THE COMPOSITION OF THE BLOOD OF THE SHORE CRAB, *CARCINUS MOENAS* PENNANT, IN RELATION TO SEX AND BODY SIZE

II. BLOOD CHLORIDE AND SULPHATE

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INTRODUCTION

In a previous communication it has been shown that in both sexes of the shore crab blood conductivity rises steadily to a maximum at a weight of approximately 35 g. and thereafter falls with increase in body weight: on the other hand total osmotic pressure (O.P.) shows a small but steady decrease with increasing size.

It seemed worthwhile therefore to consider the ionic composition of the blood in relation to sex and body size; such information as is available does not consider the factor of size and in only one case has the influence of sex been examined (Drihlon Courtois, 1934). In the present work total blood chloride and sulphate were investigated.

MATERIALS AND METHODS

Procedures were essentially similar to those already described (Gilbert, 1959), crabs again being obtained from the shore close to the Dove Marine Biological Station, Cullercoats.

Blood was removed through a small incision in the arthrodial membrane at the base of the large chela. Using a glass pipette with a rubber teat sufficient blood was removed from a single crab for either chloride or sulphate determinations. Anti-clotting agents were not used.

The chloride ion is of such importance that in view of the conductivity results (Gilbert, 1959) variations with size might be expected. Moreover, the large amounts of chloride present, and the accuracy of the method for its determination made it likely that any differences would be detectable. Chloride was determined on 1 ml. samples by the method of Robertson & Webb (1939): the sample is treated with $AgIO_3$, the displaced iodine dissolved in KI and titrated with thiosulphate. Titrations were carried out until three consecutive readings were obtained within 0·005 ml. of one another, and the correction of Sendroy (1939) applied to the mean of the results. Since the ionic content of the blood depends on the external medium, the chloride of the sea water in which the crabs were kept was determined each day. However, only slight fluctuations were found over the course of several weeks and during the latter part of the investigation it was determined weekly.

The silver iodate was prepared in the laboratory at first, but later it was obtained commercially (B.D.H.); identical results were obtained with both preparations.

Blood sulphate

There is no accurate method for the semi-micro determination of the sulphate content of biological fluids. The most satisfactory yet developed is that of Robertson & Webb (1939) for which an accuracy of 2% is claimed; this could not be achieved in the present work. With test solutions values were usually low, and in the region of 93% of the true value, with a variation of $\pm 3\%$. It was found that if the sodium sulphate used in the titration was rapidly added to within 0·5 ml. of the expected end-point and the titration then continued as described by Robertson & Webb the variation was reduced to about $\pm 1\%$; but results were still low. The investigation was restricted to possible differences between the sexes as it was felt that the method was not sufficiently accurate for the demonstration of variations in blood sulphate with body size.

Sufficient blood could be obtained from a single crab for two and usually three determinations. The mean of these was taken.

All glassware was thoroughly washed in hot water, rinsed repeatedly in distilled water, and dried with acetone. No contamination from the sulphate present in the cleansing fluids usually used for cleaning glassware could be detected.

RESULTS

Blood chloride

For convenience the results of the determinations were converted to millimolar chloride. These values have been plotted against body weight in Fig. 1 for sixty-seven male and in Fig. 2 for sixty-three female crabs. It is obvious that blood chloride is not constant over the whole size range, and that for both sexes, the pattern is very similar to that found for blood conductivity (Gilbert, 1959). From a value of about 490 mM. for the smallest animals blood chloride increases steadily to a maximum of about 520 mM. at a body weight of about 35 g. For crabs above this weight there is a gradual decrease until it reaches a value of about 500 mM. for the largest animals.

For the statistical analysis, the data for each sex was divided into two groups, above and below 35 g., as for the conductivity results (Gilbert, 1959); reference to Figs. 1 and 2 will show that this treatment is justified. The respective regression coefficients were calculated by the method of least squares. As would be expected, all four regression lines differ significantly from the horizontal ($P < 0{\cdot}01$ for females below 35 g. and $P < 0{\cdot}05$ for the others).

An analysis of co-variance showed that there was no significant difference between the two regression coefficients for the lines of positive slope for males and females below 35 g. body weight, nor between the two regression coefficients for lines of negative slope for animals above 35 g. ($P > 0{\cdot}05$ in each case). Throughout the size range females tended to have higher values of blood chloride than males. Below

35 g. body weight this difference is highly significant ($P<0.01$); but it was not significant for crabs above this weight ($P>0.05$).

In view of the conductivity results (Gilbert, 1959) this was rather surprising. Above 35 g. body weight the males had a higher conductivity, but there is no difference in the chloride content of the blood; below 35 g. body weight there was no difference in the conductivity yet the males have a lower chloride value than the females.

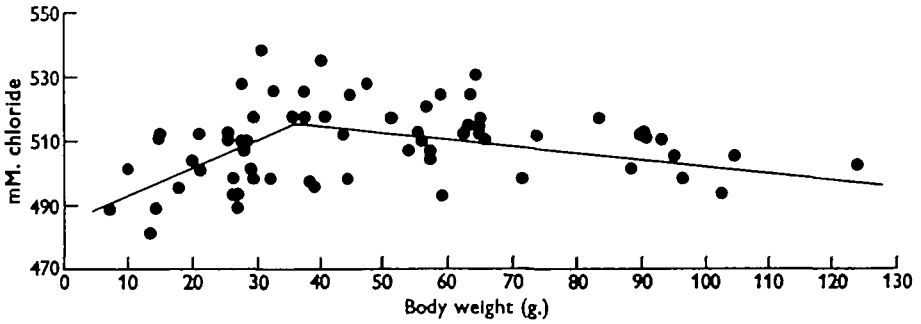

Fig. 1. Body weight and blood chloride in male crabs. Linear regression lines were calculated (1) for animals below 35 g. body weight, and (2) for animals above this weight. This procedure was followed for all subsequent figures except Fig. 3.

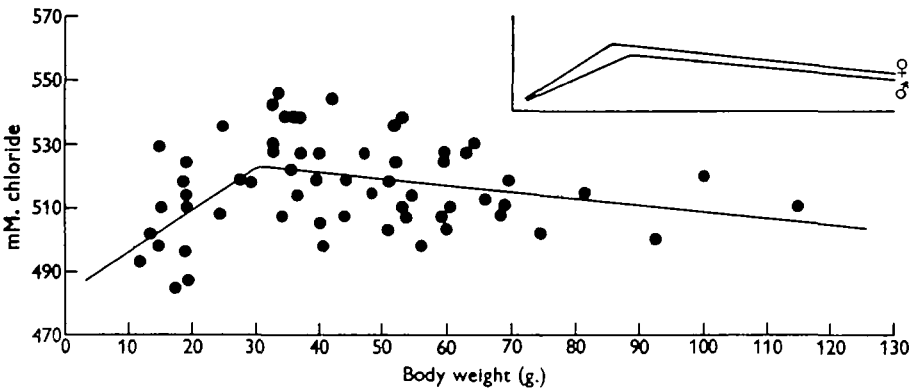

Fig. 2. Body weight and blood chloride in females. Inset: calculated regression lines for males and females.

Blood sulphate

The disparity between the results obtained for conductivity and blood chloride suggested that the concentration of some of the other ions differed between the sexes. Moreover, the chloride results suggested that such a difference would be among the anions. There is already some evidence that the blood phosphate may be higher in males than in females (Drihlon Courtois, 1934). In the present work blood sulphate was investigated in view of the greater concentration of this ion (Webb, 1940).

The results of the determinations have been converted to millimolar sulphate and plotted against body weight in Fig. 3 for thirty-one male and twenty-seven female crabs. As already mentioned, the method for the sulphate determination is not sufficiently accurate to enable differences with body size to be detected; clearly Fig. 3 shows no trend with body weight and a straightforward comparison of the two means is justified. The mean value for males is 15·1 and that for females over the same size range is 11·3. The difference of 3·8 between the two sexes is highly significant ($P < 0{\cdot}01$).

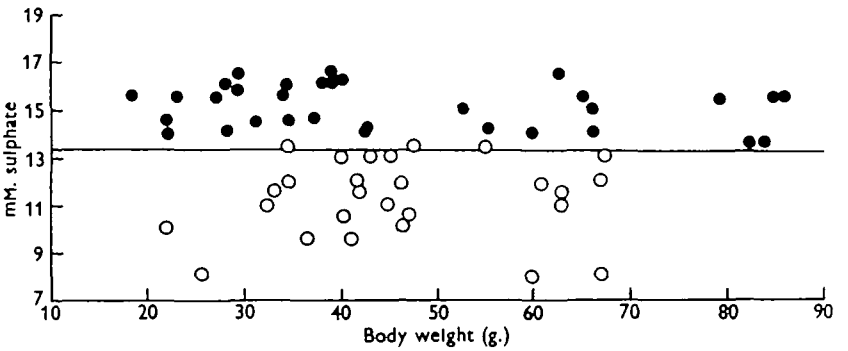

Fig. 3. Body weight and blood sulphate for male and female crabs. The horizontal line represents the population mean. ●, Males; ○, females.

DISCUSSION

Clearly failure to consider sex or body size when comparing the ionic composition of the blood of the shore crabs could be responsible for the lack of agreement between the results reported in the literature (Quinton, 1904; Duval, 1925; Bateman, 1933; Drihlon Courtois, 1934; Nagel, 1934; Webb, 1940; Shaw, 1955; and others). The sulphate content of the blood of female crabs has been shown to be significantly lower than that of males. Blood chloride varies with body size and sex, the pattern being very similar to that for the conductivity, in that the maximum value is reached at a weight of about 35 g. with a general decrease above and below this weight. In general the blood chloride of the males tends to be lower than that of the females; below 35 g. this difference is statistically significant, although it is not so above this weight. It has also been shown that there is no significant difference in conductivity of the blood between the males and females for weights above 35 g. Males tend to grow at a greater rate than females (Orton, 1936) so that at any given weight the males will be younger. If the sexes were compared by age rather than by body weight any differences would be accentuated if they were related to maturity, so that it is possible that they would be significant throughout.

A detailed investigation of the ionic composition of the blood has already been reported for animals also supplied from Cullercoats (Shaw, 1955). Crabs used by this worker were probably females of 35–40 g. body weight (Personal communication). On this estimate of sex and size Shaw's results and mine for both blood

chloride and conductivity determinations show good agreement: his figure for chloride is 524 and that for conductivity 552; for females of 35 g. body weight my values are 523 and 556 respectively. Although it would appear therefore to be justified to use the results of Shaw for crabs of this size and sex, caution is necessary since a comparison of the data of Shaw (1955) and Webb (1940) suggests that crabs of different 'races' may have different blood compositions.

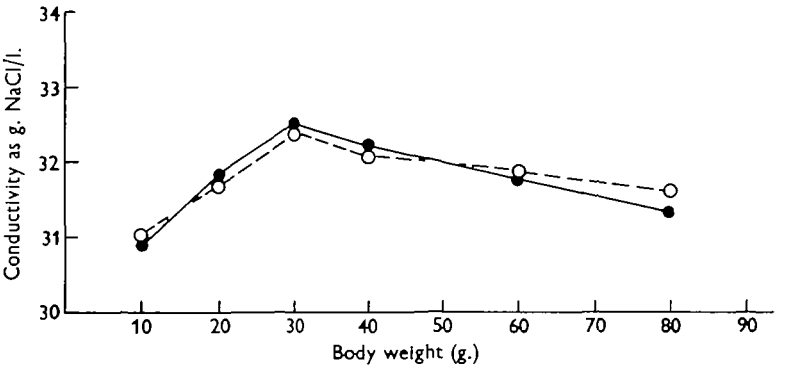

Fig. 4. Experimentally determined and calculated curves for body weight and conductivity: males only. ●—●, Experimental; O- - -O, calculated.

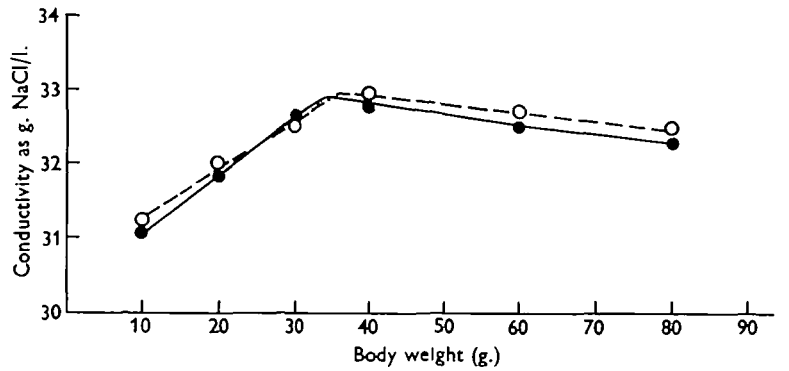

Fig. 5. Experimentally determined and calculated curves for body weight and conductivity: females only. ●—●, Experimental; O- - O, calculated.

Using Shaw's values for Na^+ K^+ Ca^{2+} Mg^{2+} for the composition of the blood and those of the present work for Cl^- and SO_4^{2-} it is possible to estimate the total millimolar blood concentration for females of 35 g. body weight. Values for the total mM. ionic content are in close agreement with total mM. concentration as obtained from the freezing-point depression, and with the values for conductivity measurements. For this weight therefore blood concentration is apparently almost entirely due to electrolyte. If the relative proportion of the different ions remains constant over the whole size range the blood conductivity can be estimated: the

estimated values for females are plotted, together with those determined experimentally in Fig. 4. Fig. 5 shows similar agreement for males, although there is more doubt about the validity of the assumption on which the estimates are based. Clearly the hypothesis that the relative proportion of the ions remains constant over the whole size range for both sexes appears to be justified.

At 35 g. body weight the total O.P. as shown by the freezing-point depression agrees well with the total O.P. as estimated from the conductivity and blood analysis of Shaw (1955) and Gilbert (1959). At other weights, however, there are considerable discrepancies. Since Figs. 4 and 5 show that it is highly probable that the relative proportion of the ions remains constant over the whole size range it follows that these discrepancies must be due to the non-electrolyte fraction; when the ions decrease this must increase and vice versa. Drihlon Courtois (1934) has already shown that under certain conditions blood protein increases as the ionic concentration decreases in individual crabs; an investigation of the non-electrolyte variation in crabs of different body size and sex will be reported in a future communication.

SUMMARY

1. The influence of sex and body weight on the concentration of sulphate and chloride ions in the blood of the common shore crab was investigated.

2. Blood chloride increased in both sexes until a maximum was reached at a weight of about 35 g.; thereafter it fell with increasing body weight.

3. Over the whole size range blood chloride was higher in females than in males. This difference was highly significant for animals over 35 g. body weight; below 35 g. however, there was no significant difference between the sexes.

4. Over a restricted size range blood sulphate of males was significantly higher than that of females.

5. Results of the present work have been discussed in relation to those reported earlier for conductivity and total O.P.

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