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STUDIES ON THE GLOMERULAR FILTRATION RATE OF RAINBOW TROUT (*SALMO GAIRDNERI*)*

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INTRODUCTION

The rainbow trout (*Salmo gairdneri*) and its anadromous form, the steelhead trout, maintain their *milieu intérieur* hypotonic to the environment after transfer to sea water (Houston, 1959, 1960). Such a transfer involves the physiological adjustment from a condition of continual hydration and electrolyte depletion to the opposite circumstance of a sustained tendency towards dehydration and excessive electrolyte intake.

Part of this physiological adjustment probably involves a reduction in urine flow of a magnitude comparable to the differences seen between stenohaline freshwater and marine forms. Further, it has been suggested that such a reduction in urine flow may be achieved by a lowering of the glomerular filtration rate (G.F.R.) (Smith, 1951; Fontaine, 1956).

The purpose of this study was to examine the glomerular filtration rates of freshwater-adapted and sea-water-adapted rainbow trout and also the effects of adrenocortical and posterior pituitary hormones on filtration in the freshwater form.

MATERIALS AND METHODS

These studies were conducted in two separate series of experiments during winter and summer months. During the winter, the freshwater fish were kept in running dechlorinated tap water at 6° C., and the sea-water-adapted fish in 80% standard sea water at the same temperature. The first group of sea-water-adapted trout was acclimated for 10 days in the sea water before the determination of G.F.R. A second group was maintained in sea water for 1 month and then returned to fresh water for 10 days before use. Fish used during the summer months were kept under similar conditions, but at 10° C. All fish were unfed for at least a week previous to G.F.R. estimations.

Fish were removed from their respective holding facility, injected intraperitoneally with 50 mg. inulin in 0·5 ml. isotonic saline (NaCl, 0·78%) and placed individually in plastic tanks containing exactly 1 l. of aerated fresh water or sea water at the same temperature. Five hours after the injection of inulin, a 20 ml. sample of the tank water was taken. This was considered to be the zero-hour sample and similar 20 ml. aliquots were taken at 5, 10 and 15 hr. thereafter.

Zero-hour and terminal blood samples were also taken from separate inulin-injected groups of fish. The blood was collected in heparinized tubes and immediately centrifuged. Protein was precipitated from the plasma samples with cadmium sulphate

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(Fujita & Iwatake, 1931, as modified by Smith, Finkelstein, Aliminosa, Crawford & Graber, 1945) and the supernatant was filtered through washed cotton.

Duplicate 2 ml. samples of the plasma filtrate and similarly filtered samples of the tank water were analysed for inulin according to the Schreiner (1950) modification of the direct resorcinol method of Roe, Epstein & Goldstein (1949). Standard solutions of inulin were included with each determination.

The total inulin excreted, after correction for the quantities removed in previous samples, was calculated for each period and expressed in mg./kg. body weight of fish. From these values, and the plasma inulin concentration, the total volume of plasma filtered up to the end of each period was calculated as follows:

$$\text{Total plasma filtered (ml.) at any given time} = uv/p,$$

where u = urine inulin concentration (mg./ml.), v = volume of urine (ml.), and p = plasma inulin concentration (mg./ml.). Although the volume of urine excreted by the trout was not known, the product (uv) was represented by the total weight of inulin excreted at any time.

The accumulated volume (ml.) of plasma filtered was plotted against the time in hours. Each regression was fitted by the method of least squares, and the various regressions were compared by the analysis of covariance (Snedecor, 1956). From the slopes of the lines the glomerular filtration rates were calculated in ml./kg. body weight/day.

Sodium and potassium concentrations in plasma and in muscle were measured in freshwater-adapted and sea-water-adapted fish. All measurements were made with a Zeiss PF 5 flame photometer. Concentrations were expressed as milliequivalents per litre of plasma and as milliequivalents per kilogram wet weight of muscle. Muscle samples were dried to constant weight at 110° C. and the percentage water content was determined.

All hormones were injected intraperitoneally at the time of the inulin injection. Commercial preparations of oxytocin and vasopressin ('Pitocin' and 'Pitressin', Parke-Davis) were administered in aqueous solution, corticosterone (Sigma) was prepared as a stabilized suspension in isotonic saline, and aldosterone (D-aldosterone-free alcohol, Ciba) was given in oil solution.

RESULTS

Several investigators have demonstrated the presence of an extra-renal, as well as a renal, excretory pathway in the teleost fishes (Smith, 1930; Keys, 1931; Krogh, 1939; Holmes, 1959). Therefore, when the G.F.R. is measured by analysis of the environment, rather than of the urine *per se*, the possibility of extra-renal secretion or filtration of inulin must be examined. This source of error was evaluated in preliminary studies by the estimation of the inulin output from freshwater trout in which the cloaca was closed by a 'purse-string' ligature immediately after the injection of inulin. Although the rate of appearance of inulin in the tank water was significant, it was extremely low (mean = 0·42 mg./kg./hr.) and highly variable. It is possible, indeed probable, that this low rate of appearance of inulin was due to leakage from the tissue damaged at ligation. Therefore, we considered the extra-renal excretion of inulin to be negligible. Another possible source of error in this type of estimation may result from the

Table 1. *The effect of transfer to* 80% *sea water on the glomerular filtration rates of rainbow trout* (Salmo gairdneri)

(All fish were kept in running dechlorinated tap water or 80% standard sea water at 6° C.)

	No. of fish	Body weight (g.)	Regression*	S_{yx}†	S_b‡	'r'§	'P' value on 'r'	Covariance of filtration rates: Degrees of freedom	Covariance of filtration rates: 'F' value	Covariance of filtration rates: 'P' value on 'F'
Fresh water	14	186·0 ± 5·9	$Y = 6{\cdot}53X + 1{\cdot}81$	15·68	0·37	0·92	< 0·001	—	—	—
Sea water (from fresh water)	10	163·5 ± 8·4	$Y = 0{\cdot}42X + 0{\cdot}68$	3·96	0·11	0·52	< 0·001	1,92	180	< 0·001
Fresh water (from sea water)	9	152·3 ± 4·6	$Y = 7{\cdot}68X - 1{\cdot}95$	16·13	0·48	0·94	< 0·001	1,88	3·61	> 0·05 < 0·1

* $Y = bX + a$, where Y = cumulative volume of glomerular filtrate in ml./kg. body weight, b = G.F.R. in ml./kg. body weight/hr., X = time in hours and a = the ordinate intercept.

† S_{yx} = standard deviation from the regression.

‡ S_b = standard error of the regression coefficient (b).

§ r = correlation coefficient.

Table 2. *The sodium, potassium and water composition of plasma and muscle of freshwater-adapted and sea-water-adapted rainbow trout* (Salmo gairdneri)

(All fish were maintained in dechlorinated tap water or 80% standard sea water at 6° C.)

	No. of fish	Plasma: Na (m-equiv./l.)	Plasma: K (m-equiv./l.)	Muscle: % water	Muscle: Na (m-equiv./kg. wet muscle)	Muscle: K (m-equiv./kg. wet muscle)
Fresh water	16	145·0 ± 1·6	2·33 ± 0·20	78·46 ± 0·32	7·13 ± 0·26	108·4 ± 0·8
Sea water (10 days)	10	160·0 ± 2·7*	2·42 ± 0·20	78·26 ± 0·22	11·70 ± 0·70*	112·3 ± 1·9

* $P < 0{\cdot}001$ with respect to the corresponding freshwater value.

Table 3. *The effect of neurohypophysial and adrenocortical hormones on the glomerular filtration rate of freshwater rainbow trout* (Salmo gairdneri)

(All fish were kept at 10° C. and were intraperitoneally injected with the indicated doses of hormones.)

Group	No. of fish	Body weight (g.)	Regression* ($Y = bX+a$)	S_{yx}†	S_b‡	'r'§	'P' value on 'r'	Covariance of filtration rates: Degrees of freedom	'F' value	'P' value on 'F'
Control	9	173·8 ± 10·9	$Y = 7{\cdot}04X+2{\cdot}9$	17·36	0·49	0·93	< 0·01	—	—	—
100 mu. vasopressin	10	152·0 ± 11·9	$Y = 10{\cdot}96X+3{\cdot}9$	29·07	0·82	0·91	< 0·01	1,72	16·41	< 0·01
100 mu. oxytocin	9	144·7 ± 13·1	$Y = 11{\cdot}85X+1{\cdot}7$	37·62	1·12	0·88	< 0·01	1,68	16·03	< 0·01
50 mu. vasopressin + 50 mu. oxytocin	7	159·7 ± 14·8	$Y = 10{\cdot}29X-1{\cdot}1$	29·63	0·98	0·89	< 0·01	1,60	9·65	< 0·01
25 μg. aldosterone	9	160·2 ± 13·7	$Y = 7{\cdot}74X+1{\cdot}9$	14·74	0·44	0·95	< 0·01	1,68	1·08	> 0·2
5·0 mg. corticosterone	9	143·4 ± 7·6	$Y = 5{\cdot}25X-0{\cdot}4$	20·13	0·61	0·83	< 0·01	1,68	5·37	< 0·025

* $Y = bX+a$ where Y = cumulative volume of glomerular filtrate in ml./kg. body weight, b = G.F.R. in ml./kg. body weight/hr., X = time in hours and a = the ordinate intercept.

† S_{yx} = standard deviation from the regression.

‡ S_b = standard error of the regression coefficient.

§ 'r' = correlation coefficient.

production by the fish of some 'inulinoid' chromogenic material. This was investigated by means of saline-injected controls. The average rate of appearance of non-inulin chromogen from saline-injected fish was equivalent to about 0·03 mg. inulin/kg./hr. (equivalent to less than 0·95 ml. filtrate/kg./day) and was not significant ($P > 0·5$). We consider, therefore, that the inulin clearances reported here represent valid estimations of the G.F.R.

In all groups of trout studied the cumulative total volume of glomerular filtrate varied directly with time according to the equation $Y = bX + a$, where $Y =$ the volume of glomerular filtrate in ml./kg. body weight, $X =$ the time in hours and $a =$ the ordinate intercept. It follows, then, that b, the slope of the line described by the equation, will represent the rate of clearance of inulin, in ml. plasma cleared per hour.

Freshwater rainbow trout maintained at 6° C. showed a highly significant rate of inulin excretion which represented a G.F.R. of 156·7 ± 8·9 ml./kg./day (Table 1). Adaptation of the fish to 80% sea water at 6° C. for 10 days resulted in a reduction in G.F.R. to 10·1 ± 2·6 ml./kg./day (Table 1). This represented a very significant decline to only 6·8% of the freshwater value. Further, during the period of acclimation to sea water, the water and electrolyte composition of the tissues had returned to values approximating those found in the freshwater fish (Table 2).

Trout which were maintained in 80% sea water at 6° C. for 1 month and then transferred back to fresh water at the same temperature showed a restoration of the previously observed filtration rate (184·3 ± 11·5 *vs.* 156·7 ± 8·9 ml./kg./day). Although the mean rate was higher than the freshwater controls the level of significance was borderline (Table 1).

Rainbow trout maintained at 10° C. had a slightly higher mean G.F.R. (169·0 ± 11·8 ml./kg./day), than those maintained at 6° C., but the two values were not significantly different (Table 3). Treatment of freshwater fish with a single intraperitoneal dose of 100 mu. vasopressin increased the G.F.R. to 263·0 ± 19·7 ml./kg./day. Similar treatment with 100 mu. oxytocin enhanced the G.F.R. to an even greater degree (284·4 ± 26·9 ml./kg./day). Both these rates were significantly higher than the intact freshwater control value (Table 3). The combination of 50 mu. vasopressin and 50 mu. oxytocin also increased the G.F.R. (247·0 ± 23·5 ml./kg./day). This value was not significantly different from the values obtained when the hormones were administered separately (Table 3).

There was no detectable effect on the G.F.R. of freshwater trout following a single intraperitoneal dose of 25 μg. aldosterone. However, treatment with 5·0 mg. corticosterone significantly reduced the G.F.R. to 126·0 ± 14·6 ml./kg./day (Table 3).

DISCUSSION

Dr R. M. Holmes (1961) has recently been able to collect urine from catheterized rainbow trout for periods up to 500 hr. Under these conditions, freshwater rainbow trout, in the same weight range as in the present study, showed urine flows of 75–90 ml./kg./day and urine chloride concentrations of 5–12 mM./l. This would suggest, on the basis of our estimated G.F.R., and at a plasma chloride concentration of 137·2 mM./l. (Houston, 1959), that 43–52% tubular reabsorption of water and 95–98% tubular reabsorption of chloride occurred in the rainbow trout in fresh water. In the same study, R. M. Holmes reported that, after adaptation of the rainbow trout to sea water, the urine

production had declined to 0·5–1·0 ml./kg./day and the urine chloride concentration had increased to 200–220 mM./l. The plasma chloride concentration of sea-water-adapted rainbow trout was found by Houston (1959) to be 140 mM./l. Using the G.F.R. value of 10·1 ± 2·6 ml./kg./day, it would seem that 90–95 % reabsorption of water and 84–93 % reabsorption of chloride occurred in the rainbow trout adapted to sea water. Apparently, therefore, part of the homoiostatic mechanism associated with the adaptation of rainbow trout to sea water consists of a reduction in the G.F.R. together with an increased renal tubular reabsorption of water. These factors could well account for the drastic antidiuresis observed at this time.

The rapid re-establishment of the high G.F.R. upon return of the rainbow trout from sea water to fresh water suggests that the low G.F.R. observed in the sea-water-adapted fish was due to physiological rather than morphological or developmental changes (cf. Nash, 1931; Ford, 1956).

Shortly before, during and after the spawning season rainbow trout do not show the marked reduction in urine flow normally associated with adaptation to sea water. The antidiuresis can be induced, however, by intramuscular perfusion of these fish with posterior pituitary extract equivalent to 0·04 i.u. vasopressin/oxytocin per kilogram body weight every 6–8 hours (R. M. Holmes, 1961). The present study does not indicate that a mammalian neurohypophysial hormone is capable of causing this reduction in non-spawning trout; however, neither does it establish the converse. Almost all studies of the effects of mammalian posterior pituitary hormones on the water diuresis of lower vertebrates indicate a diuretic effect at low doses and an antidiuretic effect at high doses (Richards & Schmidt, 1924; Burgess, Harvey & Marshall, 1933; Adolph, 1936; Uranga, 1960; Holmes & Adams, 1963). In the present experiments, however, the doses were several times greater than those used by R. M. Holmes, yet their effects suggest diuresis. Furthermore, vasopressin has not been found to occur in fish. The antidiuretic material extractable from teleost pituitaries is instead arginine vasotocin (Heller & Pickering, 1961). During the period immediately after transfer of the rainbow trout to sea water there is certainly a depletion of pituitary antidiuretic material, probably arginine vasotocin (Carlson & Holmes, 1962). Since corticosterone was the only hormone to cause a reduction in the G.F.R. of freshwater rainbow trout under the conditions of the present study, it is possible that the antidiuretic response reported by R. M. Holmes was due to the stimulation of ACTH release by some neurohypophysial hormone. This would be consistent with the observed increase in adrenocortical activity during the smoltification of salmonid fishes (Fontaine & Hatey, 1954; Olivereau, 1960), a period of halophilia and increased ability to withstand transfer to sea water (Baggerman, 1960; Houston, 1960).

SUMMARY

1. Variations in the glomerular filtration rate (G.F.R.) and the renal tubular reabsorption of water are probably important factors in the homoiostatic mechanisms associated with the euryhalinity of the rainbow trout (*Salmo gairdneri*).

2. The observed reduction in urine flow after the adaptation of rainbow trout to sea water can be largely accounted for on the basis of the reduced G.F.R.

3. Intraperitoneal injections of mammalian preparations of vasopressin, oxytocin and vasopressin/oxytocin into freshwater trout significantly increased the G.F.R.

4. The intraperitoneal injection of aldosterone into freshwater fish had no detectable effect on G.F.R.

5. The similar administration of corticosterone significantly reduced the G.F.R. to 73% of the intact freshwater control value.

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REFERENCES

ADOLPH, E. F. (1936). Control of urine formation in the frog by the renal circulation. *Amer. J. Physiol.* **117**, 366–79.

CARLSON, I. H. & HOLMES, W. N. (1962). Changes in the hormone content of the hypothalamo-hypophysial system of the rainbow trout (*Salmo gairdneri*). *J. Endocrin.* **24**, 23–32.

BAGGERMAN, B. (1960). Factors in the diadromous migrations of fish. *Symp. Zool. Soc. Lond.* no. 1, 33–60.

BURGESS, W. W., HARVEY, A. M. & MARSHALL, E. K. (1933). The site of the antidiuretic action of pituitary extract. *J. Pharm. Exper. Therap.* **49**, 237–49.

FONTAINE, M. (1956). The hormonal control of water and electrolyte metabolism in fish. *Mem. Soc. Endocrin.* no. 5, 69–82.

FONTAINE, M. & HATEY, J. (1954). Sur la teneur en 17-hydroxycorticostéroïdes du plasma de saumon (*Salmo salar*, L.). *C.R. Acad. Sci., Paris*, **239**, 319–21.

FORD, P. (1956). Studies on the development of the kidney of the Pacific pink salmon (*Oncorhynchus gorbuscha* Walbaum). *Canad. J. Zool.* **36**, 45–7.

FUJITA, A. & IWATAKE, D. (1931). Bestimmung des echten Blutzuckers ohne Hefe. *Biochem. Z.* **242**, 43–60.

HELLER, H. & PICKERING, B. T. (1961). Neurohypophysial hormones of non-mammalian vertebrates. *J. Physiol.* **155**, 98–114.

HOLMES, R. M. (1961). Kidney function in migrating salmonids. *Rep. Challenger Soc., Camb.*, **3**, no. 13, 23.

HOLMES, W. N. (1959). Studies on the hormonal control of sodium metabolism in the rainbow trout (*Salmo gairdneri*). *Acta endocr., Copenhagen*, **31**, 587–602.

HOLMES, W. N. & ADAMS, B. M. (1963). The effects of adrenocortical and neurohypophysial hormones on the renal excretory pattern of the water loaded duck (*Anas platyrhynchos*). *Endocrinology* (in the Press).

HOUSTON, A. H. (1959). Osmoregulatory adaptation of the steelhead trout (*Salmo gairdneri* Richardson) to sea water. *Canad. J. Zool.* **37**, 729–48.

HOUSTON, A. H. (1960). Variations in the plasma level of chloride in hatchery-reared yearling Atlantic salmon during parr–smolt transformation and following transfer to sea water. *Nature, Lond.*, **185**, 632–3.

KEYS, A. B. (1931). Chloride and water secretion and absorption by the gills of the eel. *Z. vergl. Physiol.* **15**, 364–88.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals.* Cambridge University Press.

NASH, J. (1931). The number and size of glomeruli in the kidneys of fishes with observations on the morphology of the renal tubules of fishes. *Amer. J. Anat.* **47**, 425–46.

OLIVEREAU, M. (1960). Étude volumétrique de l'interrénal antérieur au cours de la smoltification de *Salmo salar* L. *Acta endocr., Copenhagen*, **33**, 142–56.

RICHARDS, A. N. & SCHMIDT, C. F. (1924). A description of the glomerular circulation in the frog's kidney and observations concerning the action of adrenalin and various other substances upon it. *Amer. J. Physiol.* **71**, 178–208.

ROE, J. H., EPSTEIN, J. H. & GOLDSTEIN, H. P. (1949). A photometric method for the determination of inulin in plasma and urine. *J. Biol. Chem.* **178**, 839–45.

SCHREINER, G. E. (1950). Determination of inulin by means of resorcinol. *Proc. Soc. Exp. Biol., N.Y.*, **74**, 117–20.

SMITH, H. W. (1930). The absorption and excretion of water and salts by marine teleosts. *Amer. J. Physiol.* **93**, 480–505.

SMITH, H. W. (1951). *The Kidney, Structure and Function in Health and Disease.* New York: Oxford University Press.

SMITH, H. W., FINKELSTEIN, N., ALIMINOSA, L., CRAWFORD, B. & GRABER, M. (1945). The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* **24**, 388–404.

SNEDECOR, G. W. (1956). *Statistical Methods.* Ames, Iowa, U.S.A.: Iowa State College Press.

URANGA, J. (1960). Diuretic response in the bullfrog (*Rana catesbiana*) to the injection of oxytocin. *Acta endocr., Copenhagen*, suppl. 51, **35**, 139.