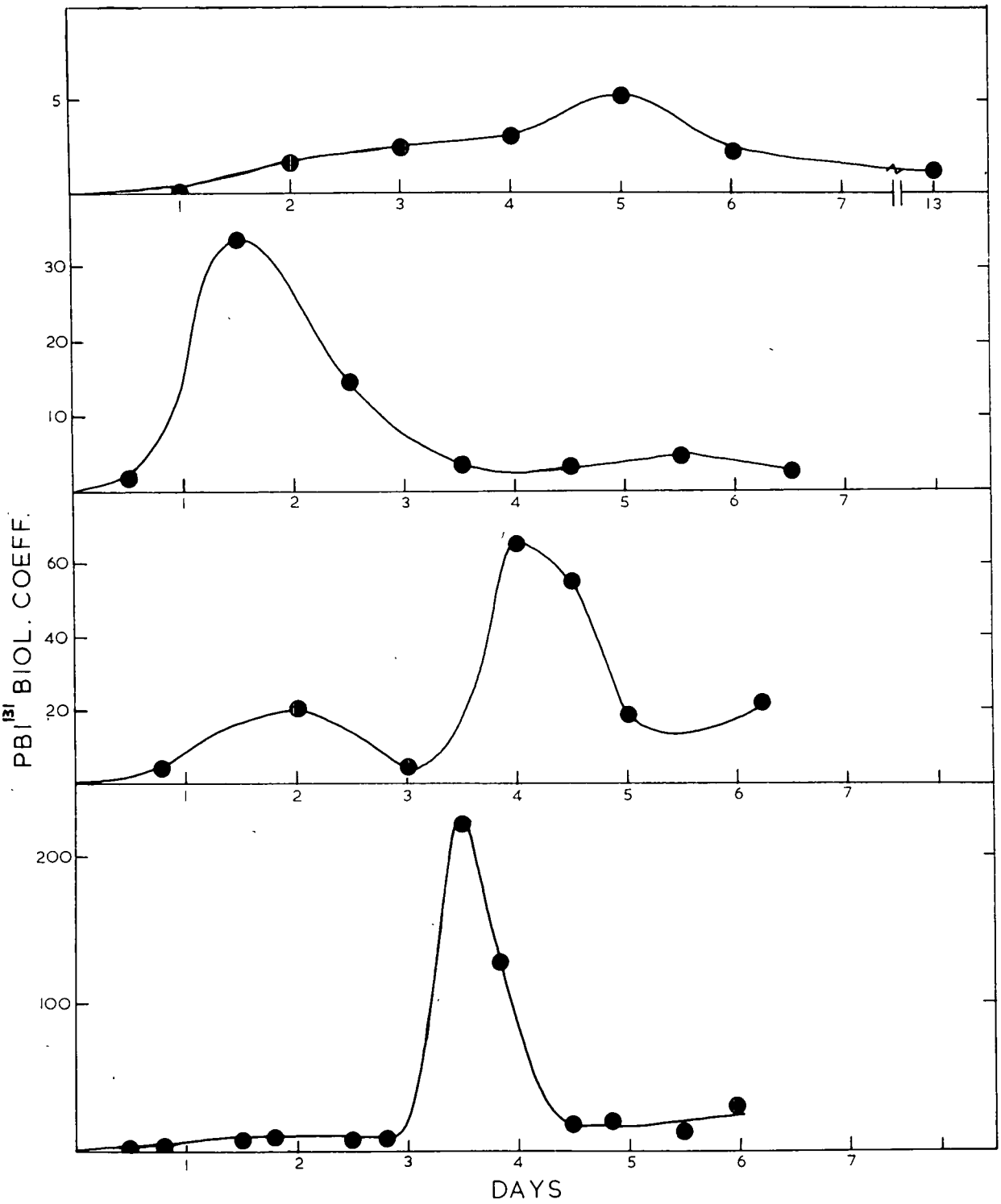
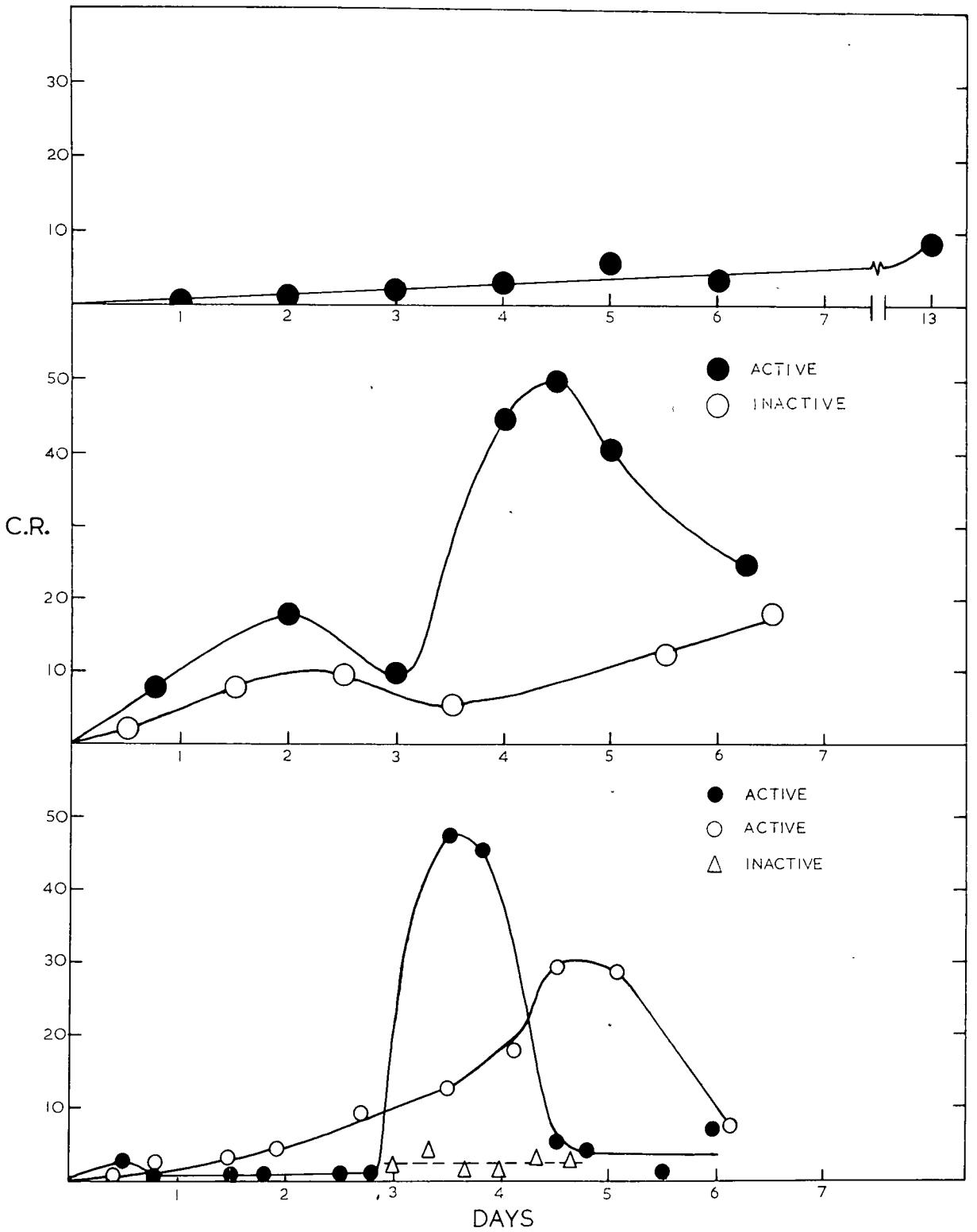


Fig. 4. Changes in PBI^{131} concentrations in plasma samples of juvenile Oncorhynchus.


PBI[131] BIOL. COEFF.
5
1
2
3
4
5
6
7
13
30
20
10
1
2
3
4
5
6
7
60
40
20
1
2
3
4
5
6
7
200
100
1
2
3
4
5
6
7
DAYS

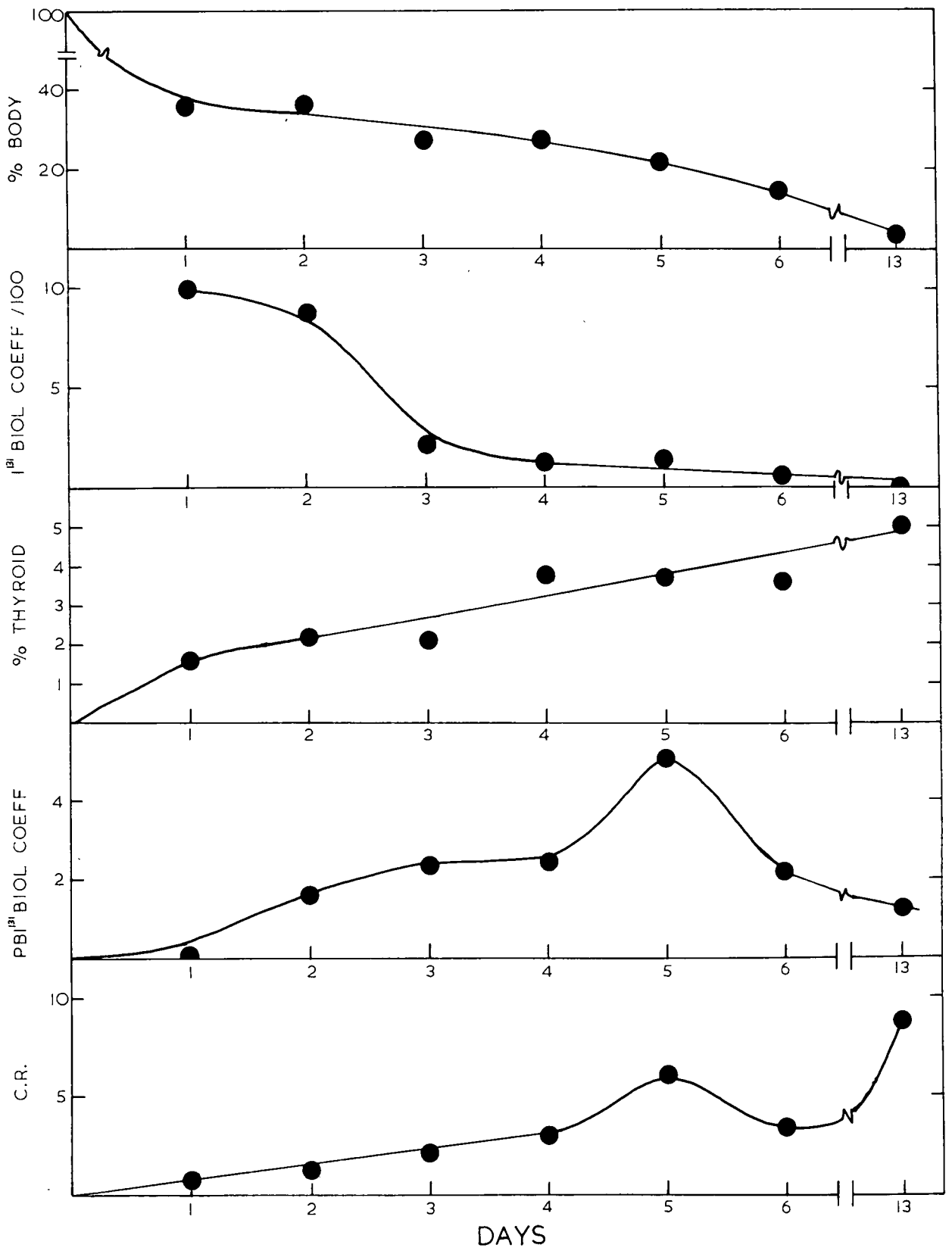
To follow page 17.

Fig. 5. Juvenile Oncorhynchus conversion curves.


30
20
10
1
2
3
4
5
6
7
13
50
40
C.R.
20
10
ACTIVE
INACTIVE
1
2
3
4
5
6
7
50
40
30
20
10
ACTIVE
ACTIVE
INACTIVE
1
2
3
4
5
6
7
DAYS

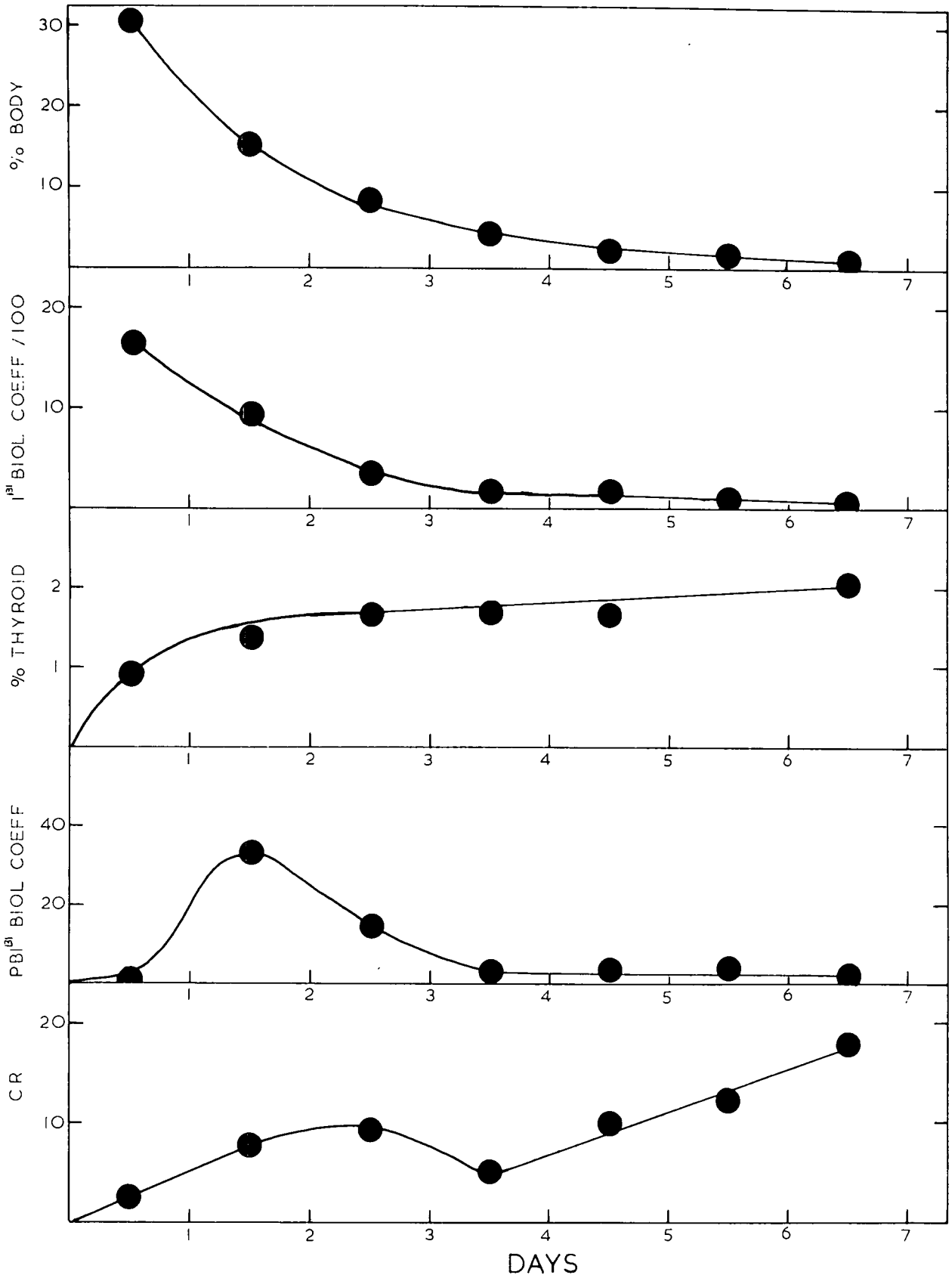
To follow page 17.

Fig. 6. I^{131} metabolism in underyearling coho salmon (inactive thyroid).


100
40
20
% BODY
1
2
3
4
5
6
13
10
5
I^{131} BIOL COEFF /100
1
2
3
4
5
6
13
5
4
3
2
1
% THYROID
1
2
3
4
5
6
13
4
2
PBI^{131} BIOL COEFF
1
2
3
4
5
6
13
10
5
C.R.
1
2
3
4
5
6
13
DAYS

To follow page 17.

Fig. 7. I^{131} metabolism in underyearling chum salmon (inactive thyroid).


% BODY
30
20
10
I^{131} BIOL. COEFF. /100
20
10
% THYROID
2
1
PBI^{131} BIOL COEFF
40
20
C R
20
10
1
2
3
4
5
6
7
DAYS

also measured (Table V; Fig. 12), and consistent seasonal trends demonstrated. At migration, the per cent retention in the body was always low but during the summer and winter climbed to a very high value in coho and sockeye smolts especially, implying a very slow loss of iodine at this season. A similar more restricted trend may also have been present in pink. In chum, however, the retention was very low throughout the entire season until the final determination was made in mid-November. At this point, two completely aberrant fish had very high retention of approximately 20% at 108 hours.

Plasma levels of I^{131} were also measured at certain seasons and the rate of loss represented as a biological half-life (Table V). In contrast to the great extremes noted for biological half-lives for loss of I^{131} for the body as a whole, calculations of half-lives for the plasma fell between closely defined limits, usually 20 - 30 hours. In chum salmon, there was little difference between the half-life for loss of I^{131} from the body and that from the plasma. In other species, the body values were usually considerably longer than those for the plasma.

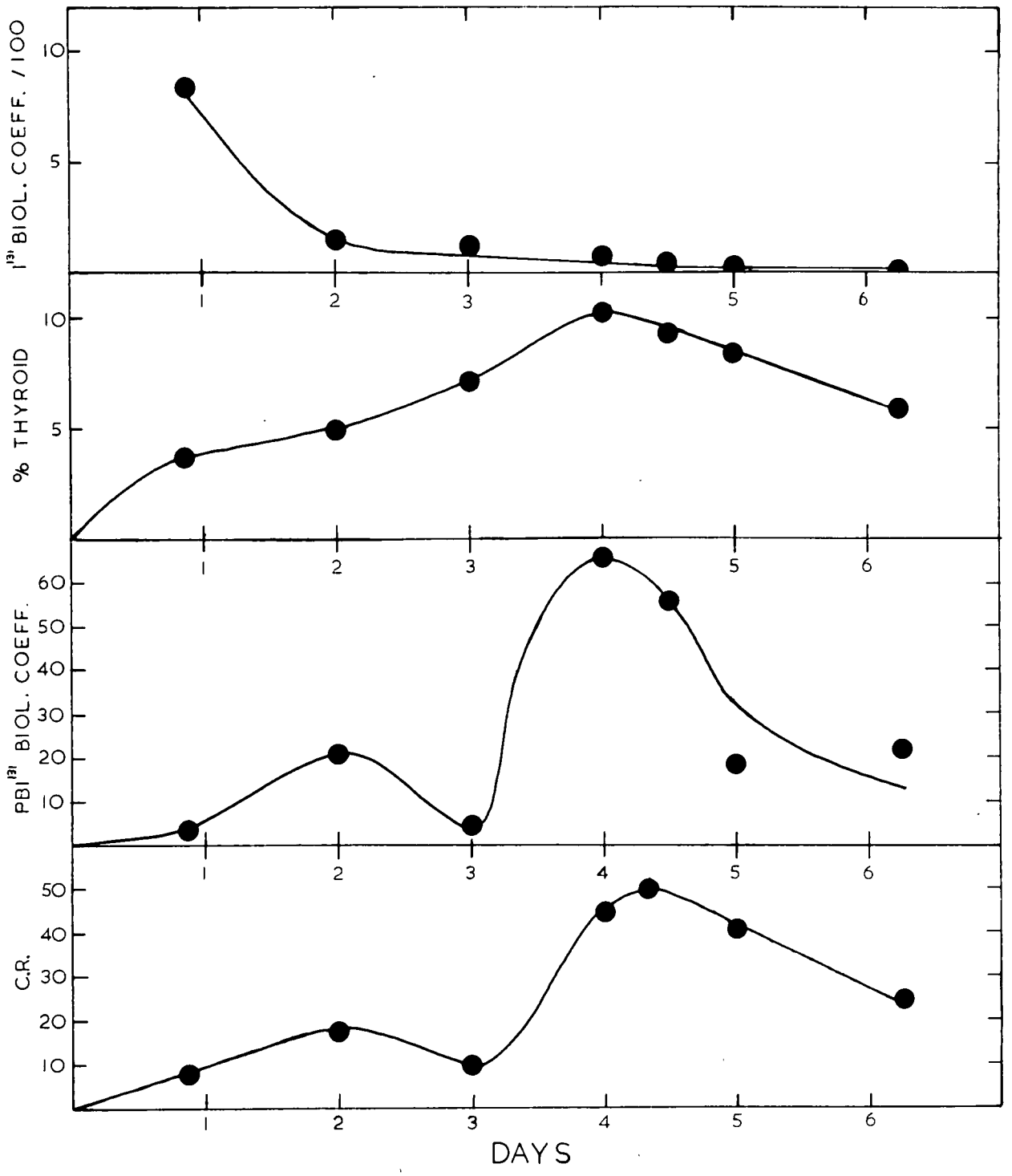
3. Data on Technique

a. Dose Size

Excessive amounts of I^{131} can injure the thyroid (La Roche and Leblond, 1954; Harris, 1959). Relatively large doses of I^{131} ($1\mu c/gm$) were used to obtain appreciable PBI^{131} levels, and it was therefore considered necessary to investigate the effect of I^{131} dose on thyroid activity. Varying doses were administered to underyearling chum still living in fresh water. The results were as shown in Table I. It can be seen that there was relatively little variation in Conversion $R_a$tio for both sockeye and chum over a considerable dose range. In the chum salmon, the thyroid activity was rising sharply at the time when determinations were made and this could account for the low intermediate value (dose $8\mu c$). That the C. R. remained

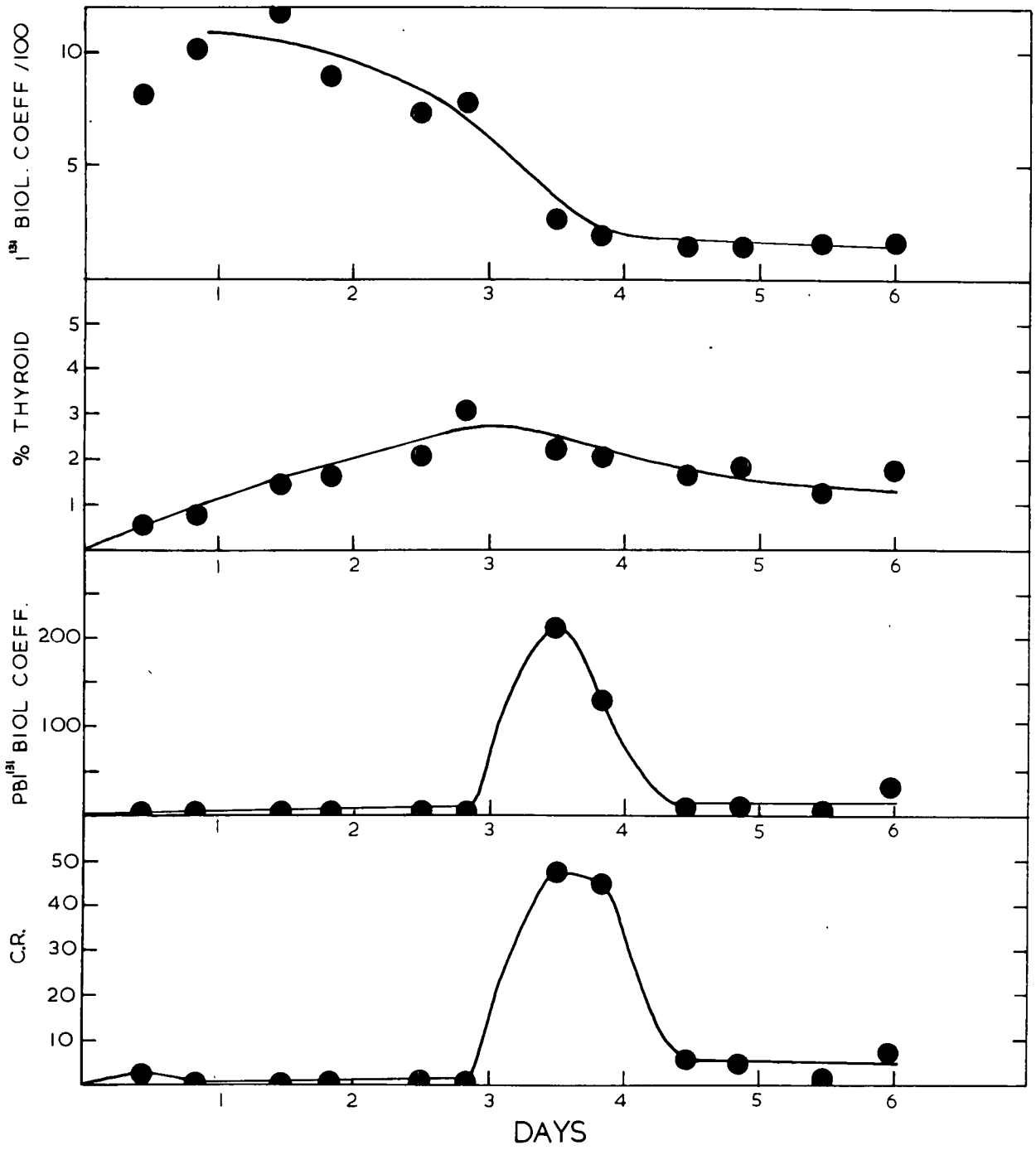
To follow page 18.

Fig. 8. I^{131} metabolism in underyearling chum salmon (active thyroid).


I^{131} BIOL. COEFF. / 100
10
5
1
2
3
5
6
% THYROID
10
5
1
2
3
5
6
PBI^{131} BIOL. COEFF.
60
50
40
30
20
10
1
2
3
4
5
6
C.R.
50
40
30
20
10
1
2
3
4
5
6
DAYS

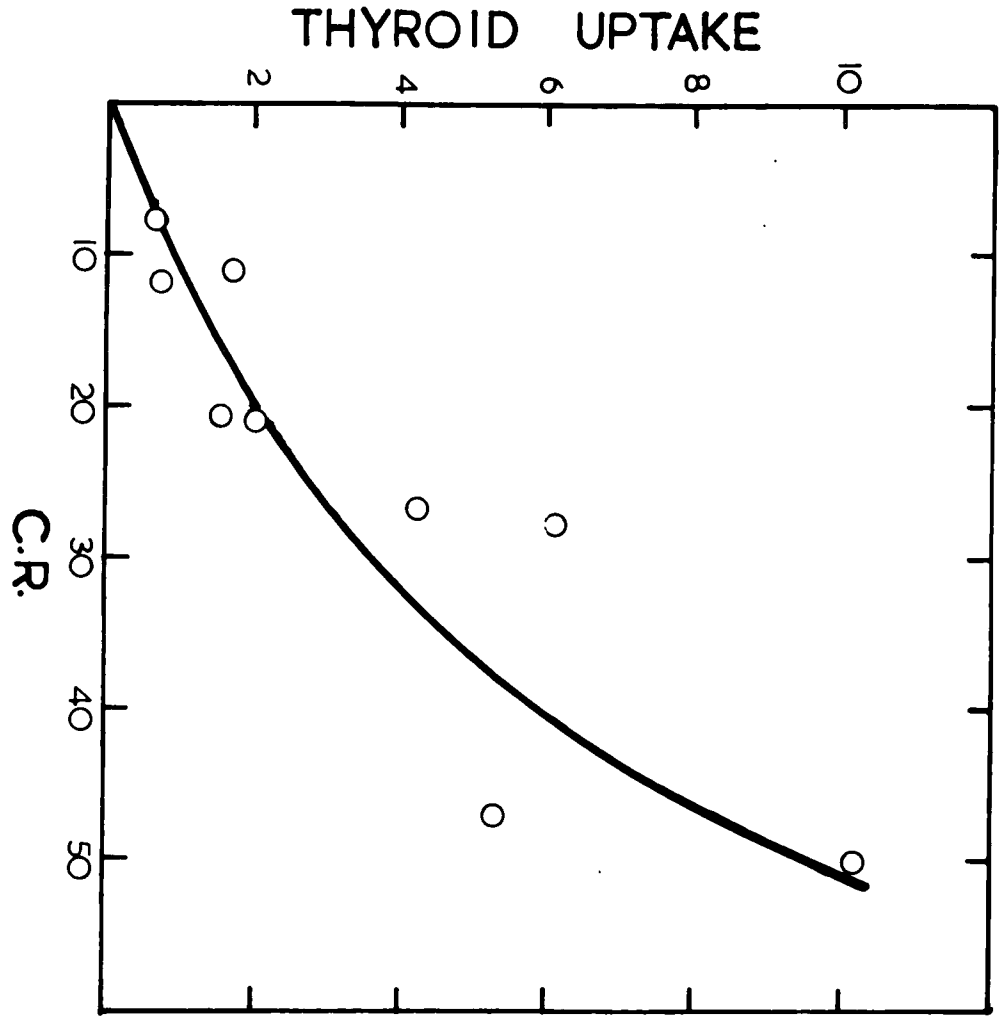
To follow page 18.

Fig. 9. I^{131} metabolism in sockeye smolts (very active thyroid).


I^{131} BIOL. COEFF /100
10
5
1
2
3
4
5
6
% THYROID
5
4
3
2
1
PBI^{131} BIOL COEFF.
200
100
C.R.
50
40
30
20
10
DAYS

To follow page 18.

Fig. 10 Relationship between Conversion Ratio and thyroid uptake of injected I^{131} in underyearling chum salmon.


THYROID UPTAKE
2
4
6
8
10
C.R.
10
20
30
40
50

constant implied that during the 72 hour period after injection (i) the radioactivity had had no detrimental effect on thyroxine synthesis and (ii) the amount of carrier-free I^{131} (0.79×10^{-5} μgm/μc) was within the realms of a 'tracer dose' and had not flooded the system with iodine to the extent that its metabolism was altered. In chum salmon, at least seven microcuries could be given per gram of fish without detriment. This compared favourably with the dose-level used (1 - 2 μc/gm).

b. Plasma Sample

When several consecutive blood samples were removed from the same fish, it was noted that the second C. R. was usually lower than the first (Table II). This was due to both a rise in I^{131} concentration and a drastic lowering of PBI^{131} concentration. The following factors could have contributed to this.

(i) Rapid clotting (as is known to occur in fish) at the wound, such that thyroxine^{131} with the attached proteins could not escape through the clot. On the other hand, the inorganic I^{131} in the free state could initially pass through unimpeded.

(ii) There was almost certainly a local reduction in blood volume on sampling. This would be supplemented by extracellular fluid, which, for the same reasons of permeability, would be rich in I^{131} and low in PBI^{131}.

Consequently, only the initial gush of blood was sampled and great care taken not to squeeze the fish unduly in the hope of obtaining a larger sample. If an adequate dose was injected (2 μc/gm), it was possible to obtain consistent C. R. values with fish of 0.6 gram providing plasma samples of 0.001 gram.

c. Diurnal Variation

Subsequent investigations on chum (Table III) revealed a possible diurnal variation in C. R., reflecting different relative output

Fig.11. Seasonal variation in Conversion Ratio values in juvenile Oncorhynchus held in fresh water.

COHO smolts

SOCKEYE smolts

Underyearling PINK

Underyearling CHUM

To follow page 19.

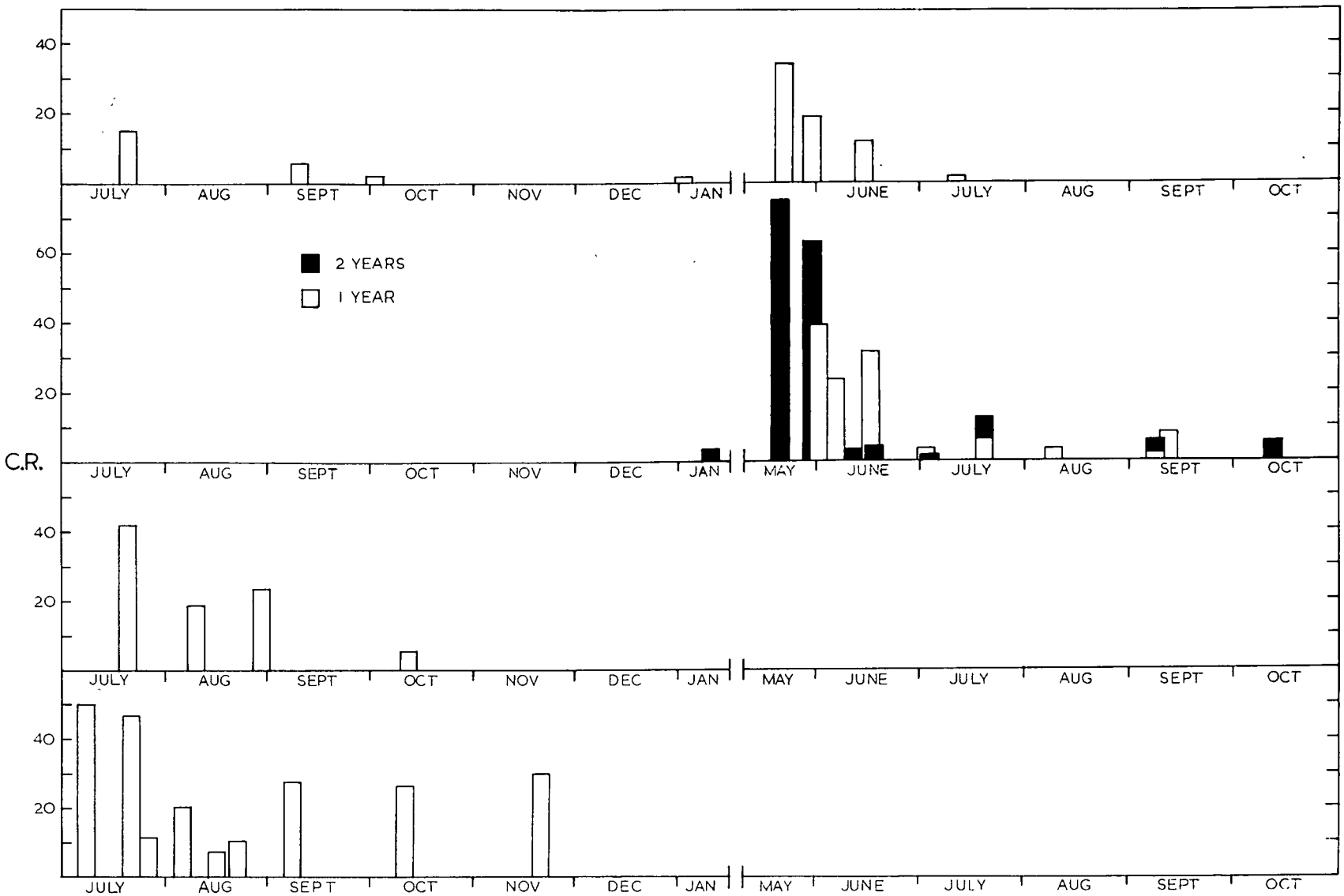

C.R.
2 YEARS
1 YEAR
40
20
60
40
20
40
20
40
20
JULY
AUG
SEPT
OCT
NOV
DEC
JAN
MAY
JUNE
JULY
AUG
SEPT
OCT

Fig. 12. Seasonal variation in the per cent retention (after 108 hrs) of injected I^{131} in the bodies (less thyroid) of juvenile Oncorhynchus held in fresh water.

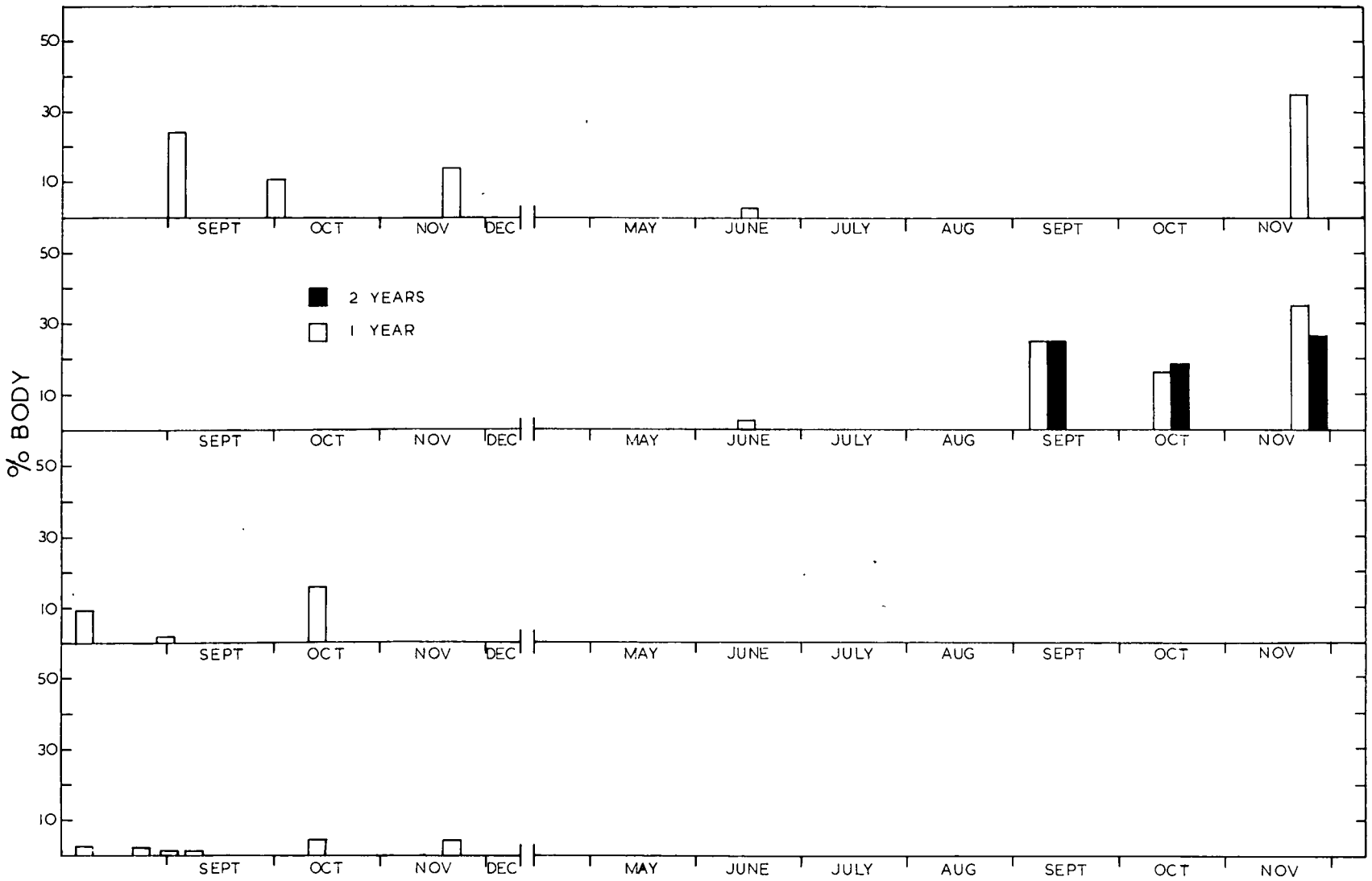
COHO smolts

SOCKEYE smolts

Underyearling PINK

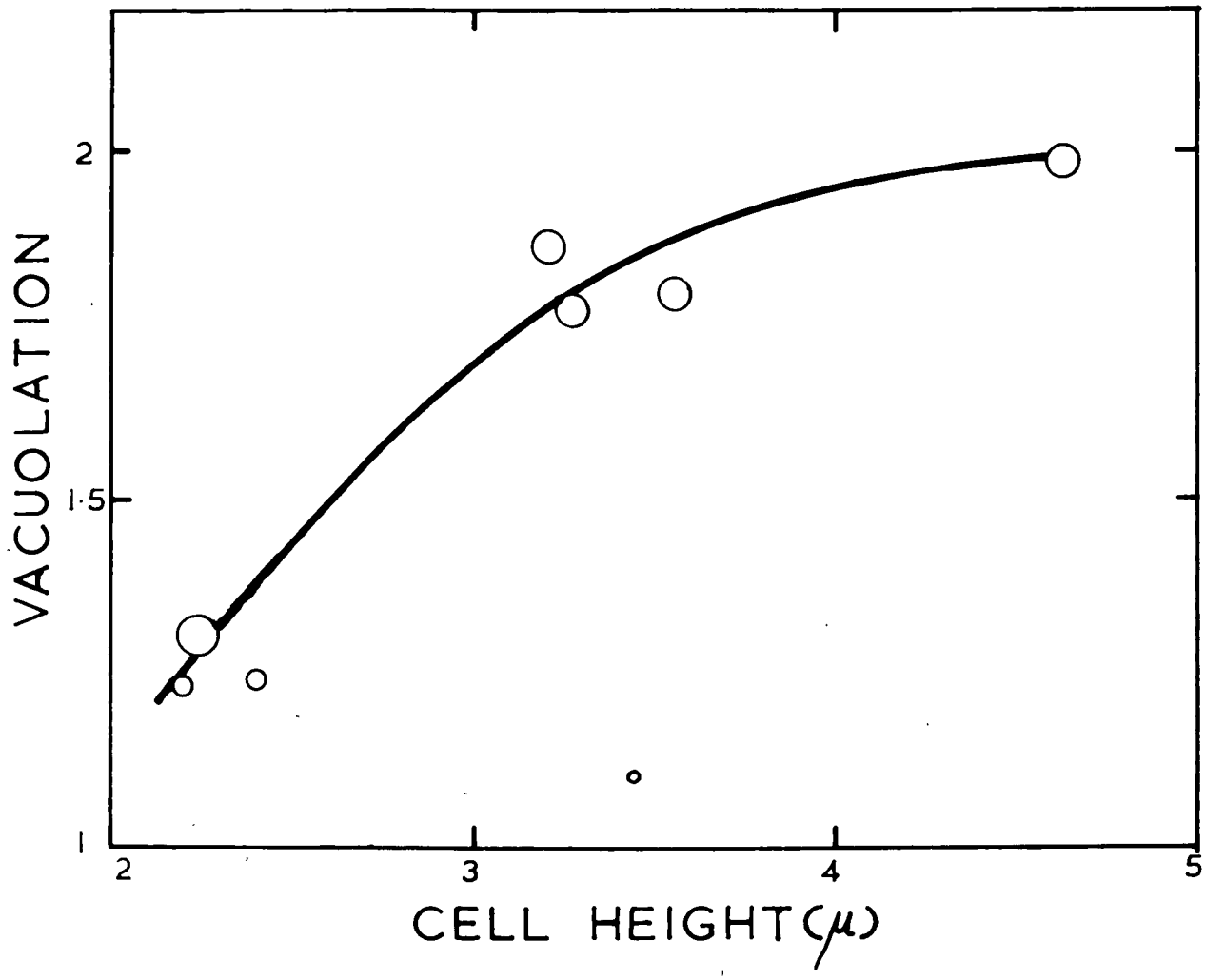
Underyearling CHUM

To follow page 19.


% BODY
50
30
10
SEPT
OCT
NOV
DEC
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
2 YEARS
1 YEAR

To follow page 19.

Fig. 13. Relationship between extent of vacuolation of colloid and lowest cell height in sockeye smolts.


VACUOLATION
2
1·5
1
2
3
4
5
CELL HEIGHT (μ)

levels of PBI^{131} at various times of day (high in the evening, low in the morning). In view of this, all fish were killed between 8:30 A.M. and 10:30 A.M., unless otherwise stated.

B. HISTOLOGICAL

The following were quantified or semi-quantified in sockeye smolts ranging from 2 to 80 grams.

(i) Lowest cell height
(ii) Tallest cell height
} (iii) Mean cell height (= Average of 1 & 2)

(iv) Greatest external follicle diameter
(v) Greatest external follicle diameter at 90° to the first diameter
} (vi) Mean external follicle diameter (=Average of 4 & 5)

(vii) Percentage of follicles containing no colloid

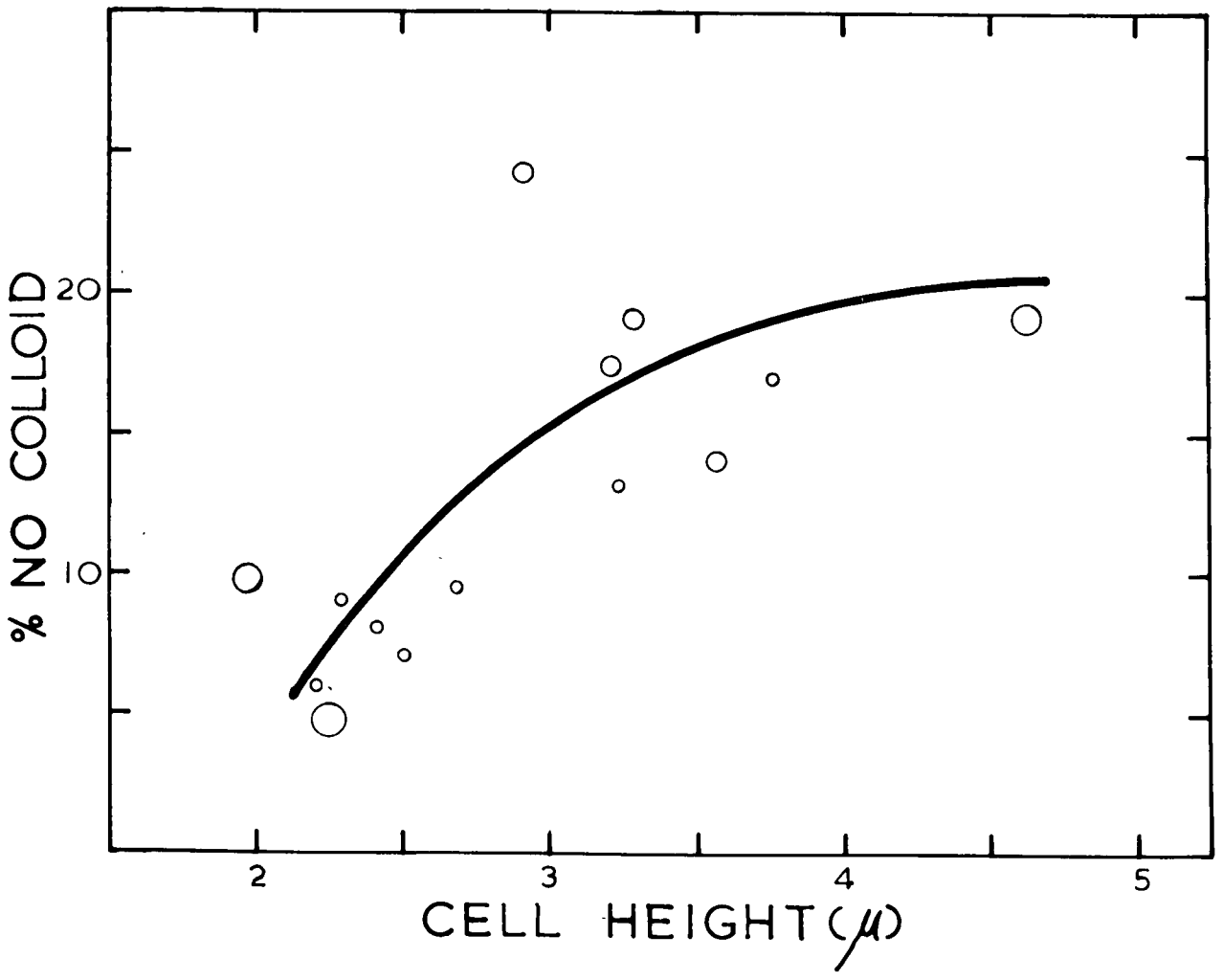
(viii) Extent of vacuolation
(ix) Depth of staining
} By arbitrary assignment to one of 8 classes

Determination were made on 100 follicles (per individual fish), selected from the second basibranchial region and means were calculated (Table X). Since, in the literature, cell height has been the most widely used criterion, its relationship was tested against the other thyroid characters (Fig. 13, 14, 15 and 17). Lowest cell height was used as this minimized the chance of the plane of section going through the base and not the height of the cell, thereby giving a falsely high value.

It may be noted that extent of "vacuolation" and "per cent of follicles with no colloid" showed remarkably good agreement with the cell height measurement. In view of possible variations in technique and the arbitrary nature of the estimation, it was not surprising that the point scatter was wide in the "depth of stain" regression. This negative regression line did however reveal that as the thyroid became more active (cell height), then the staining reaction was lost. Cell height and mean follicle diameter also

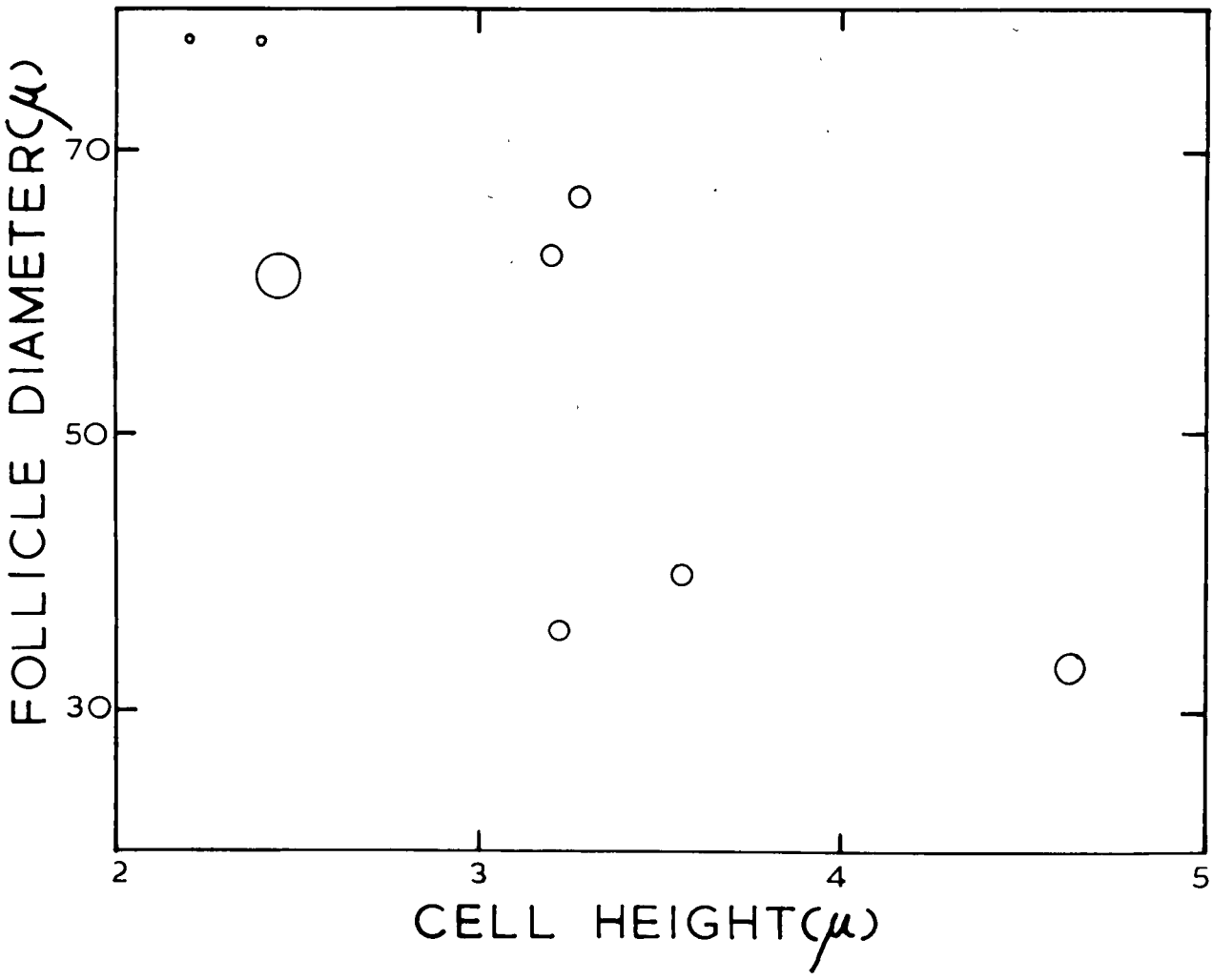
To follow page 20.

Fig. 14. Relationship between per cent of follicles containing colloid and lowest cell height in sockeye smolts.


% NO COLLOID
20
10
2
3
4
5
CELL HEIGHT (μ)

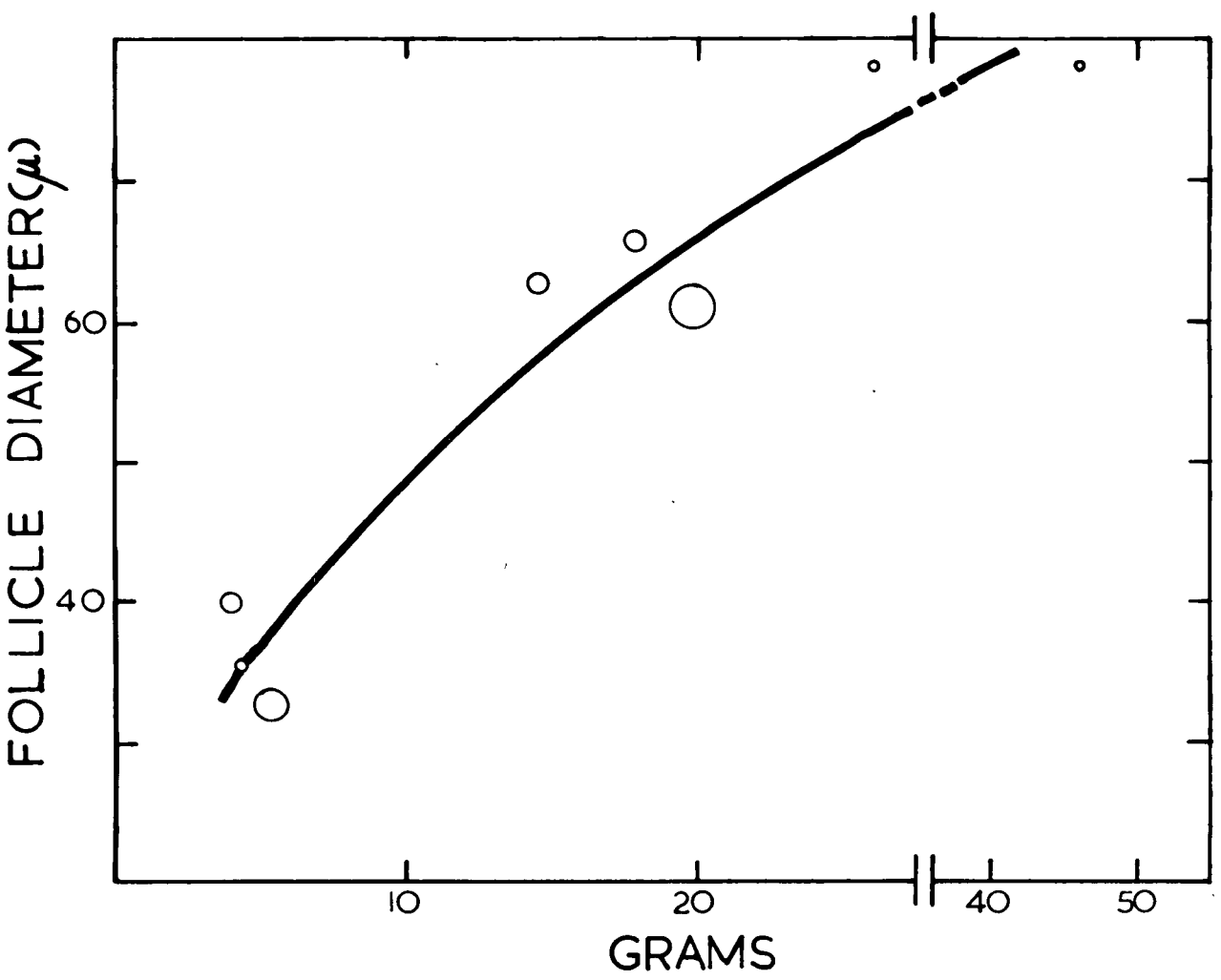
To follow page 20.

Fig. 15. Relationship between mean follicle diameter and lowest cell height in sockeye smolts.


FOLLICLE DIAMETER(μ)
70
50
30
2
3
4
5
CELL HEIGHT(μ)

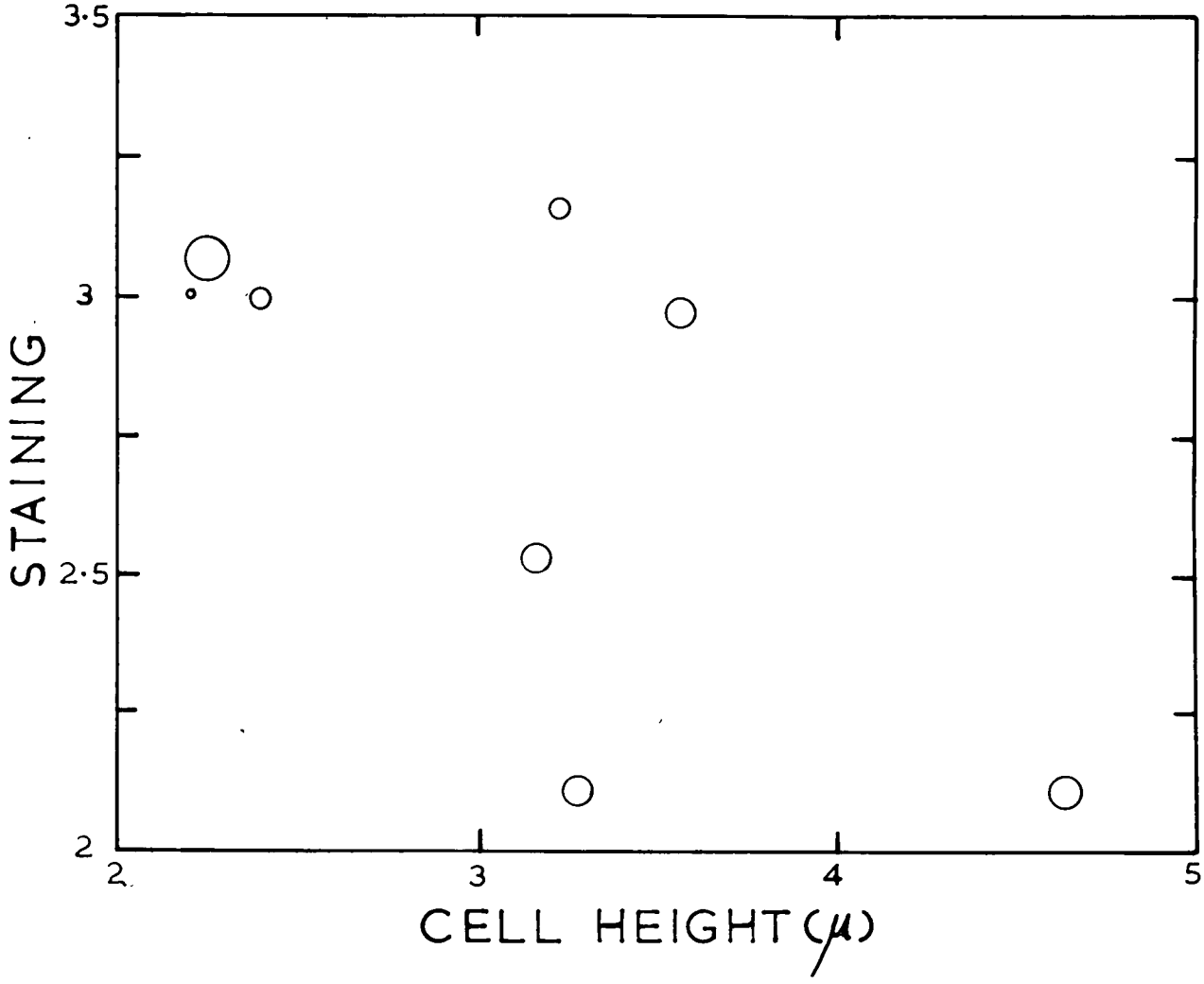
To follow page 20.

Fig. 16. Relationship between mass and mean follicle diameter in sockeye smolts.


FOLLICLE DIAMETER(μ)
60
40
10
20
40
50
GRAMS

To follow page 20.

Fig. 17. Relationship between depth of staining and lowest cell height in sockeye smolts.


3·5
3
2·5
2
STAINING
2
3
4
5
CELL HEIGHT (μ)

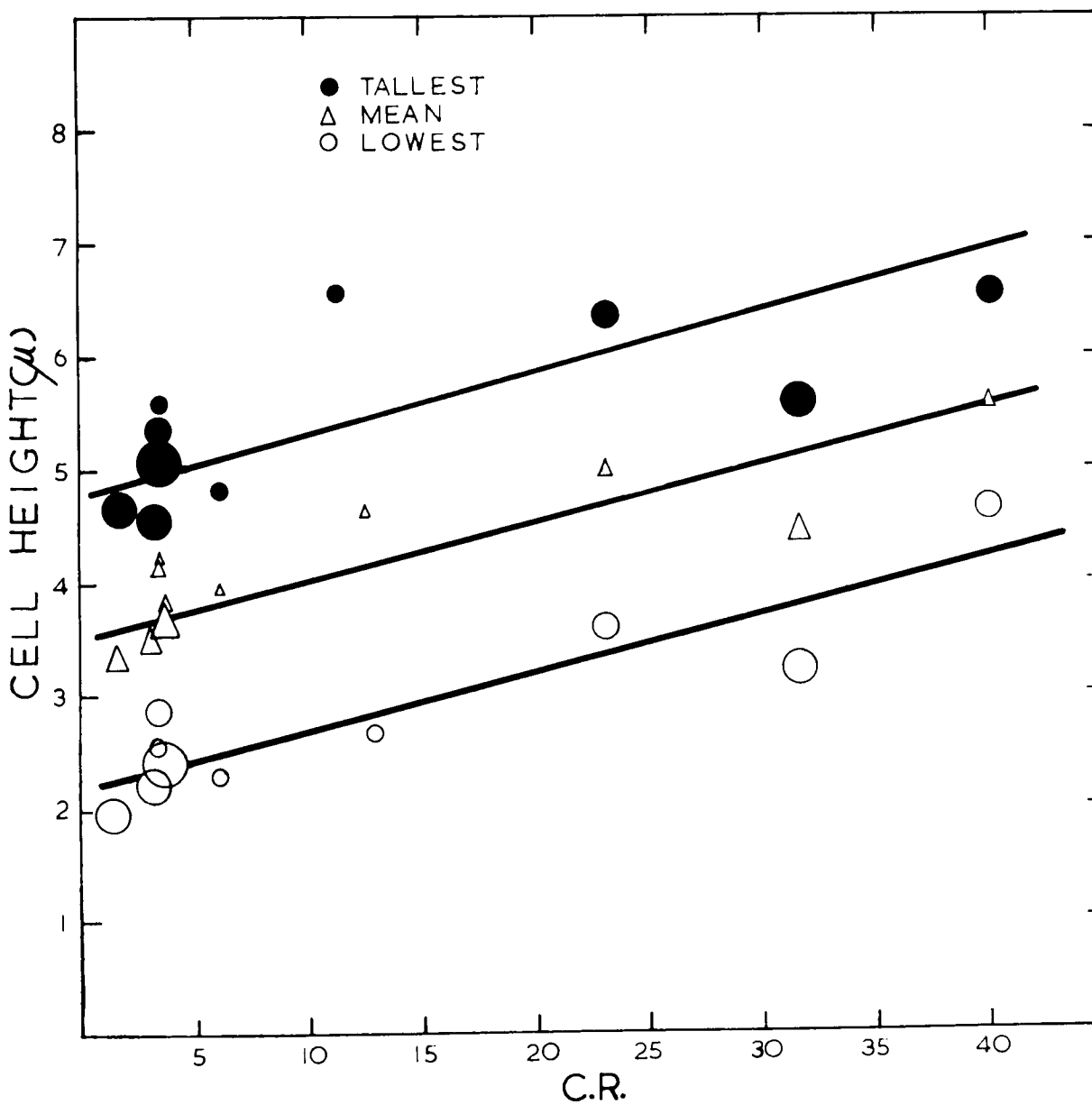
showed negative regression and also with a very wide scatter. However, there was an unfortunate bias in the sample, whereby generally smaller fish had the highest cell height. It was therefore decided to test the relationship between mean follicle diameter and mass of fish (Fig. 16). A much tighter regression resulted implying that changes in follicle diameter, as a result of increased thyroid activity, could be masked by dependence on the mass of the specimen. To test the exact nature of possible interaction between thyroid activity, follicle diameter and mass of fish, individuals of the same size but with different thyroid activities would have to be used.

Many of the above measurements were taken on thyroids in which radioiodine determinations (C. R. method) had already been made. In sockeye smolts, relationships were tested between lowest, mean, and tallest cell height and C. R. Regression coefficients were not calculated, but it can be seen that there was generally good agreement between cell height and C. R. with a slightly greater scatter where largest cell height was measured (Fig. 18). This could be due to the sectioning artifact mentioned earlier.

The agreement between mean cell height and C. R. was further substantiated by seasonal observations on sockeye, coho and chum salmon (Tables XII - XV; Fig. 19 - 21). It will be noticed in chum underyearlings (Fig. 20) that cell height also showed a consistent trend with the per cent uptake of I^{131} by the thyroid (another estimate of thyroid activity).

To follow page 21.

Fig. 18. Regression of Conversion Ratio and lowest, tallest and mean epithelial cell height in sockeye smolts.


TALLEST
MEAN
LOWEST
CELL HEIGHT(μ)
C.R.
1
2
3
4
5
6
7
8
5
10
15
20
25
30
35
40

To follow page 21.

Fig. 19. Seasonal changes in Conversion Ratio and mean cell height in two-year-old coho smolts.

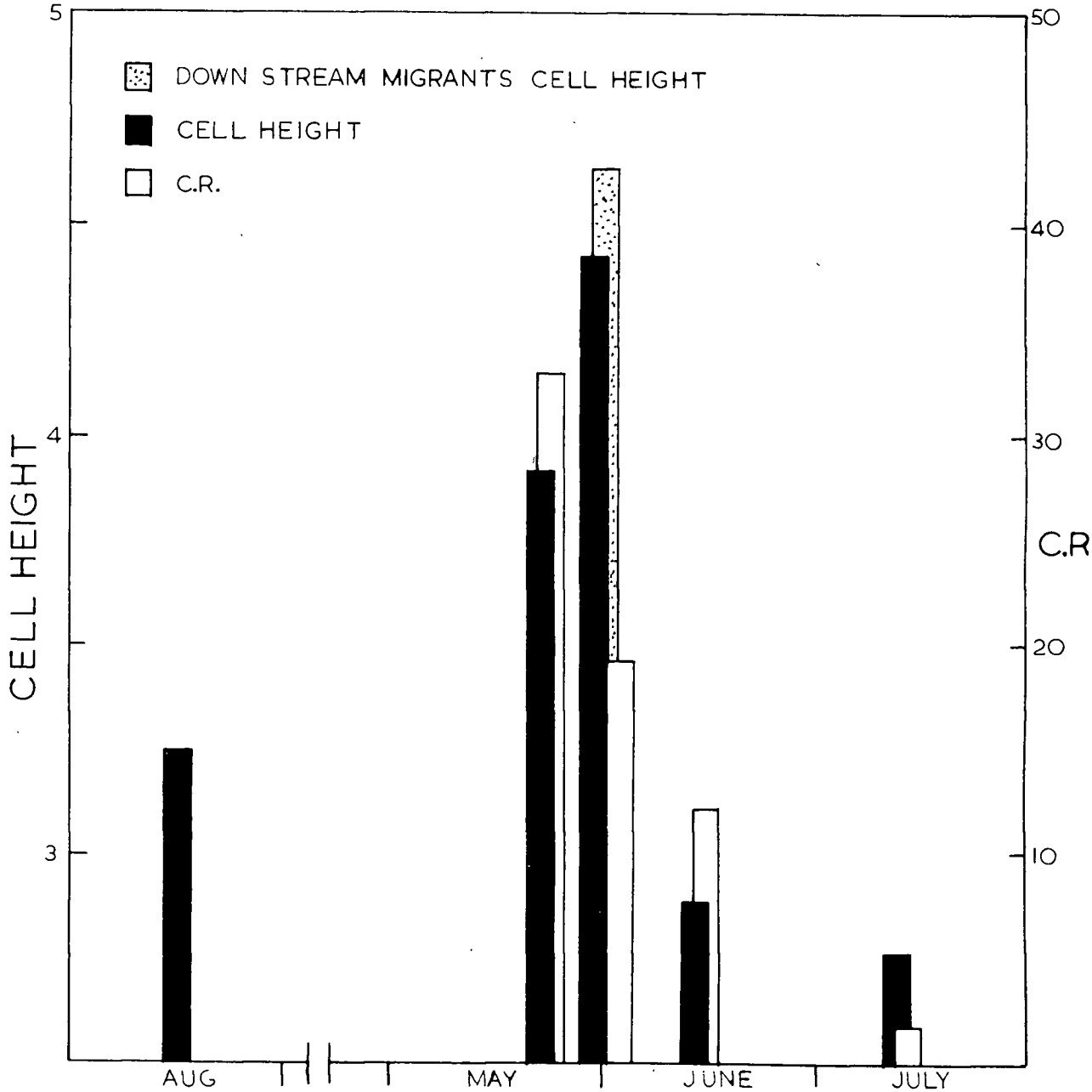

DOWN STREAM MIGRANTS CELL HEIGHT
CELL HEIGHT
C.R.
CELL HEIGHT
C.R.
5
4
3
50
40
30
20
10
AUG
MAY
JUNE
JULY

Fig. 20. Seasonal changes in Conversion Ratio, mean cell height and thyroid uptake in underyearling chum salmon.

To follow page 21.

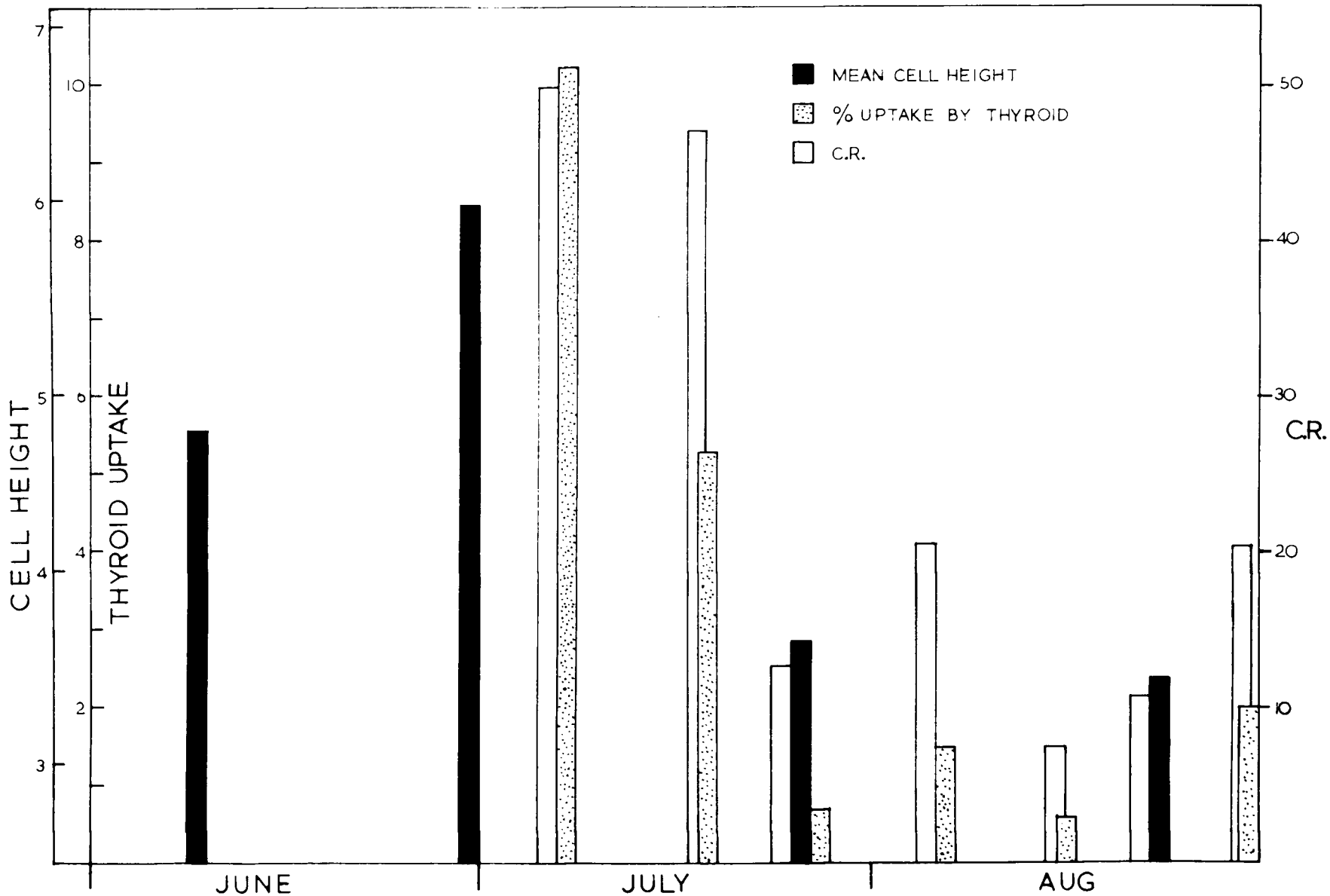
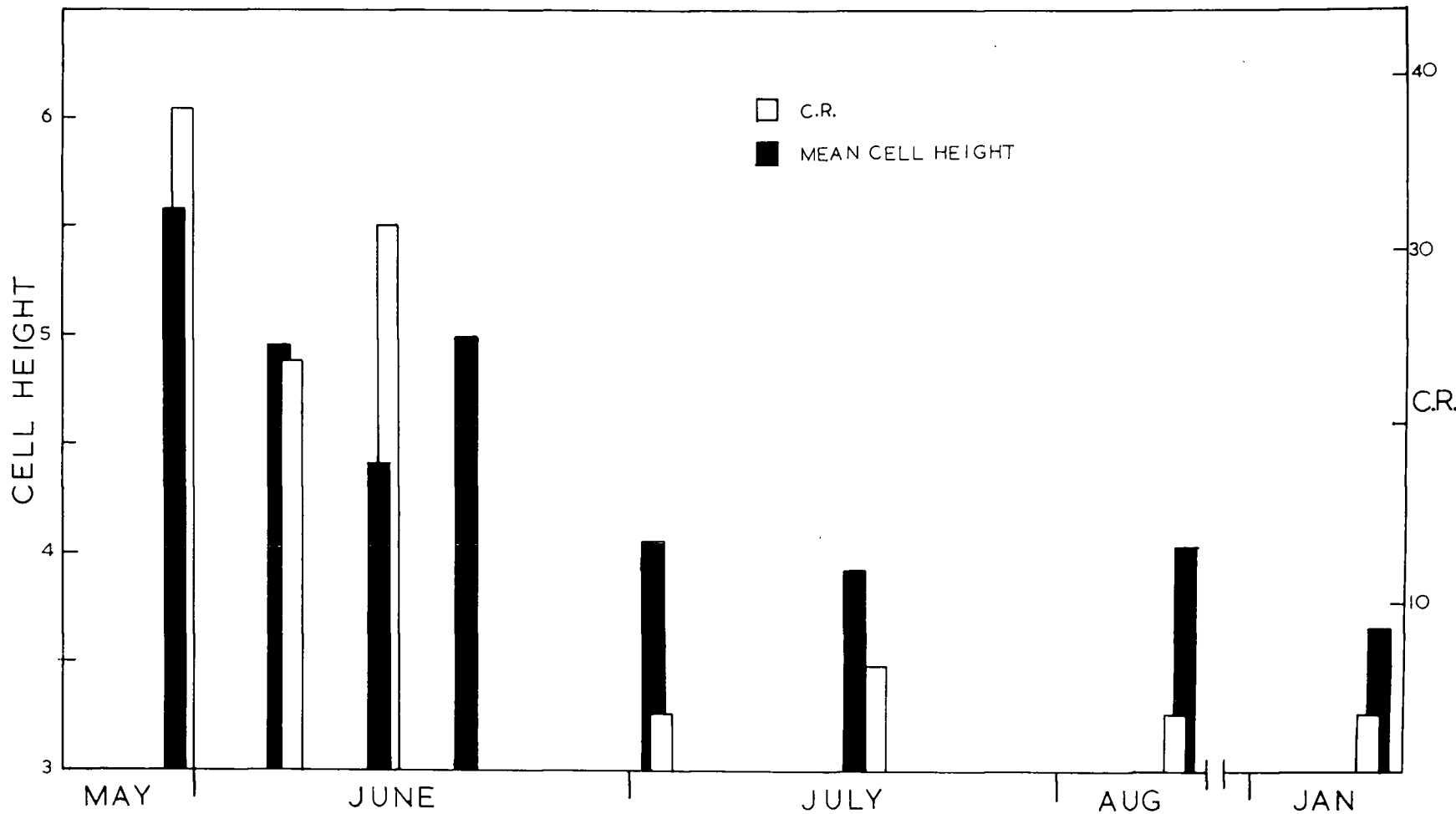

MEAN CELL HEIGHT
% UPTAKE BY THYROID
C.R.
CELL HEIGHT
THYROID UPTAKE
C.R.
7
6
5
4
3
10
8
6
4
2
50
40
30
20
10
JUNE
JULY
AUG

Fig. 21. Seasonal changes in Conversion Ratio and mean cell height in sockeye smolts.

To follow page 21.


C.R.
MEAN CELL HEIGHT
CELL HEIGHT
6
5
4
3
40
30
C.R.
10
MAY
JUNE
JULY
AUG
JAN

V. DISCUSSION

A. IODINE METABOLISM AND EVALUATION OF THYROID ASSAY METHODS

When I^{131} is injected into the coelom it rapidly enters the blood stream. Maximum blood concentration occurs in flounder and Pacific salmon within 3 or 4 hours after injection (Hickman, 1959; Baggerman, 1960). Current studies on juvenile Oncorhynchus suggest that maximum blood concentration occurs before ten hours, but no time period shorter than this was investigated in detail.

After uptake into the serum the I^{131} becomes uniformly dispersed and can leave by a variety of routes.

(i) It may enter red blood cells. As Leloup and Fontaine (1960) have pointed out the extent to which this occurs is quite variable and in the Atlantic salmon and other amphibiotic forms may be prevented by a widespread but loose binding of I^{131} to proteins in the slow albumin or the α-1-globulin zone.

(ii) It may be taken up by the thyroid which concentrates iodine against a steep gradient. This is dealt with later.

(iii) It may be selectively withdrawn into certain organs of the body. In some salmonids, a very marked concentration can occur in the ovary (Leloup & Fontaine, 1960).

(iv) The I^{131} may be eliminated permanently via the gut, the gills or the kidneys. The relative rôles of these potential sites of I^{131} excretion have not been investigated in any detail, but in flounder a very considerable percentage is thought to be lost via the gills (Hickman, 1959).

Assuming that each of these various sites of I^{131} elimination is operating at a consistent level, then the depletion of I^{131} in the serum will show a regular pattern, and in terms of measured amounts of I^{131} in the blood will

show an exponential relationship with time (Fig. 1). Consequently, the rate of I^{131} removal is best determined in terms of a biological half-life ($\frac{1}{2}t$), which may vary with species and season. In the accompanying figures, each point represents the "I^{131} Biological Coefficient/100" at a particular time for a mean of 10 to 12 fish.

Indication of excretion rate is also given by comparing the I^{131} Biological Coefficients after a particular time period. Such values depict relative differences in excretion but are less meaningful.

Excretion from the entire body (less thyroid) can also be measured (Fig. 2). This is extremely variable from species to species and in juvenile Oncorhynchus depends largely on the season. It will be noted in under-yearling chum and coho that in terms of the per cent dose retained in the body, there are marked differences even at the same time of year ($\frac{1}{2}t$ chum = 29 hrs; $\frac{1}{2}t$ coho = 90 hrs). This is explained by a greater affinity of the peripheral tissues for I^{131} or iodine in general and is discussed later at some length.

It is from this continually diminishing level of I^{131} in the plasma and the body as a whole that the thyroid I^{131} concentration occurs. If the sole rôle of the thyroid were to accumulate iodide, then the hypothetical curve would show logarithmic form, the exponential fall off in rate of I^{131} accumulation being a direct function of the fact that I^{131} blood concentration is also falling in an exponential manner.

However, it is the function of the thyroid to synthesize and secrete thyroxine and triiodothyronine which combine in the blood with a thyroid-binding protein (TBP). This is believed to be an α-1-globulin in mammals (Barker, 1955), although in some teleosts it appears that the binding is not complete (Leloup & Fontaine, 1960). This loss of I^{131} as the protein-bound form (PBI^{131}) will exert a continual influence on the thyroid uptake

curve. The hormone synthesized will progressively contain a greater and greater relative proportion of PBI^{131} as more and more I^{131} is taken up by the gland. Therefore, assuming total thyroxine output (PBI^{127} & PBI^{131}) is occurring at a constant rate, there will be an ever increasing amount of PBI^{131} lost from the gland. At a critical time period the loss of PBI^{131} will eventually exceed the ever diminishing uptake of I^{131}. Under these conditions the total level of I^{131} (protein-bound and inorganic) will show a peak. Leloup & Fontaine (1960) have demonstrated that this peak turnover of iodine occurs earlier in the active thyroid. This is supported by data presented below (Fig. 3). Therefore, ignoring any discrepency in excretion rate, it seems that (i) affinity of thyroidal tissue for iodine, (ii) rate of hormone synthesis and (iii) output of PBI^{131} can all influence the thyroid uptake curve. However, merely by measuring I^{131} uptake by the thyroid it is impossible to analyse separately these various simultaneously operating phases of I^{131} metabolism, but different states of activity may be associated with different shaped curves.

In Fig. 3A and 3B, the thyroid is inactive and the uptake curve shows a gradual climb implying a steady increase in I^{131} uptake and a little loss of PBI^{131}. The latter was borne out by observation (Fig. 4A and 4B). In a thyroid with heightened activity the curve shows a peak at approximately 120 hours (Fig. 3C) and the concentration of I^{131} in the gland may rise up to 10% of the injected dose (Fig. 3D). In Fig. 3D and 3E, extremely active thyroids are being considered. These show a distinct peak which in the case of chum salmon occurs as early as 96 hours. In the very active sockeye this may be as early as 72 hours. This rapid turnover of iodine is possibly reflected by (i) the relatively low maximum concentration of I^{131} in the gland and (ii) the very drastic rise of PBI^{131} in the plasma with which it coincides (Fig. 4D). Thus data on Oncorhynchus show that the level of I^{131}

in the thyroid can increase in a very characteristic manner which is dependent on three phases of intra-thyroidal metabolism all operating synchronously. Most meaningful appears to be the shape of the curve. A comparison of the sockeye and chum (Fig. 3C and 3D) shows that peak values may vary even in active glands. The slopes of thyroid uptake curves are probably more meaningful and as mentioned earlier, the biological half-life for loss of radioactivity from the thyroid has been used as an indication of activity in the trout by Fromm & Reineke (1956) and for other species (Leloup & Fontaine, 1960).

The closest approximation to absolute thyroid activity would be to measure the rate at which thyroxine is produced by the thyroid. As yet there is no direct measure of this quantity. By chemical methods it has been possible to determine the concentration of thyroxine and triiodothyronine in the blood. However, this cannot be used to measure thyroxine output as peripheral utilization is continually depleting this concentration.

On the other hand, one could measure the rate of production of radioactive hormone from the gland (Fig. 4). This quantity is just as prone to peripheral utilization as the non-radioiodine form. However, in the early stages of I^{131} metabolism, the relative amount of PBI^{131} added to the plasma from the thyroid is greater than that catabolised and is therefore not as susceptible to the above effect. This can be illustrated by the following hypothetical situation in which the hormone level in the blood is constant, but is being both synthesized and peripherally catabolised at a uniform instantaneous rate.

Shortly after injection, a very small percentage of the total hormone output will be radioactive. Owing to the considerable dilution occurring when this PBI^{131} becomes mixed with the stable hormone, only a small fraction will be lost due to peripheral catabolism. Thus the plasma level of PBI^{131}

will rise and will be further augmented by the ever increasing ratio of PBI^{131} to PBI^{127} free for liberation by the gland. After the peak in thyroid uptake, however, this ratio will become progressively smaller and less PBI^{131} will be released per unit time. At the same time, owing to the increase in PBI^{131} concentration in the plasma, more will be lost peripherally. Eventually, the rate of loss of PBI^{131} from the plasma exceeds its addition and this accentuates the fall in PBI^{131} level. These aspects of PBI^{131} metabolism cause the "PBI^{131} Biological Coefficient/time" curve to show a distinct peak, which is evident for all species.

The graphs are representative of both iodine uptake and hormone synthesis and secretion. As such the following points may be noted.

(i) In the inactive thyroid both the climb and fall are very slow (Fig. 4A). This is indicative of slow peripheral catabolism and low production. The maximum PBI^{131} concentration in the plasma is extremely low.

Figure 4B shows the plasma PBI^{131} concentrations in what is considered to be a relatively inactive chum thyroid. In general, the level of radioactive hormone is low but the early minor rise and fall is baffling. Relatively high values of PBI^{131} have been noticed within one or two days of injection in other instances and the only explanation is that it is an after effect of injection treatment. It is therefore an artifact, disrupting the normal smooth continuity of thyroid metabolism.

(ii) In the active thyroid (Fig. 4C and 4D), the absolute height of the peak is considerable and rise and fall is extremely sharp. This is indicative of an active thyroid gland, in which both thyroxine output and catabolism are proceeding very rapidly.

(iii) The height of the peak represents the concentration of PBI^{131} in the plasma when the output of radioactive hormone by the thyroid is equal to the rate of its catabolism. This peak value is one which will depend on

the maximum extent to which PBI^{131} replaces PBI^{127} in the thyroxine produced and also on the rate at which thyroxine is produced. Thus this peak value will depend on output of hormone, uptake of I^{131} and extent of synthesis incorporating I^{131}. This peak value is therefore representative of the several phases of iodine metabolism and may be considered a good indication of the rate of thyroxine output.

However, comparisons between peak PBI^{131} values, only have significance if to each thyroid there was the same availability of I^{131}. This difficulty is partly removed by calculating PBI^{131} plasma concentration in terms of per cent of the injected dose. However, the I^{131} pool is continually being depleted as the result of renal and extrarenal loss. Consequently, the most logical procedure would be to relate the PBI^{131} concentration peak to the rate of I^{131} loss from the blood. This could be estimated most precisely in terms of the half-life or rate constant.

Such determinations involve many measurements. A quicker and less precise, but at the same time quite meaningful method, would be to measure the relative concentrations of PBI^{131} and I^{131} at a time corresponding to peak PBI^{131} concentration in the plasma.

This may be expressed as follows:

$$\frac{PBI^{131}\ \text{Biological Coefficient}}{I^{131}\ \text{Biological Coefficient}}$$

for a single blood sample from the same fish this may be represented as

$$\frac{PBI^{131}\ (\text{c.p.m.})}{I^{131}\ (\text{c.p.m.})}$$

or, expressed in terms of a percentage of the total iodine in the blood.

$$\%\ \text{CONVERSION RATIO} = \frac{PBI^{131}\ (\text{c.p.m.})}{PBI^{131}\ (\text{c.p.m.}) + I^{131}\ (\text{c.p.m.})} \times 100\%$$

Such a ratio may be determined at any time after injection. Examples of Conversion Ratio curves for several species are given (Fig. 5). The Conversion Ratio curves can be easily interpreted in terms of I^{131} metabolism by consideration of Fig. 6, 7, 8, and 9 (Tables VI - IX).

In active thyroids, there is a peak in the C. R. occasioned by high PBI^{131} at a time of low I^{131} concentration. In very active thyroids the peak is very sharp (Fig. 8). In inactive thyroids, though the PBI^{131} concentrations peaks, no peak occurs in the C. R. and the ratio gradually rises. This is because the rate in fall of PBI^{131} concentration in the plasma is lower than the loss of I^{131} (Fig. 6 and 7).

In practice most observations were made at 108 hours after injection as this corresponded quite closely to most of the peaks in the active thyroid. In the very active thyroids, examination should be made up to 24 hours earlier otherwise a falsely low thyroid level will be recorded (Fig. 8). It is also equally important not to use C. R. measurements made too long after injection. This will give a ratio which is falsely high as the rate of loss of PBI^{131} never approaches that of I^{131} loss (Fig. 6) and in theory the ultimate C. R. after a sufficient length of time would be 100%.

Thus the success of the Conversion Ratio is dependent on a certain knowledge of the overall I^{131} metabolism of the individual. This is a criticism which can be levelled at any radioiodine determination and does not detract from its applicability.

There are, however, certain possible disadvantages associated with the method

(i) It assumes the binding of thyroxine and triiodothyronine with plasma globulins to be complete and assumes that no organic binding of I^{131} with plasma proteins occurs. As Leloup & Fontaine (1960) have pointed out variability in the former is a possibility, while the latter combination

is a certainty in amphibiotic fish. But, it is also shown that under the experimental conditions the latter combination is split by trichloracetic acid.

(ii) There may also be variability in extent of I^{131} penetration into erythrocytes. This has been shown to vary with species and season (Leloup & Fontaine, 1960). One way to eliminate such discrepency in the inorganic count would be to count the red blood cells with this fraction or to haemolyse them prior to precipitation.

Despite these possible anomolies (which could be allowed for), it is considered that the C. R. method takes into account most aspects of I^{131} metabolism and is free from disadvantages associated with measurement of I^{131} uptake by the thyroid. It is a quick and sensitive method and can be used even to measure diurnal variation in thyroid activity, if such does exist. It requires only small blood samples and in large specimens does not necessitate killing the fish if sampling is done by lateral puncture of the haemal artery.

In almost every instance it agrees with data obtained by histological techniques (Fig. 18 - 21). In the case of underyearling chum salmon retained in fresh water, remarkable agreement (on the basis of means of 10-- 12 fish) is also shown with per cent I^{131} uptake by the thyroid (Fig. 19). Also significant in these fish is the consistent loss of I^{131} from the body throughout the season (Table V; Fig. 12).

Most important is the good agreement between determinations of thyroid activity by radioiodine and by histological techniques, especially cell height. There is also good agreement between cell height and other histological characters. Certain anomolies have been observed by other workers. In particular, Pickford (Pickford & Atz, 1957, p. 129) mentions the discrepency between cell height and staining reaction in Fundulus.

A less critical but far quicker estimate would be in terms of the extent of "vacuolation". In particular, estimation of the "% of follicles with no colloid" could be used to very great advantage for quick determinations and seems a generally reliable and precisely quantified measure.

Far less reliance should be placed on estimates of depth of stain, follicle shape, and especially mean follicle diameter. The latter is almost certainly dependent in part on size in sockeye smolts.

The best method is that of Fortune (1955). As described earlier, this accounts for cell height and extent of colloid, both of which are reliable and measurable characteristics. However, the method appears tedious. Taking into account the above data and the central role played by the epithelial cell in iodine uptake, initial iodination and thyroxine release, it is suggested that the simplest and most straightforward method is to measure the epithelial height. Such a method has often been used but has rarely been compared to more reliable radioiodine techniques and thereby never substantiated before.

In conclusion, all methods for determining thyroid activity can be usefully employed in poikilotherms. Data presented reveals good agreement between histological and radioiodine determinations. Chemical methods may also be helpful in completing the picture of iodine metabolism, but could possibly be misleading in determinations of level of hormone output, though this has not been borne out by any personal observations.

The value of reliance on histology is particularly evident for field determinations. The C. R. method is chosen where an accurate assessment of thyroid overall activity is required and is the generally preferred measure. However, it would be inapplicable for small fish where the individuals have to be kept alive. In this case, thyroid uptake of iodine, though not so reliable, could be adapted to an 'in vivo' technique.

Despite above criticism, sudden increase in thyroxine output appears in many cases to cause an increased plasma thyroxine level (Leloup & Fontaine, 1960), while stable inorganic iodine determinations are of value in assessing general iodine depletion of the tissues or the environment. All methods have their use, but must be used with discrimination and interpreted as much as possible in terms of the complete iodine metabolic pathway.

B. THE ROLE OF THE THYROID IN ANADROMY

Seasonal fluctuation of thyroid activity in fish has been confirmed by many workers - Hoar (1939) and Leloup & Fontaine (1960) in the Atlantic salmon; Hoar & Bell (1950) in the Pacific salmon; Hoar (1952) in the alewife and smelt; Buchmann (1940) in the herring; Barrington & Matty (1954) in the minnow and Swift (1955, 1958) in the brown trout.

In certain species, especially amphibiotic salmonids, peaks in thyroid activity have been correlated with migration disposition. In the Atlantic salmon and rainbow trout, there is evidence to suggest that thyroxine is causal to many aspects of smoltification. It is significant that the effects of thyroxine on the "pseudosmolt" are often less marked than those in nature, despite a frequent hormone dosage above normal physiological level. The stimulatory role of thyroxine has therefore not been fully established and it is certainly not stimulatory to all aspects of smoltification.

One line of evidence not supporting such a role was supplied by Hoar & Bell (1950) in Oncorhynchus on the basis of histology. It was found that although sockeye and coho might have more active glands at the time of migration, pink and chum had inactive glands, but would become extremely hyperplastic in postmigrants retained in fresh water. Late pink and chum migrants also showed increased thyroid activity.

Such observations suggested that the thyroid was acting in a compensatory role. The thyroid was considered to offset osmotic unbalance occasioned by increased salt loss, induced by retaining a fish already adapted to marine life for an extended period in fresh water. However, doubt was cast on the validity of the histological observations. Nevertheless, data presented here suggest a very good agreement in juvenile Oncorhynchus between histological and radioiodine determinations. On that basis, the earlier work by Hoar & Bell has been accepted as representative of the thyroid state and has been combined with current histological and radioiodine data to give a more complete comparative picture for Oncorhynchus, which is as follows.

In all fry, the thyroid is quiescent. During this stage, sockeye may move from stream to lake and chum and pink move to the sea. Some of the late sea-running pink and chum may become slightly more active and on retention in fresh water become extremely hyperplastic. In chum salmon, this hyperplasticity temporarily drops in mid-August but is then maintained at a high level until November. In pink the thyroid activity is high in June, but moderately low in October. It may be noted that chum and pink in their first year of marine life have quiescent or mildly active glands.

On the other hand, sockeye and coho normally remain in fresh water and migrate as yearlings. They both have an extended parr life and during this period thyroid activity is low, though in underyearling coho a moderate fluctuation in activity may be observed (Fig. 11). At smoltification, the gland shows a very marked increase in activity. However, unlike chum and pink, retention in fresh water induces not an active but an extremely inactive thyroid state, which occurs very suddenly in June. It is also interesting to note that sockeye can survive in fresh water for at least four years and that in their third year they show a cycle of thyroid change

comparable to that in the younger fish.

Such observations could suggest a compensatory rather than a stimulatory thyroid role in alleviation of osmotic stress (Hoar & Bell, 1950; Hoar, 1952). It should be remembered, however, that the thyroid affinity for iodine is well adjusted to the concentration of iodine in the environment. In a fish destined to move into iodine-rich marine conditions, retention in iodine deficient fresh water could lead to a goitrogenic condition, offset by greater hyperplasticity. Thus the hyperactivity of the chum thyroid could be apparent rather than real. But both hypotheses would suggest thyroid hyperactivity as being secondary to other changes.

There is certain evidence to support the osmotic theory. In part, many of the changes observed in smoltification are superficially either adaptations enabling the young salmon to be displaced downstream or a form of "preadaptation" for "anticipated" marine life. Most evident is the loss of parr marks and acquisition of the "mirrored" sides of the pelagic fish. Preadaptation for an environment of very high osmotic pressure is of greater importance. In general, there is adaptation towards water retention and ion loss. This is reflected by a thickened epidermis, the development of chloride secreting cells in the gills, increased salinity tolerance and a marked salinity preference. Should these aspects be functioning while the animal is in fresh water, then osmotic stress in the form of demineralization might be expected. Such demineralization has been shown to occur in the eel. As Hoar (1959) has pointed out, a loss in the inorganic constituents also precedes the seaward migration of Salmo salar (Fage & Fontaine, 1958), Oncorhynchus masou (Kubo, 1955) and Salmo gairdneri (Houston , 1960) and may be a characteristic feature of all such migrations.

Data presented here on the variation in retention of I^{131} in the body also suggest demineralization, if one assumes that I^{131} loss is in part a

reflection of overall ion loss. Coho and sockeye had a very low I^{131} retention at the time of high thyroid activity, but later when thyroid activity dropped, this changed to low retention. In chum, there was a persistent very rapid loss of any I^{131} injected and this was coincident with a very high thyroid activity. Pink salmon showed an intermediate condition. Like chum salmon, they had a low retention in September but in October it was much higher. Again I^{131} retention paralleled thyroid activity.

In such instances it might be argued that since I^{131} retention is generally high when thyroid activity is low, the low body retention could be due to a rapid I^{131} turnover by the thyroid. But when one considers that the per cent uptake in chum salmon by the thyroid at 108 hours may vary from 0.6% to 10%, with no perceptible change in iodine loss, and also that the PBI^{131} level in the blood is but a minute fraction of the original dose, then such an explanation seems unlikely.

If it is assumed that loss of I^{131} reflects general ion loss, then the "demineralization theory" is partly substantiated. But, chum salmon in August (Fig. 11 and 12) have both an inactive thyroid and a very low body retention and this alone would tend to suggest that the two phenomena are not directly related. However, from the beginning of September to mid-November, the C. R. in these fish did not vary. It is possible that the initial rise and fall in C. R. value prior to this stabilization reflects a period of homoiostatic adjustment.

Indirect evidence negating such a role of thyroxine was presented by Hickman (1959) who showed in the starry flounder that thyroxine was associated with increased loss of ions and not their retention. In addition, Baggerman (1960) claimed that the high thyroid activity in chum salmon retained in fresh water was not reduced by immersion in salt water. Baggerman's work was done on the basis of thyroid uptake. In an exploratory

study, her experiment was repeated using the C. R. method. The results were far from conclusive but did suggest that not only may salt water have no inhibiting action on the thyroid, but could occasion an appreciable rise. These experiments are to be repeated.

The relationship between thyroid activity and osmoregulation is far from settled. There are many experiments demonstrating changes in thyroid activity when fish are transferred from fresh to salt water and also with the reverse procedure. Such data would suggest a compensatory and probably transitory rise in thyroid activity whenever the osmotic pressure of the surrounding medium is drastically altered. However, this does not preclude the possible stimulatory role implied by Baggerman's work.

This is a view frequently expressed by Fontaine (1950) whereby thyroxine stimulates or is closely associated with ion loss. This loss then brings about more profound neuroendocrinological changes. These changes in conjunction with appropriate external conditions give rise to changes in behaviour favouring displacement of the parr to the sea. In many ways this is an attractive theory as it combines both aspects of smoltification, "marine preadaptation" and "downstream displacement", into a single integrated physiological and ethological change.

Although Fontaine's idea lack direct support, they pose no serious criticism when related to Atlantic salmon. As applied to Pacific salmon, certain anomolies are evident when chum and pink are considered which have histologically low thyroid activity at migration. The survival value of very high thyroid activity in a stimulatory capacity at a time well past their normal time of migration is difficult to interpret and one wonders if it has any biological meaning in the normal life cycle.

Concerning the role of thyroxine, little can be said other than it is part of the neuroendocrinological transition which must occur when the

physiologically drastic process of smoltification occurs. It is closely associated with smoltification but its specific role is still unknown. Changes at the neuroendocrinological level rarely occur singly but as a complex and integrated association. It could be that in terms of such changes, the thyroid plays a very subordinate role and that early investigations of smoltification in salmonids, by placing great weight on this relatively easily quantified gland, have inadvertently led science away from the main governing factors in the neuroendocrine system.

Irrespective of its function, however, changes in thyroid activity can be used as indications of migration disposition in the genus Oncorhynchus.

C. SPECULATION ON SMOLTIFICATION AND THE PHYLOGENY WITHIN THE GENUS ONCORHYNCHUS

Certain differences exist between species of Oncorhynchus in both thyroid activity and body retention of I^{131}, which, as a possible reflection of total ion loss, might be indicative of marine preadaptation. In both respects chum are extreme. Although thyroid activity is low at the time of downstream migration, retention in fresh water causes a rise maintained until death. This is accompanied by drastic demineralization. These features suggest an irreversible adaptation to marine life compatible with their obligatory seaward migration.

Accepting Neave's theory (Neave, 1958) concerning the role of pleistocene glaciation in the origin to speciation of the genus Oncorhynchus, it is not difficult to envisage possible conditions under which chum could have evolved. Severe glaciation, restricting partially diadromous fish to localities of reduced food and fresh water, would favour selection for development of an extended phase of marine life. The advantages afforded by faster growth in a medium of higher osmotic pressure (Canagaratnam, 1959)

would only be offset by the physiological need for reproduction in fresh water (Tchernavin, 1939). The high thyroid activity and extreme ion loss resulting from prolonged retention of the fry in fresh water suggests inability to readapt to fresh water. If one accepts the Oncorhynchus ancestor as being only weakly amphibiotic, then it is quite conceivable that more complete smoltification as evidenced in sockeye and coho was never attained in chum salmon.

In sockeye and coho, smoltification is transitory with respect to thyroid hyperfunction and I^{131} loss from the body. The change is precisely timed and normally occurs after a prolonged period of juvenile life in fresh water. It probably develops as the result of external influence on an appropriately primed neuroendocrine system. In many respects some of the changes parallel those in chum salmon and it is speculated that smoltification now seen in sockeye and coho is a reflection of genetically controlled marine preadaptation, which has more recently been incorporated as a mechanism for displacing the parr to the sea. Expression of marine behaviour in the fresh water habitat could in itself provide the basis on which selection might act. Thus though sockeye and coho have a more elaborate smoltification, its transitory nature might suggest a closer affinity of these fish towards a possible fresh water or partially diadromous ancestor.

Pink are intermediate between sockeye and chum. The low thyroid activity at migration and elevation on prolonged retention in fresh water is characteristic of chum. But the decline in thyroid activity and increase in I^{131} retention in the body is reminiscent of the transitory hyperfunction of sockeye. It is postulated that a sockeye ancestor isolated under glacial conditions, as severe as these considered to govern the evolution of chum salmon, could have led to a secondary obligatory migration now witnessed in

pink.

In conclusion, it might be stated that coho, sockeye and chum could all have evolved independently from an Oncorhynchus ancestor under a variety of glacial conditions. However, pink may have been secondarily evolved from sockeye.

In many respects, this conclusion supports the phylogeny suggested by Hoar (1958), who, on the basis of a study of juvenile behaviour, accepted the fresh water ancestry proposed by Tchernavin (1939). Coho were claimed to represent the primitive stream-dwelling ancestor most closely, while from a sockeye-like ancestor, probably living in lakes, more extreme migrants as chum and ultimately pink were evolved.

Both physiological and ethological evidence suggest that coho and sockeye are more primitive with respect to tendency to stay in fresh water. However, on physiological evidence alone, chum could be independently evolved from the ancestral form while pink seem much closer to sockeye than Hoar indicated. It is likely that adequate fossil evidence will finally resolve the problem of the phylogeny of the genus Oncorhynchus.

VI. SUMMARY AND CONCLUSIONS

From radioiodine metabolism and histological studies on juvenile Oncorhynchus, the following conclusions are drawn.

(i) That histological and radioiodine determinations show good agreement.

(ii) That the Conversion Ratio method is a valid one and is representative of the metabolic pathway of iodine, but should not entirely replace other methods.

(iii) Comparative studies (in conjunction with the data of Hoar & Bell, 1950) reveal marked seasonal change in thyroid activity associated with downstream migration.

(iv) In coho and sockeye this occurs simultaneously with smoltification, a process which both "preadapts" the fish for marine life and also brings about its downstream displacement. Evidence for drastic physiological change at this time is the rapid loss of I^{131}, probably indicative of greatly increased deficiency of all ions. Sockeye and coho can readily revert to the fresh water physiological state of low thyroid and high ion retention and enjoy a relatively high survival in fresh water.

(v) In chum, the mechanism operates differently. These young fish are never really adapted to fresh water, but undergo their final transition in the sea. Evidence that such change has become inherent, is the irreversible thyroid hyperplasticity and ion loss induced by retaining them in fresh water.

(vi) Pink are superficially similar to chum in all respects but do show readaptation to fresh water in terms of low thyroid activity and reduced I^{131} loss when fresh water retention is prolonged.

(vii) Thyroxine has been assigned no specific role. It appears to be

closely correlated with I^{131} loss and may have an osmoregulatory role but there is no direct evidence to support this in Oncorhynchus. In the present treatment, it has been considered as a physiological indication of migration disposition. It may even be a reflection of continued existence in a iodine deficient fresh water environment.

(viii) In view of the lack of available evidence, detailed speculation on the evolution within the genus would be futile. However, combining physiological data with the geological presentation by Neave, general statements can be made. It is suggested that chum, coho and sockeye could have evolved independently from the ancestral Oncorhynchus species and derived their varied life histories during glacial isolation. Pink, however, may have been secondarily derived from sockeye.

(ix) Smoltification has been considered a specialized process combining marine preadaptation with a mechanism for downstream displacement. It could have been derived by delaying the type of marine preadaptation shown to occur in postmigrant chum salmon held in fresh water.

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VIII APPENDIX

TABLE I

Effect of I^{131} dose on Conversion Ratio in underyearling chum salmon

Mean mass (S.D.)	Mean C.R. (S.D.)	Dose injected (µc)	No. of fish
3.8 (1.47)	13.0 (3.56)	2	11
5.0 (2.18)	12.0 (5.60)	4	11
4.8 (2.39)	7.60 (5.81)	8	12
5.7 (1.29)	15.80 (8.15)	16	8
5.7 (2.52)	15.35 (5.05)	40	9

TABLE II

Decrease in Conversion Ratio in second plasma sample in sockeye smolts

No.		C.R.	Body mass (gm)	Plasma mass (mgm)	% change I^{131}/mgm	% change PBI^{131}/mgm	% change C.R.
A	1	73.0	25.00	41.3	3.53	6.9	3.01
	2	70.8		29.2	increase	decrease	decrease
B	1	57.7	23.35	26.6	66.30	16.8	29.65
	2	40.6		21.6	increase	decrease	decrease
C	1	49.7	18.80	38.65	0.379	14.2	5.84
	2	45.8		30.00	increase	decrease	decrease
D	1	18.06	13.50	16.5	0.234	89.0	86.80
	2	2.37		35.6	increase	decrease	decrease
E	1	36.9	16.80	27.0	10.80	86.2	77.90
	2	8.2		40.0	decrease	decrease	decrease
F	1	4.15	33,00	57.0	4.12	21.6	10.60
	2	3.71		61.0	decrease	decrease	decrease
G	1	40.70	23.80	45.0	215.5	93.8	96.6
	2	1.33		50.0	increase	decrease	decrease
H	1	1.97	22.20	64.0	0.90	3.4	5.3
	2	1.87		48.0	increase	decrease	decrease

TABLE III

Diurnal variation in Conversion Ratio in chum salmon

Killed August 15th.		Killed July 25th.	
Conversion Ratio			
10:00 a.m.	10:00 p.m.	11:00 a.m.	11:00 p.m.
5.2	10.6	1.0	12.2
7.1	9.2	3.2	51.8
9.2	11.6	7.2	9.1
8.0	11.2	7.1	9.3
11.0	7.1	3.6	8.1
13.2	9.8	25.6	24.5
19.6	12.1	7.0	14.2
3.2	13.4	37.5	12.5
6.6	-	3.5	9.6
2.2	-	1.3	17.4
1.5	21.4	30.9	14.2
3.0	10.0	-	8.9
Mean = 7.7	11.6	11.8	16.0
Significantly different by ranking at 0.01% level.		Significantly different by ranking at 0.05% level.	

TABLE IV

Seasonal change in Conversion Ratio in juvenile *Oncorhynchus* held in fresh water

COHO underyearlings and smolts	Aug. 15	Sept. 7	Oct. 1		Jan. 1	May 20	May 30	June 13	July 11		Date
	15.3	6.0	2.4		1.7	34.0	19.3	12.2	1.9		C.R.
	7.9	4.0	0.9		0.6	12.2	11.3	3.9	1.6		S.D.
	May 29	June 6	June 15	July 2	July 18	Aug. 9	Sept. 7	Oct. 12			Date
Yearling SOCKEYE	39.4	23.6	31.6	3.4	6.1	3.4	2.5	8.3			C.R.
	12.2	15.6	30.1	1.9	2.5	2.4	0.9	3.0			S.D.
	Jan. 10	May* 20	May* 28	June 4	June 16	July 2	July 18	Sept. 7	Oct. 12		Date
Three and four-year-old SOCKEYE	3.3	75.4	62.9	3.2	3.8	1.6	12.5	6.0	5.2		C.R.
	0.9	13.2	15.8	2.2	2.1	0.2	4.6	0.1	1.9		S.D.
	July 18	Aug. 10	Aug. 30	Oct. 12							Date
Underyearling PINK	42.0	18.7	23.0	6.9							C.R.
	18.9	6.7	2.2	1.3							S.D.
	July 7	July 18	July 25	Aug. 6	Aug. 15	Aug. 22	Aug. 30	Sept. 7	Oct. 12	Nov. 20	Date
Underyearling CHUM	49.9	47.0	11.6	20.5	7.5	10.8	13.0	27.6	26.6	30.0	C.R.
	29.5	9.6	14.5	5.9	5.4	8.8	3.6	19.5	19.5	†	S.D.

* Examined 84 hours - all other determinations at 108 hours.

† Estimated from per cent uptake by thyroid.

TABLE V

Seasonal change in the per cent retention (after 108 hrs) of injected I^{131} in plasma and body (less thyroid) of juvenile Oncorhynchus held in fresh water

Date	$\frac{1}{2}t$ serum (hr)	$\frac{1}{2}t$ body (hr)	% in body at 108 hrs
A. Underyearling CHUM			
July 7	32	-	-
Aug. 6	-	24*	2.8
Aug. 22	24	23	2.2
Aug. 30	-	19*	1.3
Sept. 7	-	19*	1.4
Oct. 12	-	28*	4.4
Nov. 20	-	26*	4.2**
B. Underyearling COHO			
Sept. 3	30	89	24.0
Oct. 1	-	50*	10.8
Nov. 20	-	69*	13.9
C. COHO smolts			
June 15	24	25	2.7
Nov. 20	-	(very long)*	34.7
D. Underyearling PINK salmon			
Aug. 6	-	47*	8.9
Aug. 29	-	21.5*	1.7
Oct. 12	-	72*	15.6
E. Yearling SOCKEYE smolts			
June 14	25	23	2.5
Sept. 7	-	(very long)*	24.8
Oct. 12	-	74	16.1
Nov. 20	-	(very long)*	35.0
F. Three-year-old SOCKEYE smolts			
May 16	23	-	-
Sept. 7	-	(very long)*	25.0
Oct. 12	-	(very long)*	18.5
Nov. 20	-	(very long)*	26.3

*Calculated from per cent in body at 108 hrs.
**2 fish in this group had values of 22.7 and 20.0%.

TABLE VI

I^{131} metabolism in underyearling coho salmon (thyroid inactive)

PBI_t (hr)	C. R. Mean	C. R. S.D.	% thyroid Mean	% thyroid S.D.	I^{131} Biol. Coeff./100 Mean	I^{131} Biol. Coeff./100 S.D.	PBI ^{131} Biol. Coeff. Mean	PBI ^{131} Biol. Coeff. S.D.	% body Mean	% body S.D.
24	0.7	0.15	1.6	0.33	9.9	4.5	0.0	0.0	35.8	12.4
48	1.2	1.11	2.2	1.07	8.8	4.4	1.6	1.3	36.8	7.6
72	2.1	0.35	2.1	0.92	2.1	1.3	2.4	1.6	27.5	10.9
96	3.0	1.34	3.8	0.83	1.3	0.7	2.5	2.0	27.6	7.6
120	6.0	4.00	3.7	1.23	1.5	0.6	5.2	2.12	22.1	3.6
144	3.4	1.38	3.6	1.30	0.6	0.3	2.2	2.3	14.8	7.3
312	8.7	2.89	5.0	1.95	0.024	0.007	1.3	1.2	3.8	2.9

Sampled September 2 - 15, 1960.
Each mean comprises 10 fish.
Size range 0.85 - 2.6 gm; 4.1 - 7.2 cm.

TABLE VII

I^{131} metabolism in underyearling chum salmon (thyroid inactive)

PBI_t (hr)	C. R. Mean	C. R. S.D.	% thyroid Mean	% thyroid S.D.	I^{131} Biol. Coeff./100 Mean	I^{131} Biol. Coeff./100 S.D.	PBI^{131} Biol. Coeff. Mean	PBI^{131} Biol. Coeff. S.D.	% body Mean	% body S.D.
12	2.2	0.69	0.93	0.41	16.10	5.31	1.80	2.40	30.5	8.15
36	7.7	2.89	1.38	0.53	9.50	4.99	33.60	5.80	15.1	2.33
60	9.4	8.46	1.68	0.49	3.45	2.43	14.50	3.90	8.0	4.00
84	5.2	2.82	1.70	0.34	1.90	1.18	3.30	2.80	4.1	1.90
108	10.0	8.66	1.67	0.55	0.83	0.45	3.60	0.60	2.2	1.33
132	12.1	6.48	-	-	0.53	0.33	4.8	0.50	1.8	0.93
156	17.9	11.10	2.07	0.62	0.37	0.30	2.8	0.80	1.2	0.63

Sampled August 18 - 24, 1960.
Each mean comprises 11 - 12 fish.
Size range 1.3 - 4.3 gm.

TABLE VIII

I^{131} metabolism in underyearling chum salmon (thyroid active)

PBI_t (hr)	C. R. Mean	C. R. S.D.	% thyroid Mean	% thyroid S.D.	I^{131} Biol. Coeff./100 Mean	I^{131} Biol. Coeff./100 S.D.	PBI^{131} Biol. Coeff. Mean	PBI^{131} Biol. Coeff. S.D.
20	7.6	2.45	3.72	1.23	8.39	9.10	3.1	1.25
48	17.6	8.90	4.93	1.42	1.56	0.80	20.5	1.28
72	9.8	5.94	7.07	2.69	1.28	0.39	4.3	2.00
96	44.7	12.30	10.33	1.43	0.73	0.21	65.2	31.50
108	49.6	21.50	9.20	3.84	0.52	0.80	55.5	22.60
120	40.2	13.10	8.40	2.24	0.25	0.29	18.4	15.60
150	24.8	23.40	5.80	3.00	0.28	0.12	22.0	8.70

Sampled July 2 - 8, 1960.
Each mean comprises 3 - 8 fish.
Size range 0.4 - 1.2 gm; 3.5 - 6.0 cm.

TABLE IX

I^{131} metabolism in three-year-old sockeye smolts (thyroid active)

PBI_t (hr)	C. R. Mean	C. R. S.D.	% thyroid Mean	% thyroid S.D.	I^{131} Biol. Coeff./100 Mean	I^{131} Biol. Coeff./100 S.D.	PBI^{131} Biol. Coeff. Mean	PBI^{131} Biol. Coeff. S.D.
11.5	2.5	2.36	0.52	0.35	8.15	7.91	3.9	2.9
19.0	0.5	0.12	0.77	0.53	10.06	2.75	4.3	4.9
35.0	0.7	0.10	1.44	0.12	11.67	5.84	6.7	2.5
43.0	1.1	0.40	1.61	0.20	8.93	2.74	7.6	2.4
59.5	1.1	0.65	2.04	0.34	7.28	3.59	5.7	4.6
67.0	1.1	0.35	3.10	0.93	7.84	3.42	7.0	4.0
83.5	47.6	50.20	2.23	1.10	2.60	1.86	223.5	175.0
92.0	45.0	53.40	2.08	1.13	1.92	1.90	128.7	90.9
107.0	5.3	17.50	1.64	0.56	1.42	2.00	17.3	12.9
118.0	4.3	1.80	1.81	0.44	1.40	1.03	21.3	11.6
131.5	1.4	0.94	1.28	0.63	1.62	0.90	13.5	10.0
143.0	7.2	3.38	1.76	0.77	1.62	1.20	35.0	48.5

Sampled May 17 - 28, 1960.
Each mean comprises 3 - 6 fish.
Size range 10 - 30 gm; 10 - 18 cm.

TABLE X

Quantal and semi-quantal determinations of histological characters in sockeye smolts

Lowest cell height(μ)		Vacuolation		% follicles with no colloid		Staining		Follicle diameter(μ)		Mass fish (gm)	
Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
2.24	1.95 2.55	1.30	1.03 1.50	4.57	3 6	3.12	2.87 3.46	61.0	44.7 76.7	19.4	14.5 23.0
3.20	2.61 3.82	1.86	1.29 2.18	17.40	8 25	2.51	2.06 3.25	62.5	59.6 65.4	14.5	8.3 21.3
3.27	2.66 3.83	1.78	1.54 2.02	19.00	13 23	2.21	1.69 2.73	66.4	63.7 71.2	17.8	16.8 18.8
4.63	4.01 5.17	1.98	1.47 2.37	19.25	12 26	2.17	1.61 2.89	33.2	28.9 36.8	5.4	3.2 8.4
3.56	3.23 3.89	1.79	1.51 2.08	14.00	10 18	2.94	2.58 3.31	39.9	37.1 42.8	4.0	3.0 5.0
2.20	1.90 2.30	1.23	1.20 1.27	6.00	6 6	2.98	one fish	77.8	one fish	26.1	21.7 30.4
3.22	2.70 3.64	1.03	0.94 1.11	13.25	8 20	3.33	3.05 3.62	35.3	29.9 38.2	4.3	3.8 4.7
3.75	2.92 4.58	-	-	17.00	15 19	-	-	-	-	4.5	4.0 5.0
2.40	2.36 2.43	1.24	-	8.00	-	2.98	-	77.8	-	45.7	-
1.96	1.44 2.40	-	-	9.80	6 12	-	-	-	-	37.2	21.3 50.1
2.90	2.64 3.24	-	-	24.30	20 31	-	-	-	-	6.4	4.3 7.8
2.28	2.12 2.45	-	-	9.00	8 10	-	-	-	-	5.6	5.4 5.8
2.68	2.40 2.96	-	-	9.50	6 13	-	-	-	-	60.6	47.0 74.0
2.50	2.30 2.73	-	-	7.00	6 8	-	-	-	-	11.2	10.5 12.0

Note: In each thyroid, 100 follicles were measured and means calculated. The mean of these mean values is given above for groups of 1 - 7 fish.

TABLE XI

Cell heights and Conversion Ratio in sockeye smolts

HISTOLOGY DATA							RADIOIODINE DATA			
Mean cell heights (100 follicles/fish) (μ)						No. of fish	Mean C.R.	S.D.	PBI_t (hr)	No. of fish
Lowest	Range	Highest	Range	Mean	Range					
2.24	1.95 2.55	5.07	4.33 5.62	3.65	3.13 4.03	7	3.3	0.94	108	7
4.63	4.01 5.17	6.54	6.02 6.96	5.58	5.00 5.81	4	39.4	12.20	108	9
3.56	3.23 3.89	6.36	5.94 6.76	4.97	4.59 5.53	3	23.6	15.60	112	3
2.20	1.90 2.50	4.52	3.70 5.34	3.36	2.80 3.93	2	3.2	2.17	108	4
3.22	2.70 3.64	5.59	4.97 5.85	4.41	3.84 4.76	4	31.6	30.10	108	8
2.40	2.36 2.43	5.13	4.62 5.71	3.78	3.49 4.07	3	3.8	2.11	108	3
1.96	1.44 2.40	4.68	3.84 5.27	3.32	2.64 3.83	4	1.6	0.24	108	6
2.90	2.64 3.24	5.43	5.21 5.66	4.16	4.14 4.20	3	3.4	1.89	108	9
2.28	2.12 2.45	4.81	4.79 4.83	3.93	3.64 4.23	2	6.1	2.55	108	7
2.68	1.96 2.40	6.56	6.33 6.79	4.62	4.36 4.88	2	12.5	4.60	108	8
2.50	2.30 2.73	5.59	5.55 5.62	4.16	3.96 4.14	2	3.4	2.41	108	2

Note: See note to Table X concerning the mean cell height.

TABLE XII

Seasonal changes in Conversion Ratio and mean cell height in two-year-old coho smolts

Mean cell height(μ)	Range	No. of fish	C.R.	S.D.	No. of fish	Date
3.92	2.89 5.18	4	33.0	12.2	6	May 20
4.43	3.74 5.11	2	19.3	11.3	2	May 30
2.89	2.13 3.47	3	12.2	3.98	4	June 13
2.78	2.22 3.34	2	1.9	1.62	2	July 11
3.25	3.33 3.41	3	15.3	7.90	9	Aug. 15 *

* Underyearling fish.
Note: See note to Table X concerning the mean cell height.

TABLE XIII

Seasonal changes in Conversion Ratio, mean cell height and thyroid uptake in underyearling chum salmon

HISTOLOGY			RADIOIODINE						
Mean cell height(μ) Mean	Range	No. of fish	C.R. Mean	C.R. S.D.	% thyroid Mean	% thyroid S.D.	No. of fish	PBI_t (hr)	Date
4.82	-	1	-	-	-	-	-	-	June 8
6.08	5.72 6.44	2	-	-	-	-	-	-	June 30
-	-	-	49.9	29.50	10.2	3.85	6	108	July 7
-	-	-	47.0	9.61	5.3	1.63	11	108	July 18
3.65	3.58 3.89	4	11.6	14.50	0.7	0.19	12	108	July 25
-	-	-	20.5	5.89	1.5	1.39	12	108	Aug. 6
-	-	-	7.5	5.36	0.6	0.16	12	108	Aug. 15
3.45	2.97 3.87	3	10.8	8.85	1.7	0.62	12	108	Aug. 22
-	-	-	20.6	13.30	2.0	0.94	12	108	Aug. 30
-	-	-	27.6	19.60	6.1	2.57	12	108	Sept. 7
-	-	-	26.6	19.50	4.5	0.51	7	108	Oct. 12

Note: See note to Table X concerning the mean cell height.

TABLE XIV

Seasonal changes in Conversion Ratio and mean cell height in two-year-old sockeye smolts

Mean cell height(μ)	Range	No. of fish	C.R.	S.D.	No. of fish	Date
5.58	5.00 5.81	4	39.4	12.20	9	May 29
4.96	4.59 5.33	3	23.6	15.60	8	June 6
4.42	3.84 4.76	4	31.6	30.10	8	June 13
5.00	4.05 5.83	3	-	-	-	June 19
4.16	4.14 4.20	3	3.4	1.89	9	July 2
3.93	3.64 4.23	2	6.1	2.55	7	July 18
4.05	3.96 4.14	2	3.4	2.41	3	Aug. 9
3.66	3.13 4.03	7	3.3	0.94	7	Jan. 10

Note: See note to Table X concerning mean cell height.