THE OSMOTIC PRESSURE OF THE COLLOIDS IN FISH SERA

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(*Received 3rd May*, 1933.)

(With One Text-figure.)

INTRODUCTION.

In the organism the effective osmotic pressure may, and often does, differ greatly from the total osmotic pressure. In the vertebrates, which alone have been sufficiently studied, the major internal fluid exchanges take place across membranes which are very far from being perfectly semi-permeable; the membranes of the serous cavities, the capillary walls, the meninges and the glomerular membrane are more or less completely permeable to crystalloids. The principal, if not the only determinants in the fluid exchange across these membranes are the hydrostatic pressure and the osmotic pressure contributed by those solutes which cannot penetrate these membranes—*i.e.* the colloid osmotic pressure.

Starling's (1896) classical development of the concept of a balance between hydrostatic and colloid osmotic pressure in the vascular system, with the corollary of hydrostatic filtration and colloid osmotic reabsorption respectively at the arterial and venous ends of the capillaries, and the application of similar ideas to glomerular function (Starling, 1899), give the colloid osmotic pressure perhaps greater physiological significance than the total osmotic pressure. Unfortunately, however, studies of colloid osmotic pressure have been almost entirely confined to mammals and a very few birds and Amphibia. The present paper is the result of investigations with fishes, in which problems in fluid balance are raised in acute form because of the aquatic habitat.

METHODS.

With the exception of the cod, all fishes used were in large aerated tanks for several days before blood samples were taken; cod blood was obtained from specimens in live cars within a few hours of capture. The "starved" eels had been in large tanks of running sea water or tap water for about 2 months without food; both fresh and sea water tank temperatures varied between 12 and 16° C.

Blood was drawn by a hypodermic syringe from the heart in most of the eels, in one tench and in the single pike. In the remainder of the eels the blood was

collected from the dorsal aorta after severing the tail. In the plaice, the cod and the remainder of the tench, the blood was drawn from the efferent branchial vessels. In all cases the blood was delivered at once into sterile ice-cold centrifuge tubes and stored at 0° C. until centrifuged, no anti-coagulant being used. The blood was centrifuged at 3500 r.p.m. for 15 min. not later than 1 hour after drawing; the upper two-thirds of the serum were pipetted off and used for the determinations which were begun at once in most cases. So far as possible sterility was maintained throughout.

Colloid osmotic pressure was determined by the second method of Krogh and Nakazawa (1927), following the technique of Turner (1932). Some slight modifications in the apparatus permitted the use of as little as 0·2 c.c. of serum for a single estimation; in all cases, however, determinations were run in duplicate or triplicate. Refractive index was determined at 17·5° C. with a Zeiss Pulfrich refractometer which was calibrated with distilled water and standard salt solutions at the same temperature; in several samples refractive index was measured with a Zeiss large model Loewe interferometer using the 1 c.c. chamber. All determinations of refractive index were done in duplicate or triplicate. Freezing-points were done in a few cases with a cryoscope using 2 c.c. of blood; the Beckmann thermometer was read to 0·001° C. and super-cooling was limited to 0·3° C. by seeding with ice crystals. Chloride determinations were done by means of Rehberg's (1926) micro method.

Table I.

Species	Habitat	No.	Serum colloid osmotic pressure mm. H_2O	Serum refractive index, at 17·5° C.	Serum chloride mg./ 100 c.c.	Blood freezing-point depression °C.
Pleuronectes platessa	S.W.	1	128	1·34237	577	0·732
			124	1·34233	572	—
		2	104	1·340558*	—	—
			110	1·340560*	—	—
			—	1·34052	—	—
		3	114	1·34162	558	—
			119	1·34156	561	—
			—	1·34150	—	—
Tinca vulgaris	F.W.	1	94	1·34082	378	0·524
			107	1·34090	382	0·522
		2	103	1·34090	375	—
			114	1·34094	372	—
		3	96	1·34167**	—	—
			89	1·34170**	—	—
Gadus morrhua	S.W.	1	109	1·34182	—	—
			115	1·34186	—	—
		2	112	1·34249	—	—
			116	1·34244	—	—
Esox lucius	F.W.	1	91	1·34102	—	0·514
			87	1·34100	—	—

* Interferometric determination.
** Slight haemolysis in these.
S.W. sea water; F.W. fresh water.

RESULTS.

The data for the fishes other than the eels are summarised in Table I, and those for the eels are given in Table II. The tables require little explanation. In each case all the determinations were carried out on the same sample and, so far as possible, on the same day. In several instances, however, it was necessary to store serum samples overnight (at 0° C.); controlled experiments showed that sterile serum at 0° C. remains unchanged, at least in refractive index, for as long as 5 days. For example, tench serum stored in this way and tested daily for 5 days always checked within the limit of error of the refractometer, the value at the end of the fifth day being 1·34172 as compared with 1·34169 one hour after withdrawal from the fish.

The values given in Table I fall within a rather closely defined range and, taken alone, would indicate that the teleosts in general are characterised by a serum colloid pressure of about 100 mm. water pressure. The values for the eel in Table II, however, are far out of the range.

Table II. *Anguilla vulgaris.*

Habitat	Length of starvation	Serum colloid osmotic pressure in mm. H_2O	Serum refractive index, at 17·5° C.
Fresh water	7 days	258	1·34737
		253	1·34739
		255	1·34730
Sea water	7 days	278	1·34926
		283	1·34929
		266	1·34899
		269	1·34902
Fresh water	About 60 days	110	1·34221
		114	1·34224
Sea water	About 60 days	134	1·34350
		141	1·34358
		145	1·34442
		140	1·34450

DISCUSSION.

The determinations recorded here allow the construction of a table which gives at least a rough picture of the colloid osmotic pressures characteristic of most of the major classes of the vertebrates. Such a tabulation is attempted in Table III in which only rough averages are given. The column "range of normal extremes" omits the figures reported by a few authors which are in obvious disagreement with all other work and which are open to criticism of technique. The authorities for the values for mammalian bloods are too numerous (over one hundred!) for inclusion here; most of them may be found in P. Meyer's (1932) review.

From the standpoint of comparative physiology, the table is still very incomplete and the absence of values for the marsupials, urodeles and the whole class of reptiles

is particularly regrettable. As it stands, however, the blood colloid osmotic pressures of the various mammals would seem to be very much alike, while, with a single exception, the values for birds, amphibians and fish lie on a much lower plane, less than half that of the general level for mammals. The exception is the normal eel which exhibits a serum colloid osmotic pressure which falls within the mammalian limits. It may be, of course, that other exceptions will be found when more species are studied, but the divergence of the eel is so striking as to command attention.

Table III. *Average colloid osmotic pressures of sera of normal vertebrates.*

Class	Species	Mean colloid osmotic pressure mm.H_2O	Range of normal extremes reported	Authority
Mammalia	Man	330	280–480	—
	Pig	330	300–350	—
	Dog	310	230–470	—
	Goat	300	300–310	—
	Cat	300	240–330	—
	Sheep	300	290–340	—
	Rabbit	290	230–350	—
	Horse	280	230–350	—
	Ox	280	260–300	—
	Rat	260	220–290	—
	Guinea pig	250	230–280	—
Aves	Hen	150	140–160	Kylin & v. Pein, 1931
	Dove	110	80–120	Nakazawa, Seki & Inawashiro, 1930; Kylin & v. Pein, 1931
Amphibia	Frog (*Rana catesbiana*)	103	96–115	White, 1924
	Frog (*Rana temporaria*)	70	? –140	Krogh & Nakazawa, 1927
Pisces	Eel, S.W.	280	?	Present determinations
	Eel, F.W.	255	260–280	,,
	Plaice, S.W.	115	107–126	,,
	Cod, S.W.	113	112–114	,,
	Tench, F.W.	101	93–109	,,
	Pike, F.W.	89	?	,,

S.W. sea water; F.W. fresh water.

The present measurements confirm fully the conclusion drawn from refractometric studies recently (Keys, 1933) that the normal eel is characterised by a colloid osmotic pressure of the order of 250 mm. H_2O. Prévost and Dumas (1821) found the total (dry) solid content of eel blood to be 15·4 per cent., from which Schulz and von Krüger (1925, p. 1129) calculated the solid of the serum to be about equal to that of mammalian serum.

It might be supposed that the high colloid osmotic pressure and high refractive index of eel serum are due to colloid substances other than proteins, particularly in view of the fact that few, if any, animals contain so high a fat content as the eel (see, *e.g.*, Vieweger, 1928). That the lipoid content of the blood is high is probable; we have repeatedly observed eel serum in which macroscopic oil droplets were visible. However, in mammalian blood at least, lipoids can have at most a small effect on the

colloid osmotic pressure (see Meyer, 1932, p. 50). Halliburton (1886) reported the serum of the (fresh water) eel to contain an average of 6·7 per cent. protein. This would correspond to a colloid osmotic pressure of about 250–300 mm., if the proteins of eel serum are similar in molecular weight to those of the mammals.

The great effect of starvation on the colloid osmotic pressure is shown in Table II. Similar effects are well known in mammals as a result of protracted protein starvation (Knack and Neumann, 1917; Mavor, 1920; Jackson, 1925), in various forms of malnutrition (Bruckman, D'Esopo and Peters, 1930; Liu, Chu, Wang and Chung, 1931), in inanition associated with alimentary disturbances (Wolferth, 1924; Landis and Leopold, 1930), and have been produced experimentally in rats fed on low protein diets (Kohman, 1920; Frisch, Mendel and Peters, 1929). De Haan (1927) observed great reduction in serum proteins in frogs as a result of inanition.

In all the studies cited above, however, a reduction of colloid osmotic pressure to less than half the normal level resulted in marked oedema and even massive ascites. The same consequences result from any great reduction in the concentration of colloids in the plasma of mammals (Govaertz, 1924; Schade and Claussen, 1924; Iversen and Nakazawa, 1927; Krogh, 1929, p. 362 *et seq.*; Peters and Van Slyke, 1931, p. 683), and may be inferred in frogs (De Haan, 1927). In the eels, however, macroscopic examination failed to reveal such consequences. Only two explanations suggest themselves; either the classical concept, that the quantity of tissue fluid is the resultant of the balance between hydrostatic and colloid osmotic pressures, breaks down here, or, in the eel, the hydrostatic pressure adjusts itself to the colloid osmotic pressure. That is to say, either the blood pressure (strictly the capillary pressure) remains relatively unchanged in starvation, in which case some unknown mechanism must compensate the tendency to excessive capillary filtration, or, as the blood protein concentration and colloid osmotic pressure fall during starvation, the blood pressure is also diminished. Although at present both these possibilities remain open, the theory of a diminishing blood pressure seems more likely than the hypothesis of an entirely new mechanism.

Vieweger (1928) starved eels and found that over 25 per cent. of the body weight was lost but that the relative chemical composition of the body as a whole remained almost constant.

As already mentioned, the prediction of a high colloid osmotic pressure in eel serum (Keys, 1933) was made on the basis of refractive index measurements. The relation between refractive index and colloid osmotic pressure in fish sera is shown in Fig. 1. The refractive increment due to non-mineral solutes was calculated as the gross refractive index minus the refraction of water, minus an allowance for the salts; the allowance was 0·00210 for the fresh water specimens and 0·00240 for the marine specimens (see Keys, *op. cit.* p. 191). Although this allowance is only a rough estimate, it should be noted that even were it seriously in error it would have but a slight effect on the general result.

The correspondence between refractive index and colloid osmotic pressure is not very precise; this is in harmony with the studies on man which point increasingly to nothing more than a rough relation between colloid osmotic pressure and total

serum protein concentration (see, *e.g.*, Krogh, 1929, p. 362; Meyer, 1932, p. 46). The refractive increment per unit of colloid osmotic pressure is roughly the same in these fishes as found in the mammals, pointing to an essential agreement between the molecular weights of the plasma proteins in the two groups.

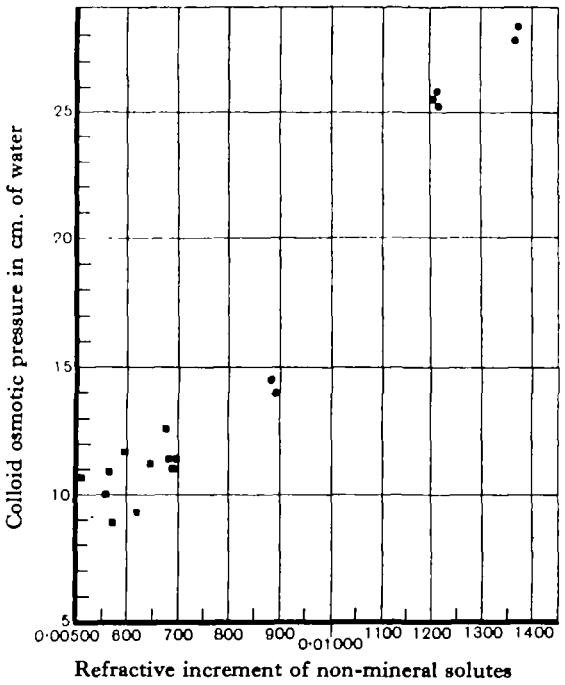

Fig. 1. Relation between colloid osmotic pressure and refractive increment due to non-mineral solutes in fish sera. Circles—values for eel serum; squares—data for plaice, tench, pike and cod.

SUMMARY.

Measurements of colloid osmotic pressure and refractive index of five species of teleost fishes are recorded. A number of observations were made on eels which had been starved for two months. With the exception of the eel, the colloid osmotic pressures were less than half the characteristic level for mammals, the average being about 100 mm. H_2O. Normal eels showed serum colloid osmotic pressures within the mammalian range, the starved eels showed values less than half as great but showed no signs of oedema.

A comparative table of colloid osmotic pressures for the vertebrates studied so far is presented.

The relation between refractive index and the colloid osmotic pressure is discussed briefly.

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