I. A CHEMICAL STUDY OF THE BLOOD OF SEVERAL INVERTEBRATE ANIMALS.*

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A study of the literature reveals the existence of comparatively few communications related to this particular field. The earliest work appears to be that of Macallum (1), dealing with the inorganic constituents of the blood of vertebrates and invertebrates. Other investigations somewhat closely related were made by Lipschütz (2), on the metabolism of fishes during starvation; Botazzi (3), on the peritoneal fluid and blood of sea animals; Jona (4), on freezing point values of the blood and body fluids of certain mammals, fish, and crustacea; Okuda (5), dealing with the quantitative determination of creatinine and creatine in some fishes, mollusks, and crustacea; and Fandard and Ranc (6), on sugar in the blood of the sea turtle.

The results embodied in these communications are greatly limited, however. The earlier workers were handicapped by the lack of accurate methods, and hence could not extend their studies to any great length. In later years the only work which has a comparative value when studied with that of the present paper is that of Denis (7), and of Wilson and Adolph (8), dealing with the determination of certain nitrogenous substances in the blood of fresh and salt water fishes; e.g., shark, ray, mackerel, carp, and a few others.

In view of the general lack of information concerning the composition of the blood of invertebrate animals, it is evident that any substantial addition to our knowledge in this field

* This paper is a part of a thesis presented to the Department of Chemistry of Stanford University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
would be of distinct interest. The immediate object of this investigation was the collection of chemical data along this line, with the added purpose of making a comparative study of the data obtained with relation to that from the more highly evolved animals. It is hoped that this study may bring to light a certain number of general relationships, which will enlarge our ideas of general metabolism, and, more remotely perhaps, assist the zoologist in tracing out some of the less clear biogenetic relationships.

The following invertebrate forms were studied.

*Ccelenterates*: The jellyfish, *Phacellophora camtschatica*.

*Echinoderms*: Two species of starfishes, *Pisaster ochraceus* and *Picnopodia helianthoides*. One species of sea urchin, *Strongylocentrotus franciscanus*.


*Crustaceans*: Two species of crabs, *Cancer productus* and *Cancer antennarius*. In this case the blood of the two species was mixed.

All the samples of blood were collected from the living animal, (a) by exposing the deeper surfaces, (b) by severing wholly or in part a blood vessel, and (c) by maceration of the tissues.

Where it was possible a specific gravity determination was made, and this was followed by a quantitative and qualitative chemical examination. The quantitative determinations made were total nitrogen, non-protein nitrogen, urea and ammonia nitrogen, amino-acid nitrogen, preformed and total creatinine, uric acid, sugar, cholesterol, chlorides as sodium chloride, calcium as calcium oxide, total solids, and ash. The qualitative tests included the reaction to litmus, the biuret, Millon's, Hopkins-Cole, xanthoproteic tests, and that for loosely combined sulfur.

In general the chemical examination was made directly after the blood was collected. Oxalate or citrate was added only in those cases in which experience had shown that coagulation would occur before the analysis could be undertaken. The samples were kept in tightly stoppered containers which were placed on ice. Toluene was added when conditions made this step advisable. Quantitative determinations were usually run in dupli-
cate, sometimes in triplicate. Analyses were repeated in all cases where there was the least doubt of the accuracy of the results. Relatively complete analyses were made, when the quantity of blood in a given sample permitted this to be done. In several cases repeated analyses of the blood from the same species of animal, collected at different times, were conducted. Whole blood was used in every case, and was composite, from two or nineteen or twenty forms of the same species being represented in the samples analyzed.

The methods followed in the collection of the samples were different for the various species studied. While the jellyfish, Phacellophora, possesses no celomic fluid or blood, it was included on account of its zoological position. Consisting largely of amorphous jelly-like mesoglea, quite firm to the touch, it was readily reduced to a fairly homogeneous liquid by gentle maceration through cheese-cloth.

In the case of the starfish, as much as possible of the extraneous sea water was removed, and then several of the rays were clipped off a few centimeters from their distal ends. The exuding liquid was allowed to drain into vessels arranged for the purpose. In the later samples the liquid was filtered through a loose tuft of absorbent cotton.

The procedure was slightly different for the sea urchin. In this case several incisions were made in the membrane surrounding the oral cavity. The animal was drained in the manner previously described, and the liquid filtered as before.

On account of the rather definite circulatory system of the mollusks, the procedure used in the preliminary stages of the work was limited to the selection of some of the larger blood vessels. Later on, however, a quicker and easier method was devised. Rather deep cavities were cut in the foot, for example, of the abalone, Haliotis rufescens, or in the gilt-cleft of the Cryptochiton stelleri. The blood filled these cavities rapidly and was removed by means of a small pipette with a curved end connected with a suction flask. Or in the case of the abalone, a part of the shell was broken and one of the branchial vessels exposed. This was partially severed, a fairly large hypodermic needle inserted, held securely in place with a small pair of forceps, and the blood carried over into a flask by suction. For
some unknown reason this method did not always yield an abundant supply of blood. A similar method was used for clams. The shell was broken and the position of the heart determined. The pericardial membrane was then severed as well as the superior vena cava and the blood running into the cavity removed in the manner previously described. This, however, is a tedious process. In order to facilitate matters the exposed body of the clam was superficially cut in several places and then gently macerated. The liquid collected was filtered through loose cotton. Total nitrogen determinations made showed a value of 39.98 mg. per 100 cc. of blood obtained in this manner, as compared with 39.92 mg. in blood collected from the pericardial cavity. Thus the difference is slight and the maceration method is to be preferred for simplicity as well as quickness.

The methods used for collecting crab blood were not essentially different from those previously described. Coagulation of the blood of these animals proceeds very rapidly after it is drawn—fibrin frequently separating out before sufficient oxalate is dissolved to prevent it.

Methods of Analysis.

Methods for blood analysis have thus far had a rather narrow application. The protein nitrogen for vertebrate blood has varied in round numbers from 2,800 to 3,700 mg. per 100 cc. of blood. The limits of the mineral constituents perhaps could be truthfully set at 400 to 600 mg. per 100 cc. These values are in decided contrast to those obtained in this investigation from the blood of invertebrate animals. Here the limits for the protein nitrogen vary from 1.5 mg. in the twenty ray starfish to 358 mg. in the crab, and for the mineral constituents from approximately 2,500 to 3,000 mg. per 100 cc. in the various species studied. In fact, comparatively speaking, these numbers are just the reverse in magnitude of those for the common vertebrates. In view of these striking differences, the presumption appears to favor the conclusion that occasional changes in the ordinary analytical procedures would be necessary. Experience in general has justified this view.
For preliminary removal of proteins the 50 per cent solution of trichloroacetic acid, used by Greenwald (9) and others, was by far the most satisfactory precipitant examined.\(^1\) In some instances one precipitation was all that was necessary, the filtrate remaining free from any insoluble matter after considerable concentration. In many cases, however, it was necessary to digest the mixture in boiling water for 15 or 20 minutes to obtain the best effects. Enough of the acid was used to produce a concentration of from 6 to 8 per cent.

The freshly prepared 25 per cent solution of m-phosphoric acid recommended by Folin and Denis was not so satisfactory. The filtrates were in general cloudy, and even digestion in boiling water, a procedure which might be questioned, failed to accomplish the end desired. Concentration seemed to be the only recourse. There is, of course, the possibility always present of a union between the acid or some of its derivatives and the protein to form soluble products. This effect is very likely to take place when m-phosphoric acid is used, according to Folin and Denis, particularly where the acid is not fresh. Heating would of course favor this condition. Whether trichloroacetic acid would react similarly does not seem to be known, but experience seems to be against such a possibility. In any case any solution of the protein would vitiate the whole procedure.

Picrate-picric acid solution proved a most excellent precipitant, but its special character limited its use.

Alcohol was also used, but evaporation always yielded appreciable quantities of protein.

0.01 N acetic acid as a general precipitant was to be criticized in much the same way as m-phosphoric acid.

Kaolin as an absorbent of protein was satisfactory.

For total nitrogen, the Kjeldahl-Gunning method was followed, a small quantity of copper sulfate serving as catalyst.

Non-protein nitrogen was determined by the method of Folin and Denis (10) and also by applying the Kjeldahl method to the protein-free filtrate. On account of the difficulty attendant on the absorption of

\(^1\) The recently described protein precipitant for use in blood analysis, tungstic acid, recommended by Folin and Wu (Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81), was not used, since the investigation was nearly completed when the method came to the notice of the author.
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color by the silicious insoluble matter, most of the determinations were made by the latter method. In most cases a sufficient supply of blood permitted this choice.

The determination of urea nitrogen was made by the methods of Folin and Denis (11) and Van Slyke and Cullen (12). Latterly the first of these methods was preferred, but with certain modifications. Instead of an emulsion of soy bean, a urease preparation was used either in the form of a powder or of a phosphate solution, according to Van Slyke and Cullen. The blood with the urease was digested at 45-50°C. for 30 minutes or longer, the remaining procedure being similar to that of Folin and Denis, except that 10 cc. of the filtrate were aerated into a known volume of a 0.2 N sulfuric acid solution to which was added ammonium sulfate equivalent to 0.05 mg. of nitrogen per cc., explanation of which will be made later. To expel the ammonia, 15 cc. of saturated potassium carbonate solution were used. The absorbent was then Nesslerized in the usual manner, a 100 cc. volumetric flask being used. The color comparisons were made by a Duboscq colorimeter with the standard set at 20 mm. Control determinations were made by subjecting urea solutions of known concentration to the action of urease, and completing the determination in the same manner as the unknown.

The determination of ammonia nitrogen was made according to the method of Folin and Denis (13) though with considerable modification influenced to some extent by the results of Barnett (14). 10 cc. of the blood were mixed with 15 cc. of saturated potassium carbonate solution and the mixture was aerated into a definite volume of 0.2 N sulfuric acid solution containing 0.05 mg. of ammonium sulfate per cc. The absorbent solution was then Nesslerized according to the method of Folin and Denis, and a colorimetric determination made with the standard set at 20 mm. Since a definite quantity of ammonia nitrogen was used to start with in the absorbent, this was subtracted from the total. The remainder represented the ammonia nitrogen in the blood. As will be noted a similar procedure was used in the determination of urea. The advantages of aerating into a standard solution of ammonium sulfate made acid with sulfuric acid are easily seen in this determination. The accessory apparatus of polarimeter tubes and condenser is eliminated. The depth of shade of the Nessler solution can be varied to suit the eye. Finally Nesslerizations can be made in 100 cc. volumetric flasks and after a little practice the standard can be so adjusted to the unknown that the reading will vary not more than 4 or 5 mm. from 20. The same remarks may well apply for the determination of urea nitrogen. The gain in simplicity is considerable.

In general the author does not look on aeration procedures with any great degree of favor. In spite of protective tubes and capacious aeration cylinders, it was necessary occasionally to make repeated determinations. The distillation procedure is perhaps more preferable.

The observation made by Barnett (14) that the quantity of ammonia nitrogen increases in the blood on standing was followed in its practical
significance by conducting the determination as soon as possible after
the blood was drawn. That there is a decided increase in the quantity
of ammonia on standing is in general agreement with the author's findings.
This, it might be mentioned, is particularly true for whale blood.

Amino-acid nitrogen was determined by means of the Van Slyke (15)
micro apparatus. In the initial treatment of the sample, the procedure
of Bock (16) was followed. Several determinations were made, but only
two are given in the table. In sea urchin, abalone, and crab blood values
varying from 30 to 70 mg. per 100 cc. were obtained. The determinations
were not always made directly after the blood was drawn. The post-
mortem formation of amino compounds, or the action of certain substances
in the blood on the nitrite used, might account for these high values. In
any case there appear to be certain disturbing conditions.

Preformed and total creatinine were determined according to the
method of Denis (17). In some cases 10 cc. of the standard were added
to the unknown, and the determination was made. The picric acid used
was tested according to the method of Folin and Doisy (18) and was found
to satisfy the requirements. The principal difficulty experienced was the
failure of color to develop after the prescribed amount of 10 per cent
sodium hydroxide was added. Precipitates usually formed after the base
was added. In order to overcome the first of these difficulties a cautious
addition in excess of from 0.5 to 1 cc. of the base was made. Within
narrow limits a variation in the base used had no influence on the reading
when the experiment was conducted on standard creatinine solutions of the
same concentrations. Precipitates were eliminated by centrifugalization.
These difficulties appeared to be accentuated in determinations of total
creatinine.

Uric acid was determined by the method of Folin and Denis (19). For
amounts of uric acid which are found in human blood or of an approximate
magnitude, no trouble was experienced. When only traces of the acid
exist, and these in the presence of about 3 per cent sodium chloride, diffi-
culties were encountered which were not entirely overcome. In order to
render measurable the traces which appeared to be unmistakably present,
from 30 to 50 cc. of the blood were used. The crystallizing out of con-
siderable quantities of sodium chloride on evaporation appeared to vitiate
the determination. Definite quantities of uric acid were then added
to 3 per cent sodium chloride solution, but it seemed to be impossible to
recover anything like the quantity of acid added. The large excess of
sodium chloride may hinder perhaps the formation of silver urate by its mass
action effect. Indications are that the method must be considerably
modified to determine small quantities of uric acid under the conditions
described.

Sugar was determined by the method of Lewis and Benedict (20). The
picramic acid for the standard was prepared2 according to the later method

2 The work of preparation was kindly performed for the author by Dr.
E. Oertly of Stanford University.
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of Egerer (21). The only difficulty met with was in the application of the method to certain samples of blood such as that of the starfish, where the protein was very low. In this case the precipitate formed was difficult to remove by filtration. The precipitate appeared in the form of a suspension which did not readily settle.

For cholesterol, the method of Myers and Wardell (22) was used. Two modifications were introduced after several experiments. Instead of the standard "napthol green B," a standard solution of Kahlbaum's cholesterol 1.0 mg. per 100 cc. in chloroform was used. The original procedure of taking an aliquot part of the extract was changed and the whole volume was concentrated to 5 cc. This modification was made necessary on account of the small quantities of cholesterol present, even when 3 to 5 cc. of the original blood were used.

It was found that the unknown as well as the standard cholesterol solutions possessed a decided bluish tint after performing the Liebermann-Burchard reaction. Hence good comparisons could not be made with the dye used as a standard. The blue shade observed is in decided disagreement with the observations made by Luden (23) in connection with the same reaction.

A final difficulty, not fully overcome, was the formation of a reddish shade in the extracts. This increased after adding the sulfuric acid and acetic anhydride. The shade seemed to develop to the greatest extent in clam blood. In the light of Luden's work the conclusion may be drawn that this color is due to icteric substances in the blood. At any rate, the color comparisons were made most difficult on account of this condition.

Chlorides as sodium chloride were determined according to the method of Rappleye (24). The method is simple and the end-point sharp for the chlorides in most invertebrate blood. When the sodium chloride is less than 0.6 per cent, the end-point becomes somewhat uncertain. Only 1 cc. of blood was used for the determinations, the amount of the silver nitrate solution varying from 15 to 20 cc. These quantities are slightly different from those prescribed by the method. In the author's view the method is considerably more simple than that of Van Slyke and McLean, and equally as accurate.

For total solids, ash, and calcium as the oxide, 5 to 10 cc. of the blood were evaporated to dryness in a weighed porcelain crucible over a water bath, and the residue was dried to constant weight at 110-115°C. This represented the total solids. The residue was then ignited, the temperature being kept as low as possible to avoid volatilization of the alkalies—a condition not always easy to accomplish in removing the last trace of the carbon—and then weighed. This represented the ash. The latter was then extracted with 15 to 20 cc. of concentrated hydrochloric acid, and the calcium as the oxide determined in general according to the method of McCrudden (25).

Specific gravity determinations were made at 20°C. by a pycnometer bottle.
The values in Table I exhibit several differences when compared with similar determinations made on the more highly developed animals. The exceptionally high content of inorganic salts, 2,900 to 3,200 mg., and the low protein nitrogen content, 0.7 to 340 mg. per 100 cc. of the blood, may be taken in illustration.

The influence of high saline content on osmotic pressure is obvious. The relatively small amount of urea and other organic crystalloids in the samples of blood examined clearly proves that the osmotic pressure is for the most part caused by the inorganic constituents. In any case the osmotic pressure is nearly the same as sea water ($\Delta = 1.81-2.8^\circ$) (26). In the more highly developed elasmobranch fishes this pressure is undoubtedly produced to a notable degree by the very large quantities of urea present in the blood, a constituent present in only small quantities in invertebrate blood. Neither the invertebrates examined nor the elasmobranch fishes appear to be independent of the surrounding medium in this regard. Neither set of forms has any means which enable them to influence their own osmotic pressure. This is in direct contrast to the marine and fresh water teleost fishes where probably a reduced saline as well as a urea content effects a material lowering of the osmotic pressure of $\Delta = 0.7^\circ$. As pointed out by Wilson and Adolph (27), these species are partially independent of changes in the surrounding medium. Undoubtedly the gills of these fishes have been modified in some way which enables them to adapt themselves to conditions of this kind.

That the low protein content of the blood of the invertebrate animals has a decided influence on its viscosity can hardly be doubted. Compared with the viscosity of the more highly developed marine forms and the mammals it should be considerably lower. In mollusk and crustacean blood the increased protein content would be parallel to an increased viscosity, and this in turn is associated with an improved cardiac and circulatory apparatus.

The buffer effects of the protein in mammalian blood in maintaining the required hydrogen ion concentration probably prevail in the blood of invertebrates, this value approximately being from 6.4 to 6.7 pH if the alkaline reaction to litmus is considered (Table II).
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<th>20 ray starfish, Piceaster ochraceus</th>
<th>Sea urchin, Strongylocentrotus franciscanus</th>
<th>Clam, Saxidomus nitidus</th>
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<td>Cryptochiton stelleri.</td>
<td>1.030</td>
<td>197</td>
<td>3.0</td>
<td>2.00</td>
<td>0.40</td>
<td>41</td>
<td>Single specimen, 120 cc. of blood, 21×11×5 cm.</td>
<td></td>
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<tr>
<td></td>
<td>1.029</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
<td>Single specimen, 137 cc. of blood, 19×10×5 cm.</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>Single specimen, 67 cc. of blood, 18×10×4 cm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, 2, 3 composite.</td>
<td></td>
<td>231</td>
<td>25.0</td>
<td>4.03</td>
<td>0.50</td>
<td>13.00</td>
<td>0.27</td>
<td>0</td>
<td>0</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>66</td>
<td>3.09</td>
<td>4</td>
<td></td>
<td>177</td>
<td>6.5</td>
<td>1.31</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Abalone, Haliothis rufescens (red).</td>
<td>1.098</td>
<td>100</td>
<td>4.84</td>
<td>0.00</td>
<td>0.65</td>
<td>38</td>
<td>Obtained from Monterey Bay, Cal.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abalone, Haliothis rufescens (black).</td>
<td>4.730</td>
<td>87</td>
<td>3.160</td>
<td>168</td>
<td>15.0</td>
<td>1.41</td>
<td>0.29</td>
<td>0.31</td>
<td>0.78</td>
<td>0.47</td>
</tr>
<tr>
<td>Crab (two species), Cancer productus. Cancer antennarius.</td>
<td>4,685</td>
<td>73</td>
<td>2,895</td>
<td>358</td>
<td>18.0</td>
<td>0.25</td>
<td>1.15</td>
<td>1.05</td>
<td>0.20</td>
<td>0.78</td>
</tr>
</tbody>
</table>

* A sample of sea water (a mixture of 15 samples representing consecutive days) contained 1,920.5 mg. of Cl (i.e. 3,165 mg. of NaCl) and 40 mg. of CaO per 100 cc. Monterey Bay, Cal., near the Hopkins Marine Station. Period April 26, 1919, to May 13, 1919.
<table>
<thead>
<tr>
<th>Animal</th>
<th>Color of blood</th>
<th>Litmus</th>
<th>Xanthoprotein</th>
<th>Millon</th>
<th>Hopkins-Cole</th>
<th>Lead</th>
<th>Biuret</th>
</tr>
</thead>
</table>
The high values for calcium oxide, from 53 to 307 mg. per 100 cc. of blood are in accord with what one might expect in view of the considerable demand on the part of the invertebrate animals for the calcareous matter needed for the shell. The largest amount, 307 mg. per 100 cc. of blood, occurs in the Saxidomus clam. The shell of this species is much heavier than that of the associated Schizotherus clam. But if this criterion is taken to account for the difference in the amount of the calcium salts observed, it is somewhat difficult to account for the lower values 74 to 87 mg. per 100 cc. in abalone blood. A qualitative observation favors the view, however, that the proportion of the body of the clam to its shell is considerably less than in the case of the abalone.

Compared to several mammals, e.g. human blood 9.5 to 11 mg. per 100 cc., the calcium salts found in invertebrate blood may be several times as great.

The proportion of urea nitrogen to non-protein nitrogen appears to vary within rather wide limits. This is true for the echinoderms, 22 to 62 per cent of the non-protein nitrogen being represented as urea nitrogen in the blood of starfishes and 11 per cent in the one sample of sea urchin blood examined. In the mollusks and the one sample of crustacean blood the amounts vary from 7 to 70 per cent, though the mean value would fall more nearly between 10 and 20 per cent. The tendency then seems to be toward conditions similar to those which prevail in the blood of the teleost and ganoid fishes. The additional fact that the urea nitrogen is 40 per cent of the non-protein nitrogen in the flesh of the jellyfish examined has further interest.

The partition of the urea nitrogen between the form elements and the plasma was not studied. The form elements, however, comprise only a small proportion of the total nitrogen of the blood, it is believed. This appeared to be true particularly in the case of Schizotherus clam blood. The indications are that the urea is found principally in the plasma.

The ammonia, preformed creatinine, and total creatinine content of the blood of the echinoderms, mollusks, and crustaceans examined was in general considerably lower than the same constituents observed in the elasmobranch, teleost, and ganoid fishes (28). The amount of ammonia seemed to approximate more
closely that found in mammalian blood, though the creatinine and creatine are still lower even in this case. In addition to this the amount of creatine found in invertebrate blood was only a little greater frequently than the preformed creatinine. In starfish blood the creatine appears to be greatly in excess of the preformed creatinine, however.

The occurrence, at most only in minute traces, of uric acid in the blood of most of these animals agrees with the findings of Denis, in respect to the blood of the elasmobranch fishes. In the blood of the crab the amount of uric acid is relatively high, 4.7 mg. per 100 cc., and considerably in excess of that found in human blood; e.g., 0.7 to 3.7 mg. per 100 cc. In this regard the metabolism of the crab appears to be similar to that of the birds and reptiles.

The amino-acid nitrogen probably makes up a large part of the remainder of the non-protein nitrogen, though only two determinations can be given to support this statement.

Sugar was found in all the samples of invertebrate blood examined, and varies from 29 to 90 mg. per 100 cc. of the blood. These values are similar in magnitude to those of human blood, e.g. 20 to 150, and perhaps other mammals. The only other determinations of sugar made on invertebrates were those of Fandard and Ranc (29) in the blood of a fasting turtle. Their values range from 82 to 95 mg. per 100 cc. of the blood, which are in agreement with those of the author for abalone and crab blood. The extreme variation does not appear to be wide in any of the animals examined. In respect to the invertebrate blood, it is interesting to speculate on the manner in which these animals maintain the amounts of sugar observed, in the presence of so much sodium chloride. At least in man the injection of 1 percent salt solution intravenously becomes a glycuric in causing a decided increase of the blood sugar in the urine. The relatively large amounts of calcium salts in the blood of invertebrates may lessen the permeability of the excretory membranes as they do apparently in man (30).

The quantity of cholesterol found was small in all the bloods examined, the variation extending from about 1 to 6 mg. per 100 cc. of blood. Compared with the values given for human blood (30 to 60 mg. per 100 cc.) they appear to be very low.
There seems to be considerable scarcity of data for cholesterol in the blood of mammals as well as of fishes. Hence any comparison must be extremely limited.

Finally it is of extreme interest to note the occurrence of several of the combined amino-acids, e.g. tyrosine, tryptophane, and cystine, in the tissue and the blood of these marine forms (Table II). If the qualitative tests are significant, the relatively large amounts of combined cystine and tryptophane in abalone and crab blood are most interesting.

**SUMMARY.**

The following determinations were made on the blood of several invertebrate animals: Specific gravity, total solids, calcium as calcium oxide, chlorine as sodium chloride, total nitrogen, non-protein, urea, ammonia, and amino-acid nitrogen, preformed and total creatinine, uric acid, sugar, and cholesterol. These values are found in Table I.

The osmotic pressure of the blood of these animals is about the same as sea water, and in this respect they agree with the elasmobranch fishes; e.g., shark and ray. The major part of the osmotic pressure is due to the high saline content and not to the urea as in the elasmobranch fishes.

The very possible influence of low protein content in the blood on the lowered viscosity and the probable increase of this factor in the blood of the Mollusca and Crustacea is accompanied by a more highly developed circulatory system.

The urea content is relatively low, though the mean value is higher than that observed in some of the marine and fresh water fishes.

The ammonia, preformed creatinine, and creatine were generally lower than in the blood of the vertebrate fishes and other mammals.

The amino-acid nitrogen probably makes up the larger part of the non-protein nitrogen.

Sugar was found in the blood of every invertebrate examined and in relatively large quantities. In comparison with the sugar in the blood of other forms, the differences were not great.

Cholesterol was found in very small quantities. In comparison with the blood of man, the amounts were low.
The combined amino-acids, tyrosine, cystine, and tryptophane, the two latter in considerable quantities, were shown to be present in most of the blood(s), and in one case the tissue, of the invertebrate animals examined.

In conclusion the author wishes to express his great indebtedness to Professor R. E. Swain, whose many suggestions and practical assistance had a most happy influence on the success of this investigation as well as that connected with whale blood reported in the following paper.

In connection with the collection of the blood samples, the author wishes to acknowledge also the invaluable assistance of Professor Walter K. Fisher of the Hopkins Marine Station of Stanford University, Monterey, California.

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Rollin G. Myers

_J. Biol. Chem. 1920, 41:119-135._

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