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Four species of euryhaline teleosts (chinook salmon, Oncorhynchus tshawytscha, Walbaum; staghorn sculpin, Leptocottus armatus, Girard; starry flounder, Platichthys stellatus, Pallas; shiner perch Cymatogaster aggregata, Gibbons) were exposed to x-rays. The irradiated animals were maintained in either of two different environments. The effects of x-radiation were determined by observing the percent survival, plasma osmotic and electrolyte concentrations and histopathological changes of gill tissue. The results obtained are:

1. Survival of fish in either a fresh- or salt-water environment following irradiation is dose and species dependent.
2. Chinook salmon living in salt water showed increased mortalities and blood osmotic properties following irradiation. Hyperplasia of the support cell area of the gill filament was observed.
3. Staghorn sculpin showed few mortalities in salt water (<10%) but a

significant increase ($P > .01$) in plasma osmotic and electrolyte concentrations was noted in animals maintained in salt water following irradiation.

4. Starry flounder showed an increase in mortality and osmotic properties of blood plasma when maintained in salt water following irradiation. A decrease in osmotic properties was noted when animals were maintained in fresh water following irradiation and some mortalities were noted in both control and irradiated groups.
5. Shiner perch showed variable response to x-ray exposure but appears to be radiation sensitive.

Radiation Inhibition of Salt-Water Adaptation in Fish

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I. INTRODUCTION

A euryhaline fish is an osmoregulator when it can maintain the internal body fluid concentration constant even though it is living in widely divergent environments. Thus the inability of the fish to osmoregulate, whether it resides in an ocean or a fresh-water lake, will usually result in disasterous consequences. In a salt-water environment, such as the ocean, the salt content of the internal fluids tends to rise. This is due to the fact that the fish becomes dehydrated because the differential salt gradient (ocean >> plasma) allows body water to pass into the environment through permeable membranes. In a fresh-water environment the internal salt concentration (plasma >> fresh water) is reversed. The differential salt gradient allows the fish to fill with water to the point of over-hydration. To survive, the fish must hypoosmoregulate while in salt water (lose salt and conserve water) and hyperosmoregulate (lose water and conserve salt) via the kidney if in a fresh-water environment (Krogh, 1939; Black, 1951; Lagler, Bardock and Miller, 1962).

H. W. Smith (1930) showed that fish in the sea ingest large quantities of the external medium in order to maintain water balance and absorb Na^{+}, K^{+} and Cl^{-} leaving a Mg^{++} and $SO_4^{=}$ residue in

the intestine. He found that the intestinal residue and urine were dilute in salt and suggested that the marine fish is doing work via a mechanism which lies anatomically outside of the kidney.

A. B. Keys (1931) implicated the surface of the gills as the site of salt exchange and implied that this exchange took place against a salt concentration gradient. In a later histological investigation of the gill, Keys and Willmer (1932) postulated a secretory role for a certain type of cell found on the gill filament between the respiratory lamellae. The cells were called "chloride secretory cells."

D. E. Copeland (1948) investigating the physiology of the "secretory cell" found that its development depended on internal rather than external osmotic gradients. Its function as a chloride excretory cell is implied but the exact mechanism is still uncertain. Using electron diffraction patterns, Philpott (1964) established that a silver halide was secreted in the gill and in all probability it is the chloride salt.

Recently, Threadgold and Houston (1964) in an electron microscopic study of the "secretory cell" in salmon enabled the ultrastructure of the gill filament to be envisaged. Conte and Lin (1967) and Lin (1966) discuss the ultrastructure and development of the "secretory cells" using labelling techniques and electron microscopy. The "secretory cells" appear to arise from the basal

membrane region of the gill and seem to have a higher turnover time based on tritium tagged DNA when the fish is in salt water.

The biochemical mechanisms and energetics of the system are still uncertain. Enzymatic activity should show correlation with the mechanisms involved in osmoregulation because active ion transport requires that energy be supplied. Alkaline phosphatase activity was found to be increased as the osmotic gradient increased (Pettengill and Copeland, 1948). Succinic dehydrogenase activity in the gills has been demonstrated (Sexton and Russell, 1955) but efforts to correlate this activity with changes in osmotic gradient have lead to conflicting reports (Natochin and Bocharov, 1962; Conte and Tripp, 1967). Recently, Epstein, Katz and Pickford (1967) have reported a sixfold increase in adenosine triphosphatase activity in the gills of the euryhaline killifish (Fundulus heteroclitus) when living in salt water as compared to fresh water. Conte and Morita (1967) found a four to eight-fold increase in cellular proteins of the gill tissue when immunochemical techniques were applied to the gills of salt-water adapted chinook salmon in contrast to fish maintained in fresh water.

The study of hormonal control of water and electrolyte balance in euryhaline fish has lead to a few definitive conclusions. Extensive reviews by Fontaine (1954) and Hoar (1951) have been made. They conclude that the neurosecretions from the diencephalon and hypophysis of the brain and the thyroid play some role in the process of

osmoregulation and migration. Pickford and Atz (1957) urge that the distinction between transitory responses and those of basic maintenance be kept in mind at all times. Recently, Pickford, Pang and Sawyer (1966) showed the necessity of prolactin for survival of hypophysectomized killifish in fresh water. Similar results were obtained by Chartier and Olivereau (1965) using the eel.

The effects of radiation on the osmoregulatory mechanisms in euryhaline fish was first reported by Conte (1965). This work showed that the radiosensitive areas appear to be either the head-gill region or the intestinal region. However, the gill filaments and interlamallae regions show major histopathological changes with exposures of > 1000 R.[1] The cytological area being most effected appears to be identical with the region of the "secretory cells." Using partial shielding techniques, it has been shown that the control of sodium excretion (Srivastava and Tachi, 1964) and of osmotic regulation in general (Conte and Lorz, 1965) is localized in the head region. V. V. Brunst (1967) has demonstrated the radiosensitivity of the gill epithelium of the axolotl (Siredon mexicanum). Survival studies have been performed on numerous species of fresh-water fishes: goldfish (Carassius auratus) by Ellinger (1944); adult rainbow trout (Salmo gairdneri) by Welander, et al. (1949); on fingerling chinook

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[1] R = Roentgen, the unit of radiation exposure.

salmon (Oncorhynchus tshawytscha) by Bonham, et al. (1948) and on goldfish and medaka (Oryzias latipes) by Egami, et al. (1962). It must be noted that survival following irradiation has always been related to hematopoietic effects (Sletten, Lockner and Heversy, 1964; Watson, et al., 1962); infection (Shechmeister, et al., 1962) or endocrine imbalance (Etoh and Egami, 1963; Sathyanesan and Chavin, 1966).

The objective of this thesis is to examine the effect of radiation on the osmoregulatory systems in the chinook salmon (Oncorhynchus tshawytscha) and the following estuarine fishes: staghorn sculpin (Leptocottus armatus, Girard), starry flounder (Platichthys stellatus, Pallas) and shiner perch (Cymatogaster aggregata, Gibbons).

II. MATERIALS AND METHODS

A. Collection of Experimental Animals

Staghorn sculpin (Leptocottus armatus, Girard), starry flounder (Platichthys stellatus, Pallas) and shiner perch (Cymatogaster aggregata, Gibbons) were collected by otter trawl[2] in the Sally's Slough and King's Slough region of Yaquina Bay, Oregon. The trawling was performed in channels existing between the mud flats in the slough areas at low tide. This insured a maximum concentration of fish and increased the efficiency of collection. The trawls were made during the months of June through October. This proved to be the best time of the year for obtaining the size (10-15 cm) specimens used in the experiments. In most instances, short trawling times of approximately five minutes duration were used to prevent injury to the fish from overcrowding in the trawl net. This was particularly necessary to insure the survival of the shiner perch (C. aggregata). Fall chinook salmon (Oncorhynchus tshawytscha, Walbaum) were obtained from the Oregon State Game Commission Research Division Laboratory, Corvallis, Oregon where they had been reared from eggs. These animals were approximately 10-15 cm in length and were sexually immature.

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[2]Marinovich Trawl Company, Biloxi, Mississippi.

B. Maintenance of Experimental Animals

After collection, specimens of staghorn sculpin, starry flounder and shiner perch were either transported from the Marine Science Center, Newport, Oregon to the aquaria facility at the Radiation Center, Oregon State University or were maintained in aquaria at the Marine Science Center. The animals were transported in plastic garbage cans containing clean, aerated sea water.

At the aquaria facility in the Radiation Center, 20-40 animals were placed in individual 120 or 300 liter fiberglass aquaria.[3] The bottoms of the aquaria were covered with sand and crushed oyster shells to simulate a native habitat and to encourage feeding. Each species fed well on chopped squid when maintained in salt water. However, they showed little tendency to feed when maintained in fresh water.

Fall chinook salmon were transported in plastic garbage cans containing fresh water from the Oregon State Game Commission Laboratory to the aquaria facility at the Radiation Center. The animals fed well on commercially prepared fish pellets[4] whether maintained in fresh or salt water.

Prior to experimentation, all animals were adapted to their

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[3] Hallcraft Plastics Ltd., Vancouver, British Columbia.

[4] Bio-Vita Oregon Pellets, Bioproducts, Warrenton, Oregon.

environmental medium for at least three days. If a change of environment was necessary before the experiment, such as, from fresh to salt water, then a minimum of ten day's adaptation was allowed.

The photoperiod was controlled and consisted of a twelve hour day and night intervals.

The salt water used in the experiment was provided by a continuous flow, recirculating system which passed filtered sea water through a refrigeration system. The main reservoir is underground and has a 10, 000 gallon capacity. The filter consisted of diatomaceous earth, oyster shells, sand and gravel. The water is maintained at 10^{o} C $\pm$ 1^{o} before entering the aquaria room.

Salinity at the aquaria facility ranged from 21 to 27 ‰ during the course of the experiments. Although a range in salinity is far from an optimal condition, it was sufficient to insure a hyperosmotic medium. The pH of the salt water ranged from 7.6 to 7.8

The fresh water is provided by a system in which dechlorinated city tap water is refrigerated. It is a continuous flow but non-recirculating system and is temperature controlled by the refrigeration system to 10^{o} C $\pm$ 1^{o}.

C. Irradiation Procedure

The animals were prepared for irradiation by anesthetization

with 1:20,000 MS-222[5] (ethyl m-aminobenzoate) and placed in individual compartments in the exposure holder. The environmental medium (either fresh or salt water) was aerated and recirculated during the exposure period and contained 1:30,000 MS-222 anesthetic. Temperature during exposure was kept within $\pm$ 2° C of the temperature in the aquaria facility by using an ice bath.

The exposure holder used in the chinook salmon and shiner perch irradiations was constructed of 0.6 cm thick plexiglass and was placed in a larger rectangular plexiglass tray to provide efficient recirculation. Twenty-four compartments enabled twenty animals to be irradiated at one time.

The radiation field overlapped the exposure holder by 1 cm on a side giving an open field of 31 x 31 cm at 66 cm anode to water surface (top of exposure holder) distance. Additional distance to table top amounted to 4.8 cm and this included 3.6 cm of water which is approximately the vertical distance from ventral to dorsal sides of the experimental animals. The additional 1.2 cm is due to the holder within a tray arrangement. Beneath the outer plexiglass tray was a wooden table whose top was 79 cm above floor level. Conditions were those of maximum backscatter since addition of unit density material beneath the wooden table failed to contribute additional detectable scattered radiation.

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[5]Sandoz Pharmaceuticals, Hanover, New Jersey.

The exposure holder for irradiation of staghorn sculpin and starry flounder consisted of a circular, rotating (5 ± 0.5 rpm) partitioned plastic pan containing recirculated, aerated water plus 1:30,000 MS-222 anesthetic. This holder allowed five starry flounder or ten staghorn sculpin to be irradiated at one time. They were initially anesthetized with 1:20,000 MS-222.

The radiation field was 32 x 32 cm at 74 cm anode to water surface distance. A water depth of 3.0 cm approximated the dorsal to ventral thickness of the experimental animals.

D. Dosimetry

The source of radiation was a General Electric Maxitron 300 X-ray therapy unit located at the Radiation Center, Oregon State University. The machine was operated under the following conditions: 300 kVp, 20 mA, HVL selector at 2.0 mm Cu and beam monitor registering .35 x 25. The unique first and second half-value layers and unique homogeneity coefficient (Trout, Kelley and Lucas, 1960, 1962) were determined to be 1.79 mm Cu, 2.94 mm Cu and 0.609 respectively. The procedure for these determinations is given in Appendix I.

Using a model 570 Victoreen condenser R-meter with a 25 R chamber enclosed in a thin plastic sleeve, the exposure rate was determined at a distance of 1 cm from the bottom of the exposure

holder. This position approximates mid-fish distance under these experimental conditions. All irradiation procedures were followed during the dosimetric determinations including having experimental animals present in all but one compartment. Successive measurements were made in each of the compartments at two locations within each compartment. Individual readings were corrected for water temperature and atmospheric pressure. In the experimental exposure holder used for the starry flounder and staghorn sculpin irradiation, a 250 R chamber was used and readings taken in two different positions in one of the five separate compartments. Again, each of the other compartments had experimental animals in place and all other conditions were the same as those used throughout the experiment.

The exposure rates determined for the experimental animals were as follows:

chinook salmon	84.6 ± 5 R/min[6]
shiner perch	84.6 ± 5 R/min[6]
staghorn sculpin	75.0 R/min
starry flounder	75.0 R/min

Exposure rather than absorbed dose is reported for the following reasons:

1. the four degree temperature variation (8-12° C) in the

[6] Mean ± 1 standard deviation. Average taken over all compartments.

experimental holder,

2. the variation in field distribution in both of the experimental conditions and

3. the variation in thickness among the experimental animals.

Each of the above points places limits on the confidence of the absorbed dose determinations. As near as could be calculated, the following equivalencies between exposure and absorbed dose exist under the experimental conditions:

Exposure	Absorbed Dose
0 R	0 rads
1000 R	807.3 rads
2000 R	1614.6 rads
3000 R	2421.9 rads

These equivalencies were arrived at using the known average exposure at mid-fish depth and assuming the tissue to be water equivalent. Mass absorption coefficients for water and air are listed in National Bureau of Standards Handbook 85 (U. S. NBS, 1962) along with the following equation:

$$D_{medium} = 0.86 \left[\frac{(\mu\ en/\rho)_{medium}}{(\mu\ en/\rho)_{air}} \right] \qquad X = fX$$

where f is given in Table IA1 of Handbook 85 (equal to 0.948 at photon

energies of 100 keV)[7] and X is the exposure under experimental conditions. The absorbed dose from an exposure of 1000 R would then be:

$$D_{medium} = 0.86 \frac{rad}{R} \times (0.948) \times (1000\ R)$$

$$= 807.3 \text{ rads}$$

E. Analytical Techniques

Survivors from fresh- and salt-water experiments were sacrificed and plasma osmotic, sodium and chloride concentrations were measured.

The fish was first anesthetized in 1:20,000 MS-222 and the caudal fin was severed. Blood from the caudal artery was allowed to collect on a cold paraffin film and immediately transferred via polyethylene tubing into polyethylene micro-centrifuge tubes. The blood was centrifuged in a Beckman Microfuge[8] at 5500 x g for 20 seconds. The supernatant plasma was removed using polyethylene tubing and

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[7] The linear absorption coefficient is determined by dividing 0.693 by the first half-value layer and then dividing by 8.92 $\frac{gm}{cm^3}$, the density of copper, to obtain the mass absorption coefficient. The calculated value of 0.434 $\frac{cm^2}{gm}$ for the mass absorption coefficient gives an extrapolated value of 102 keV using the mass absorption coefficient versus photon energy curve on page 144 of the Radiological Health Handbook (U. S. P. H. S. 1960).

[8] Beckman Instruments, Palo Alto, California.

transferred to micro-centrifuge tubes for freezing until the time for analysis.

Gill filaments were dissected from the first gill arch and placed in 10 percent neutral buffered formalin immediately after the blood sample was taken. Those animals which showed abnormal osmotic or electrolyte concentrations were examined histopathologically.

The method of determining total osmotic concentration is given in Appendix II. Methods for plasma chloride and sodium concentration determinations are given in Appendices III and IV respectively.

III. RESULTS

A. Mortalities

Tables I and IV summarize percent survival of chinook salmon as a function of exposure when the irradiation takes place in either fresh water or salt water but the fish were returned to a fresh-water environment following irradiation. There were no moralities at the termination date in any of the exposure levels.

Tables II and III show percent survival of chinook salmon as a function of exposure level when irradiation takes place in either fresh or salt water and the fish were maintained in a salt-water environment. Some deaths are seen in the control group. However, increased mortalities occur at all exposures and the response appears to be dose dependent.

Starry flounder survival during residency in fresh- and salt-water environments following irradiation in salt water is given in Tables V and VI. Staghorn sculpin survival in fresh and salt water following irradiation in salt water is given in Tables VII and VIII.

Table IX shows the percent survival of shiner perch in salt water following irradiation in salt water

B. Physiological Changes

Total plasma osmolality and plasma sodium and chloride concentrations were measured in survivors. When the amount of sample

was limited, osmotic determinations were made in preference to sodium or chloride concentration measurements. This procedure was followed to insure at least one common basis of comparison among the various species used in the experiments.

Tables X through XVII summarize the results of plasma osmotic and electrolyte determinations.

C. Histopathological Changes

A section of the first gill arch was removed following blood sample collection and was placed in 10 percent neutral buffered formalin. The tissue was embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin in preparation for viewing with light microscopy.

Transverse and cross-sections of gill filaments from the control (0 R) and irradiated (3000 R) groups of each species were examined. The starry flounder, staghorn sculpin and shiner perch showed no detectable tissue damage even in those animals that did show increased plasma osmotic and electrolyte concentrations.

The irradiated chinook salmon maintained in salt water and showing increased osmotic properties also exhibited a hyperplasia in the inter-lamellar spaces (support cell area).

Table I. Survival of fresh-water adapted chinook salmon (Oncorhynchus tshawytscha) irradiated and maintained in fresh water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 15 Days Post-irradiation
0	20	100
1000	20	100
2000	20	100
3000	20	100

Table II. Survival of fresh-water adapted chinook salmon (O. tshawytscha) irradiated in fresh water and maintained in salt water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 13 Days Post-irradiation
0	20	33*
1000	20	40*
2000	20	15*
3000	20	15*

* All groups developed a bacterial infection (a species of the genus Pseudomonas). Therefore, the experiment was terminated at 13 days post-irradiation.

Table III. Survival of salt-water adapted chinook salmon (O. tshawytscha) irradiated in salt water and maintained in salt water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 20 Days Post-irradiation
0	22	91
1000	10	80
2000	15	33
3000	15	27

Table IV. Survival of salt-water adapted chinook salmon (O. tshawytscha) irradiated in salt water and maintained in fresh water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 20 Days Post-irradiation
0	20	100
1000	20	100
2000	20	100
3000	20	100

Table V. Survival of salt-water adapted starry flounder (Platichthys stellatus) irradiated in salt water and maintained in fresh water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 15 Days Post-irradiation
0	5	60
1000	5	80
2000	5	40
3000	5	100

Table VI. Survival of salt-water adapted starry flounder (P. stellatus) irradiated in salt water and maintained in salt water.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 15 Days Post-irradiation
0	10	100
1000	5	40
2000	5	80
3000	5	60

Table VII. Survival of salt-water adapted staghorn sculpin (Leptocottus armatus) irradiated in salt water and maintained in fresh water following irradiation.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 15 Days Post-irradiation
0	10	0
1000	10	0
2000	10	0
3000	10	0

Table VIII. Survival of salt-water adapted staghorn sculpin (L. armatus) irradiated in salt water and maintained in salt water following irradiation.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 15 Days Post-irradiation
0	20	100
1000	20	95
2000	20	90
3000	20	95

Table IX. Survival of salt-water adapted shiner perch (Cymatogaster aggregata) irradiated in salt water and maintained in salt water following irradiation.

Total Exposure (R)	Total No. of Animals Exposed	Percent Survival 20 Days Post-irradiation
0	13	92
2000	10	60
3000	10	90

Table X. Plasma osmotic and sodium ion concentration of chinook salmon (O. tshawytscha) in fresh water after irradiation in salt water.

Total Exposure (R)	No. of 20 Day Survivors Sampled	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l
0	16	290* ± 13 (269-311)	146 ± 3 (143-150)
1000	11	302 ± 7 (292-315)	146 ± 4 (139-152)
2000	10	303 ± 7 (296-319)	145 ± 5 (132-159)
3000	10	292 ± 4 (286-298)	143 ± 3 (137-148)

*Mean ± 1 standard deviation ( ) = minimum and maximum values for the entire group.

Table XI. Plasma osmotic and sodium ion concentration of chinook salmon in salt water after irradiation in salt water.

Total Exposure (R)	No. of 20 Day Survivors Sampled	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l
0	12	303* ± 6 (294-309)	145 ± 7 (132-156)
1000	7	308 ± 8 (298-321)	147 ± 10 (129-154)
2000	5	306 ± 8 (298-319)	148 ± 2 (146-150)
3000	3	319** ± 26 (300-356)	149 ± 1 (148-150)

*Mean ± 1 standard deviation ( ) = minimum and maximum values for the entire group.
** P > .01

Table XII. Plasma osmotic and sodium ion concentrations of chinook salmon in fresh water after irradiation in fresh water.

Total Exposure (R)	No. of 15 Day Survivors Sampled	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l
0	8	298* ± 7 (287-310)	147 ± 4 (141-150)
1000	10	302 ± 5 (287-316)	148 ± 4 (141-154)
2000	7	305 ± 7 (299-316)	148 ± 5 (141-154)
3000	6	303 ± 6 (294-313)	148 ± 6 (132-152)

Table XIII. Plasma osmotic and electrolyte concentration of chinook salmon in salt water after irradiation in fresh water.

Total Exposure (R)	No. of 13 Day Survivors Sampled	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l
0	7	305* ± 20 (288-349)	158 ± 4 (154-168)
1000	8	309 ± 13 (294-329)	162 ± 13 (154-172)
2000	3	299 ± 3 (294-302)	163 ± 3 (159-165)
3000	3	304 ± 9 (292-312)	159 ± 4 (155-166)

*Mean ± 1 standard deviation ( ) = minimum and maximum values for entire group.

Table XIV. Plasma osmotic and electrolyte concentration of starry flounder (P. stellatus) in salt water following irradiation in salt water.

Total Exposure (R)	No. of 15 Day Survivors Sampled	Hematocrit % Cell Volume	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l	Plasma Cl^- Concentration meq/l
0	5	19 ± 3* (13-21)	307 ± 7 (298-317)	154 ± 4 (146-159)	135 ± 14 (144-178)
1000	2	25 ± 4 (21-30)	301 ± 3 (298-304)	153 ± 6 (146-159)	152 ± 13 (139-164)
2000	4	25 ± 3 (20-30)	314 ± 6 (307-321)	155 ± 2 (152-157)	145 ± 5 (139-150)
3000	3	19 ± 1 (17-21)	356 ± 56 (305-434)	172 ± 22 (153-203)	153 ± 11 (137-165)

*Mean ± 1 standard deviation ( ) = minimun and maximum values in the entire group.

Table XV. Plasma osmotic and electrolyte concentration of starry flounder (P. stellatus) in fresh water following irradiation in salt water.

Total Exposure (R)	No. of 15 Day Survivors Sampled	Hematocrit % Cell Volume	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l	Plasma Cl^- Concentration meq/l
0	3	23 ± 2* (20-24)	261 ± 7 (253-269)	136 ± 6 (128-141)	120 ± 6 (112-126)
1000	3	27 ± 5 (19-31)	242 ± 31 (201-275)	125 ± 16 (104-141)	112 ± 16 (93 -133)
2000	2	27 ± 2 (25-28)	251 ± 4 (247-255)	130 ± 2 (128-133)	112 ± 10 (102-122)
3000	4	26 ± 2 (24-28)	235 ± 25 (194-256)	104 ± 23 (69-128)	100 ± 19 (69 -118)

*Mean ± 1 standard deviation ( ) = minimum and maximum values in the entire group.

Table XVI. Plasma osmotic and electrolyte concentration of shiner perch (C. aggregata) in salt water following irradiation in salt water.

Total Exposure (R)	No. of 20 Day Survivors Sampled	Plasma Osmotic Concentration mosmol/l
0	12	331* ± 7 (318-346)
1000	---	---
2000	3	327 ± 22 (297-350)
3000	6	338 ± 4 (332-344)

*Mean ± 1 standard deviation ( ) = minimum and maximum values for the entire group.

Table XVII. Plasma osmotic and electrolyte concentration of staghorn sculpin in salt water following irradiation in salt water.

Total Exposure (R)	No. of 15 Day Survivors Sampled	Hematocrit % Cell Volume	Plasma Osmotic Concentration mosmol/l	Plasma Na^+ Concentration meq/l	Plasma Cl^- Concentration meq/l
0	10	18±6 (11-30)	308*±11 (295-333)	154±8 (141-172)	138±8 (121-147)
1000	9	19±6 (11-30)	308± 3 (302-317)	157±1 (155-159)	138±7 (128-147)
2000	9	20±4 (9-28)	310±14 (285-327)	151±6 (146-161)	145±2 (139-147)
3000	9	19±3 (14-32)	316**±14 (297-334)	158±4 (152-163)	145±4 (139-150)

*Mean ± 1 standard deviation ( ) = minimum and maximum values for the entire group.
**P > .01

IV. DISCUSSION

Conte (1965) found that x-ray exposures of $\geq$ 1000 R to juvenile coho salmon, O. kisutsh, produced a hypertrophy and hyperplasia of gill filaments located primarily within the "secretory cell" area. However, the fish were in fresh water when irradiated and subsequently maintained in salt water. In the present experiments, this work has been extended to include several different genera and species.

In general, radiation caused hyperplasia of the support cell area when chinook salmon, O. tshawytscha, were irradiated in salt water and maintained in salt water. The hyperplasia noted was less extensive than that of previous studies (Conte, 1965) but the sacrifice period (15 days) in the present experiments differed from the earlier work (60 days). Conte and Lin (1967) have found the turnover time for epithelial cells of the gill filament (O. kitsutsh and O. tshawytscha) to be 5.8 $\pm$ 1.0 days. The degree of damage can be explained by the lesser time available for extensive proliferation of cells.

Irradiation of starry flounder, P. stellatus, in salt water and maintained in the same medium resulted in a pattern similar to the Salmonidae. That is, there occurred an increased mortality and a subsequent increase of salt content of the internal body fluids of survivors at the time of sacrifice. However, a noted difference between

the two genera was that little change in the histology of the gill filament of the flounder was found. When maintained in fresh water following irradiation in salt water, starry flounder tend to demineralize as evidenced from the decreases in plasma osmotic and electrolyte concentrations. In general, as the dose is increased the loss is more profound. Unfortunately, there is a substantial loss of salts by the control group which makes for a cautious interpretation. It has been shown that the regulation of salts is centered in the gill-head region (Conte and Lorz, 1965; Srivastava and Tachi, 1964). It has also been suggested (Copeland, 1948) that a reverse action for the "secretory cell" is in effect in fresh water, that is, it "pumps-in" salts. It is possible that the hyperosmoregulatory mechanisms are also radiation sensitive although previous experiments (Conte, 1965) and experiments on other species in this present work give contradictory evidence.

Staghorn sculpin, Leptocottus armatus, in salt water show few mortalities (<10%). In comparison to the salmon and starry flounder they are much more radioresistant. The internal body fluid concentrations are significantly different in the control and irradiated groups in salt water but no mortalities occurred. No histopathological changes were noted in the gill filaments.

Limited success with rearing and maintaining the shiner perch

C. aggregata, precludes much discussion as regards radiation effects on this species. However, it was seen that exposures of > 2000 R caused mortalities in sea water. Correlated with this observation was the finding that osmotic and electrolyte values increased. No histopathological changes were noted in the fill filaments.

In experiments where chinook salmon were irradiated in fresh water and then transferred to salt water, an increased susceptibility to bacterial infection was observed (Table II). The mortalities in the control group occurred at 12 days post-irradiation, whereas, mortalities were at 9 days post-irradiation for the 2000 R and 3000 R groups. Since both controls and x-ray exposure groups evidenced bacterial infection, it is thought that the infectious organism was transmitted by animal handling technique. The organism could not be cultured from the salt-water inlet. Radiation induced susceptibility to bacterial infection in goldfish has been reported (Schechmeister, et al., 1962; Watson, et al., 1963). This effect was not observed in animals exposed in salt water and maintained in salt water.

From observations made during the course of these experiments, I believe it is difficult to correlate statistically the body fluid concentrations of the survivors with the exposure level. Often a fish would appear normal and then die within the day. This suggests that the change in internal fluid concentration is sudden and not

gradual. Furthermore, it suggests that a depletion of normal cells or an inhibition of normal function occurs prohibiting the control of internal fluid concentration. Experiments designed to continually monitor body fluid osmotic concentrations would help elucidate the true nature of radiation effects on osmoregulation.

V. SUMMARY AND CONCLUSIONS

Four species of euryhaline teleosts were irradiated and maintained at different salinities.

It is shown that the chinook salmon suffers histopathological damage to the "secretory cell" area of the gill filament. This change is correlated with increased plasma osmotic and electrolyte properties when the fish is kept in salt water following irradiation. It does not occur for fresh-water adapted animals.

Starry flounder exhibit similar increased plasma osmotic and electrolytic concentrations when maintained in salt water following irradiation but gill tissue damage was not evident. When in fresh water following irradiation the starry flounder loses salts and this loss appears to be dose dependent.

Staghorn sculpin and, to a lesser degree, shiner perch are two species which evidenced resistance to radiation as shown by the lack of mortalities. The effect on the hypoosmoregulatory mechanisms is shown by an increase in the plasma osmotic concentrations in both species.

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APPENDICES

APPENDIX I

Determination of Unique First and Second Half-Value Layers and Unique Homogeneity Coefficient

Using a Victoreen model 575 Radocon integrating rate-meter in a fixed holder 1 meter from the surface of the floor, and at mid-field position, copper filters were added (25 cm from anode) and exposure rate recorded at various field diameters. Figure 1 shows the attenuation curves from which were determined the first and second half-value layers. Figure 2 shows the determination of unique first and second half value layers from which a unique homogeneity coefficient is determined. The homogeneity coefficient is an index of the uniformity of the x-ray beam being one for a monoenergetic beam. The unique homogeneity coefficient serves to better specify beam quality by removing field size as a variable thereby eliminating one geometrical factor.

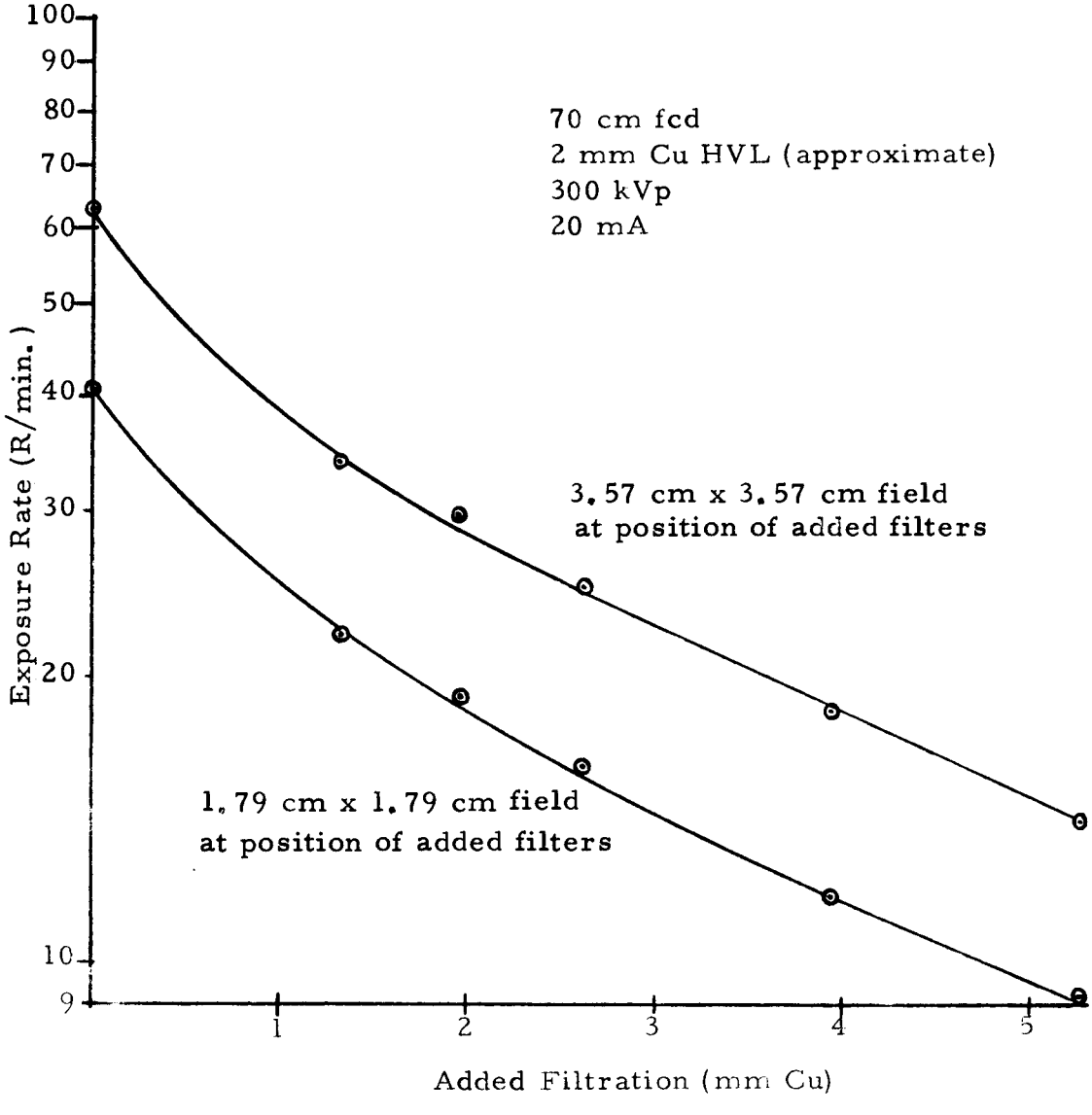

Figure 1. Half-value layer determination.

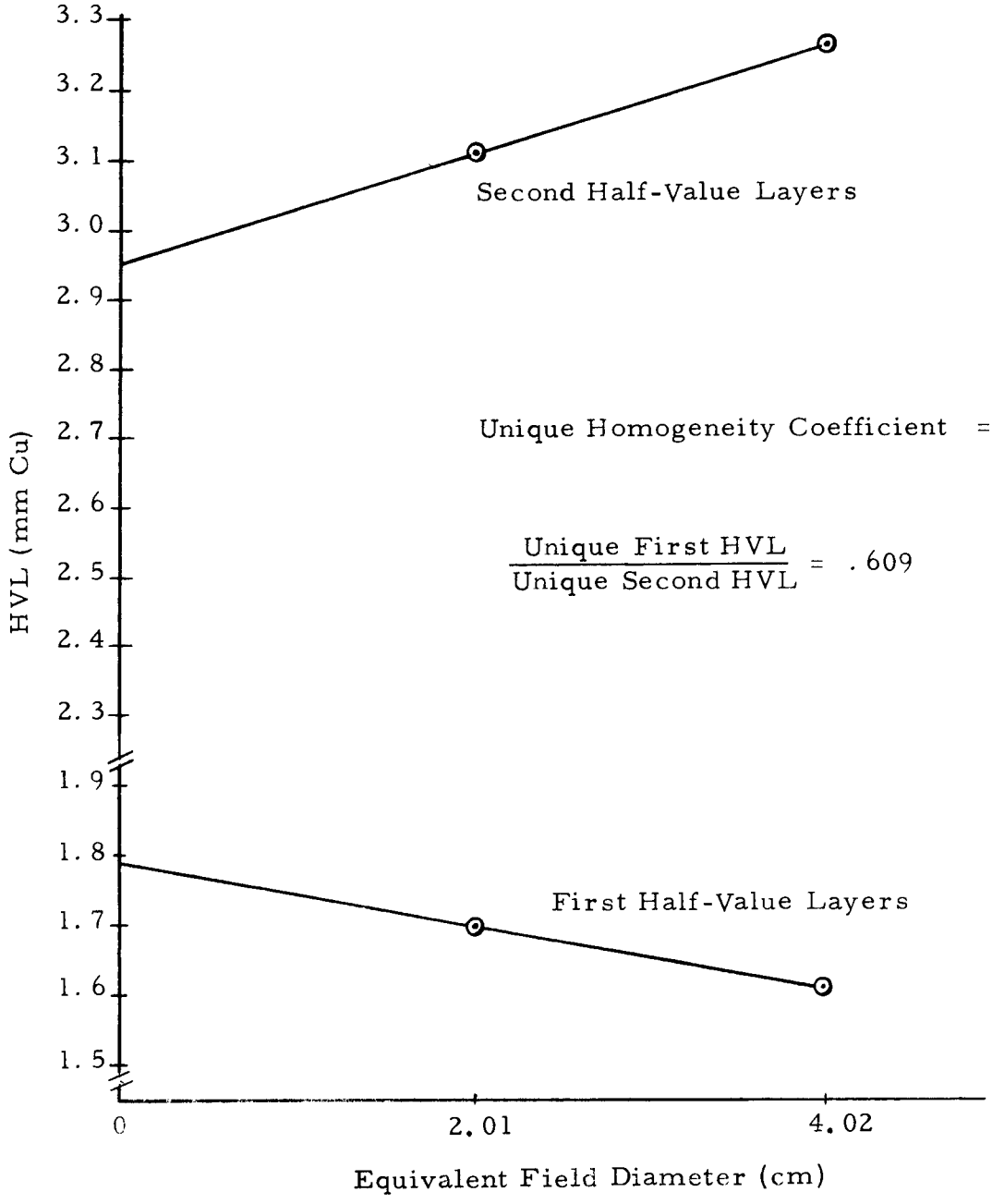

Figure 2. HVL (mm Cu) versus field diameter (cm).

APPENDIX II

Determination of Plasma Osmolality

For determining the total osmotic concentration of the clear plasma supernatant, a series of sodium chloride and mannitol standards were prepared and standard curves determined using a Mechrolab[8] vapor pressure osmometer. Figures 3 and 4 show the calibration in ΔR with change in concentration for NaCl and mannitol respectively.

Sample size for both standards and plasma was 30 µl. This allowed the thermistor bead to be washed with two drops of sample before an actual measurement was made.

In recording the osmolality the sodium chloride curve, corrected for ionic recombination, was used because some samples showed coagulation which removes the major portion of non-ionic species present.

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[8] Mechrolab, Inc. Mountain View, California.

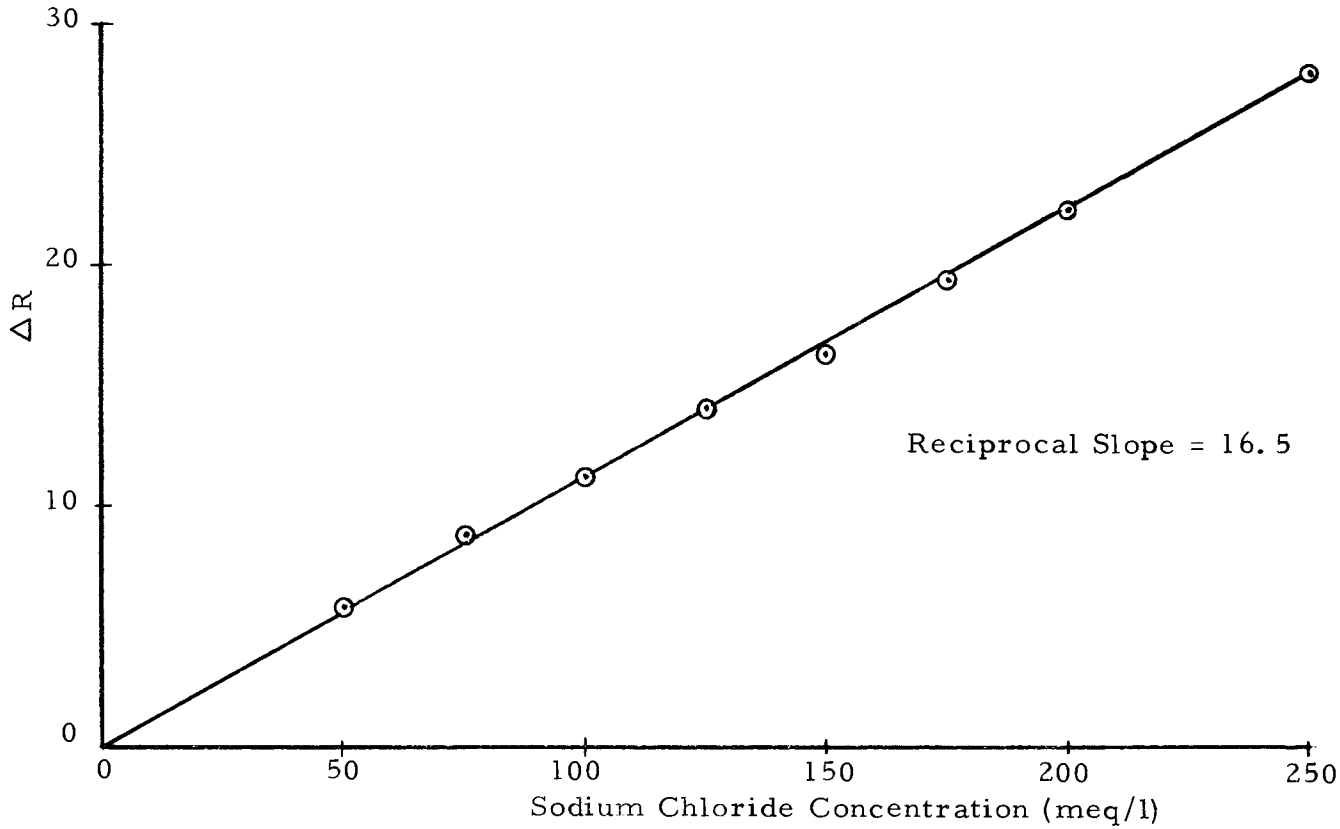

Figure 3. Calibration curve of ΔR vs NaCl concentration.

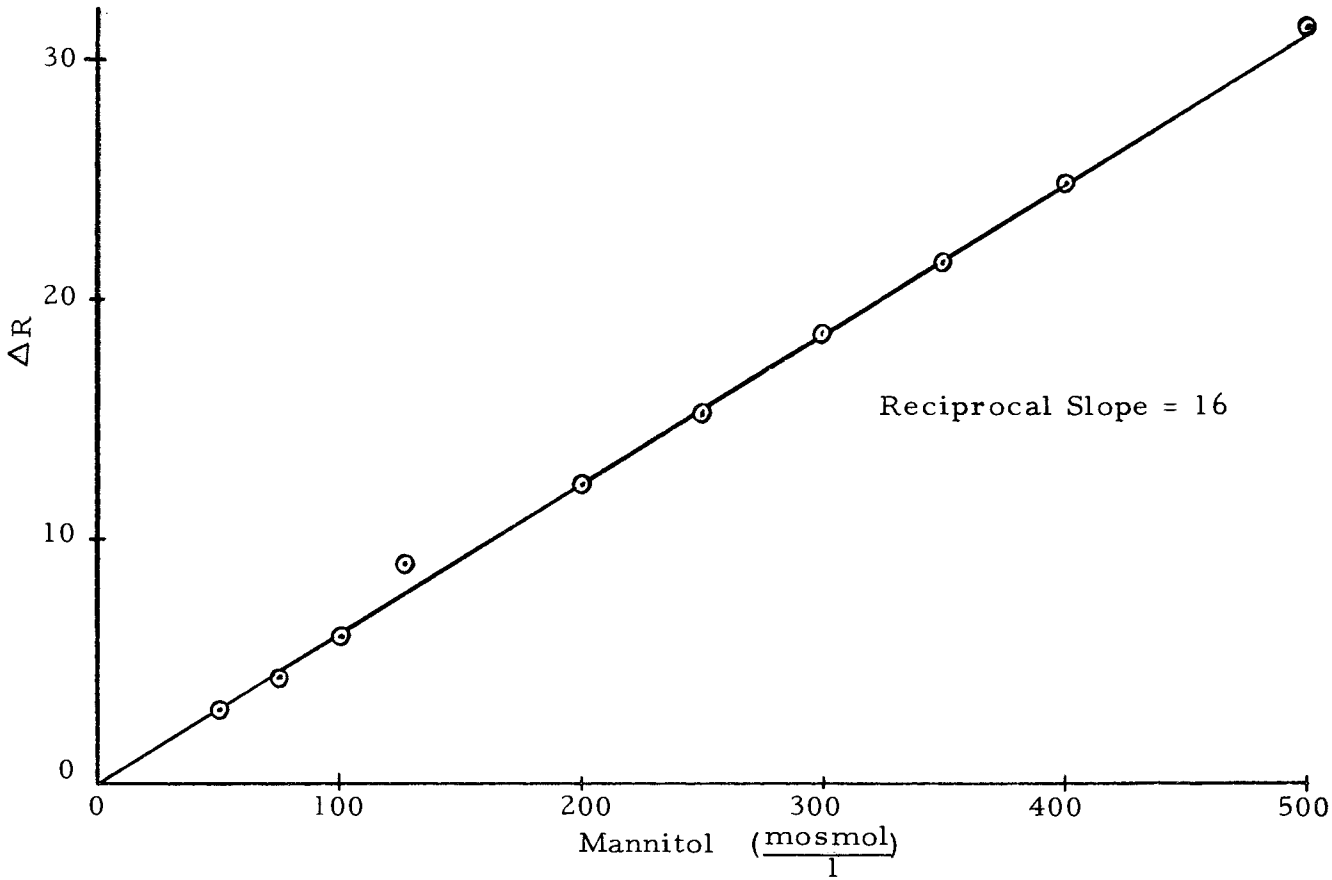

Figure 4. Calibration curve of ΔR versus mannitol concentration.

APPENDIX III

Determination of Plasma Chloride

Using the mercurimetric determination of chloride ion (Schales and Schales, 1941), 10 lambda of sample acidified by 0.03 N nitric acid and containing 100 lambda of s-diphenyl carbazone indicator solution is titrated[9] with 0.1 N mercuric nitrate. Chloride ions react with the mercuric ions to form a soluble but undissociated mercuric chloride. Excess mercuric ions at the end-point form a blue violet complex with the indicator. A 100 meq/l NaCl standard was used along with a blank (glass distilled water, nitric acid and indicator) to give the following equation for determination of chloride concentration:

$$\frac{\text{unknown - Blank}}{\text{Standard - Blank}} \times 100 = \text{meq. Chloride/l.}$$

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[9]Beckman microtitrator, Beckman Instruments, Palo Alto, California.

APPENDIX IV

Determination of Plasma Sodium

Figure 5 is a graph of percent transmission versus concentration of sodium in milliequivalents per liter. The curve was obtained with the Beckman Model B Flame spectrophotometer using standard sodium chloride solutions.

For sample measurements, 10 lambda of plasma was diluted to 5 ml, and percent transmission recorded. To obtain sodium concentration in milliequivalents per liter, the percent transmission is multiplied by the dilution factor (500) and reciprocal slope of the standard curve.

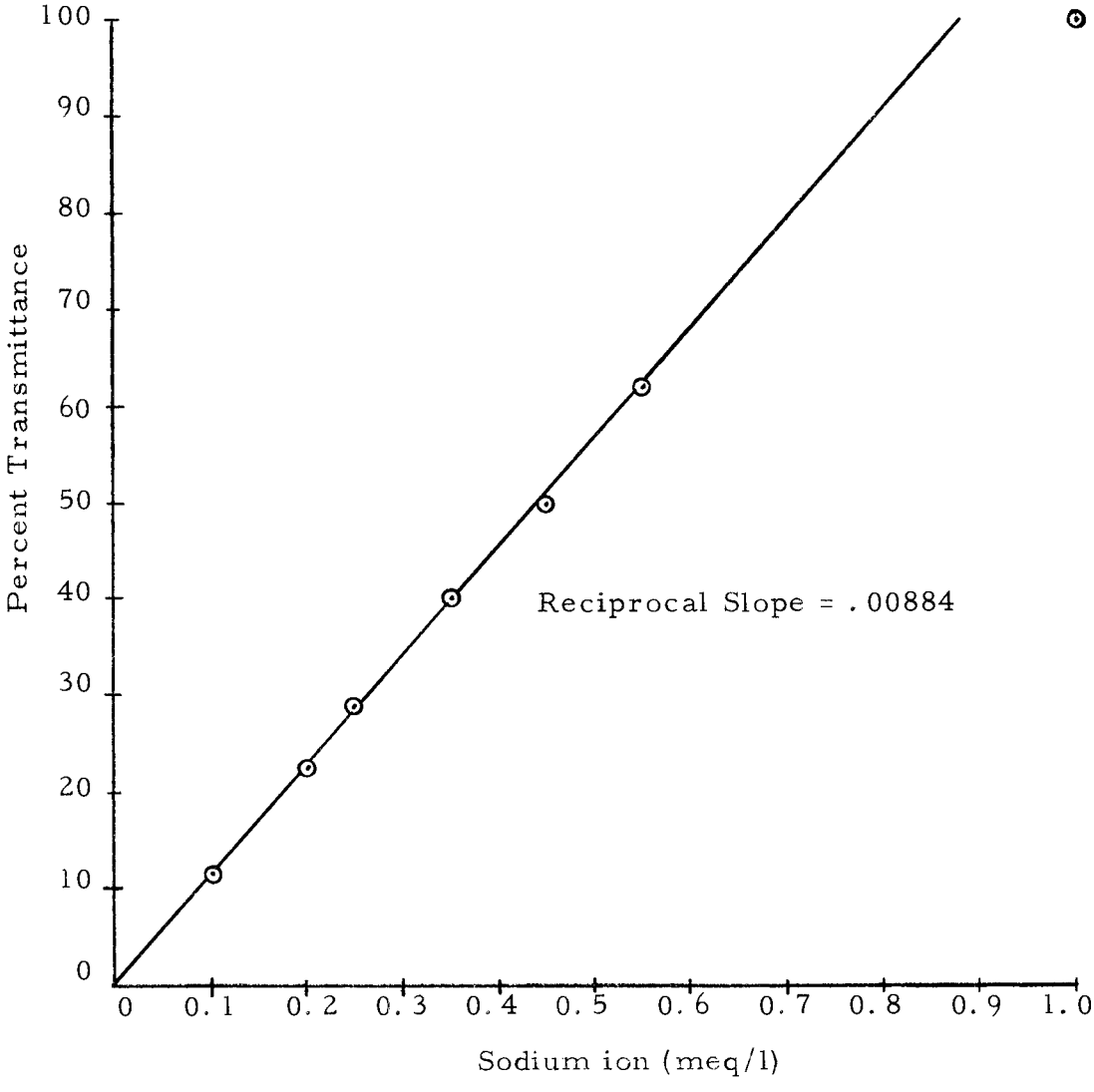

Figure 5. Calibration curve for percent transmission versus sodium ion concentration.