THE PARTITION OF NON-PROTEIN NITROGEN IN THE BLOOD OF FRESH WATER FISH.

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The introduction of microchemical methods has made possible many biological investigations which were formerly precluded by the difficulty of obtaining sufficient amounts of material. This has been the case with studies on the blood and urine of fish. The only accurate quantitative chemical data are found in the recent papers of Dr. Denis' on marine fish, where she reports results which indicate that the composition of the blood and urine is not only different from that of other animals but may even vary in different species of fish.

It has therefore seemed desirable to extend the studies to fresh water varieties in order to gain further insight into the processes of metabolism in these animals. The present study has included determinations of total non-protein nitrogen, urea, ammonia, amino nitrogen, creatine, and creatinine in the whole blood and in the plasma of several species of fresh water fish. The nitrogen partition and the distribution of materials between plasma and corpuscles displayed several unique relationships. The creatine content of the plasma was greater than that of the corpuscles. Urea was present in surprisingly small quantities in most of the bloods and was in greater concentration in the corpuscles than in the plasma. This unequal distribution is in distinct contrast with the uniform diffusion of the compound throughout mammalian tissues.

We have found that the amino nitrogen constitutes the major portion of the non-protein nitrogen in whole blood and is the largest single component in plasma. The quantities present are

1 Denis, W., J. Biol. Chem., 1912-13, xiii, 225; 1913-14, xvi, 389.
Nitrogen in Fish Blood

much greater than those found in mammalian blood. This is a complete reversal of the relationship between urea and amino nitrogen in the bloods of mammalia.

The following methods were employed. (1) Total non-protein nitrogen—Folin’s method as modified by Bock and Benedict. (2) Urea—Marshall’s method. On account of the low values obtained the method was checked in the usual ways including the addition of urea to the blood to make sure that the enzyme activity was not inhibited. The ammonia nitrogen has been subtracted. (3) Amino nitrogen—Van Slyke’s method. The filtrate from the ethyl alcohol precipitation was used as well as portions of the filtrate from the total non-protein nitrogen determinations. There was no evidence of unusual amounts of amines or other nitrogenous compounds which react slowly with nitrous acid as the correction values were similar to those obtained with other bloods. (4) Creatinine—Folin’s method. (5) Creatine—the procedure described by Denis¹ was used. The total non-protein nitrogen, urea, and amino nitrogen determinations were always carried out in duplicate, the others usually singly.

The blood was obtained from the living fish mainly from the caudal artery and vein by clipping off the tail and collecting the blood which dripped from the cut vessels. Some specimens were collected through a needle from the caudal vein while others were obtained by introducing a needle into the aorta. Potassium oxalate was used to prevent clotting. From 40 to 150 fish were necessary to furnish the desired amount of material for analysis.

To secure composite specimens of whole blood and plasma which might be entirely comparable, the blood was divided, soon after it was drawn, into two parts, one-third was put aside for the whole blood composite and two-thirds were centrifuged and the plasma was removed. The hematocrit readings were taken after centrifuging for 20 to 30 minutes. A Daland hematocrit was also used. The collections were kept in ice until sufficient quantities were obtained, when the various determinations were started. There was considerable variation in the ease with which unhemolyzed blood was obtained. No hemolysis was evident in the blood from the catfish and the croppie, while others showed slight hemolysis.

Little definite information was obtained as to the nutritive
condition of the fish. The carp and gar had been kept for some time in artificial ponds and fed regularly. The other fish were bled within a few hours after they had been brought in from the river. A considerable amount of food was found in the intestines of the catfish. The blood from the sturgeon appeared to contain a considerable amount of fat and that from the carp less.

The data obtained may be found in the accompanying table. The unusually small quantity of urea nitrogen in nearly all of the bloods is at once apparent. A large variation may, however, be

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Nitrogen</th>
<th>Urea N.</th>
<th>NH₃ N.</th>
<th>Creatinine</th>
<th>Creatine as creatinine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sturgeon (Scaphirhynchus platorhynchus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>29</td>
<td>22</td>
<td>1.1</td>
<td>1.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Plasma</td>
<td>37</td>
<td>30</td>
<td>2.5</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Gar (Lepisosteus platostomus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>31</td>
<td>21</td>
<td>5.0</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Plasma</td>
<td>32</td>
<td>22</td>
<td>5.2</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Carp (Cyprinus carpio)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>42</td>
<td>40</td>
<td>2.3</td>
<td>1.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>43</td>
<td>35</td>
<td>2.8</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Croppie (Pomozis annularis and sparoides)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>46</td>
<td>35</td>
<td>3.0</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Plasma</td>
<td>37</td>
<td>26</td>
<td>2.5</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Catfish (Ictalurus punctatus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>47</td>
<td>32</td>
<td>2.7</td>
<td>0.9</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma</td>
<td>48</td>
<td>33</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Sheephead (Aplodinotus grunniens)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood</td>
<td>50</td>
<td>49</td>
<td>1.6</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>41</td>
<td>40</td>
<td>9.5</td>
<td>1.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Urea + ammonia N.
| Total creatinine.
† The figures in the horizontal columns are from determinations of a single composite specimen of blood and its plasma, except where noted by ‡.
noted. Whether this indicates fundamental differences in the fish or merely accidental variations due to the condition of nutrition or other factors cannot be decided from the data at hand.

The amounts of urea found in most of our experiments are similar to those observed by Karr and Lewis\(^2\) in the blood of hens. The comparison is mainly of interest by way of contrast for it is well known that uric acid is the chief end-product of protein metabolism in the hen, while fish excrete only very small amounts. The urea in the urine of teleosts constitutes, however, a relatively low percentage of the total nitrogen.

We have found in only a single instance a concentration of urea as great as those reported for marine teleosts by Denis. It is interesting to note that the ganoids (sturgeon and gar) while perhaps more closely related to the marine elasmobranchs than are the teleosts, as indicated by their evolutionary development and cartilaginous skeletons, show no relationship so far as the urea concentration of the blood is concerned.

The variations in the content of urea in the blood from different families of fish exhibit an interesting parallelism with the differences in the osmotic pressures. The blood of the elasmobranchs, which contains relatively enormous quantities of urea (2 gm. per 100 cc.), has an osmotic pressure closely approximating that of sea water\(^3\) (\(\Delta = 2.3^\circ\)). In the marine teleosts, however, the blood contains but one-hundredth the amount of urea and has a much lower osmotic pressure (average \(\Delta = 0.7^\circ\)). Our results would indicate that there is a tendency toward a lower content of urea in the blood of several fresh water fish, while the osmotic pressure is, in general, still less (\(\Delta = 0.5^\circ\)). In connection with the differences in osmotic pressure in these animals it may be observed that the elasmobranchs are dependent upon the surrounding medium for the maintenance of the osmotic pressure of the blood, while the ganoids and teleosts seem to be partially independent of the medium. These facts raise several interesting speculations concerning the mechanisms involved in maintaining the osmotic pressure of the body fluids and the possibility of the diffusion of urea and other substances through the


\(^3\) Bottazzi, F., \textit{Ergebn. Physiol.}, 1908, vii, 161.
gills. The small amount of nitrogenous materials eliminated by
the kidney has already been observed.

The extremely low content of urea in the blood of fresh water
fish was further emphasized by the observation that the plasmas
contained even less than the whole血液s. The localization of
urea in the corpuscles is evident and quite unexpected. Analyses
of tissues from various animals by Marshall and Davis, Bang, and
Karr and Lewis have shown that urea is uniformly distrib-
uted throughout the body, the same in plasma as in corpuscles,
and have led to the assumption that urea may readily diffuse
throughout the body (wherever sufficient water is present) and
be maintained at a strikingly uniform concentration in all tissues.
Our results argue against a too general acceptance of such an
assumption. Furthermore, the data of Karr and Lewis show that
the tissues of the hen may vary considerably in their content of
urea. As mentioned above, the amounts found by these inves-
tigators in the blood and other tissues of the hen are similar to
the quantities found by us in the blood of fish. Hence, it seems
possible that there may be a tendency for the cells of the blood
as well as other cells of the body to retain urea when its concen-
tration becomes very low.

The percentage of urea nitrogen to total nitrogen ranges from
4 to 27 in whole blood and from 2 to 29 in plasma. The majority
of the values are surprisingly low when compared with other
animals and at once lead us to inquire as to what types of nitrog-
enous compounds constitute the large remaining portion of the
non-protein nitrogen.

Amino nitrogen was present in amounts which at once account
for most of the non-protein nitrogen of the blood. There were
found concentrations of 17 to 34 mg. of amino nitrogen per 100
cc. of whole blood, which constituted from 60 to 80 per cent of the
total non-protein nitrogen. These values are considerably higher

5 Bang, I., Biochem. Z., 1915-16, lxxii, 104.
6 Data have been reported to indicate that under pathological condi-
tions an unequal distribution of urea may be encountered.
7 A few determinations carried out by the trichloroacetic acid pre-
cipitation as described by Bock indicated that the values reported above
may be too low. A sample of the whole blood composite from the catfish
gave results for both amino nitrogen and total non-protein nitrogen about
50 per cent higher than those tabulated above.
than the figures reported for amino nitrogen in the blood of mam-

mals. Van Slyke and Meyer found 3 to 10 mg. in the blood of
dogs, depending on the state of nutrition of the animals.

Bock has recently reported intermediate values for bloods of
various mammalia.

The plasma contained less amino nitrogen than the whole blood.

Calculated from the hematocrit values, the corpuscles were found
to contain from three to five times as much as the plasma.

György and Zunz and Costantino observed a similar massing
of the amino nitrogen in the corpuscles of dogs, although the
concentration was much lower in these animals.

The relationship between the amino nitrogen and urea is prac-
tically the reverse of that in the blood of higher animals where we
find urea the chief nitrogenous constituent. The unusual concen-
tration of amino nitrogen suggests that the amino-acids as such
may play a more important part in the intermediary protein
metabolism of the fish and possibly constitute an important
means for the elimination of nitrogen. The small proportion of
total nitrogen accounted for by the materials determined by Denis
in the urine of fish (urea, ammonia, uric acid, creatinine, and
creatine) lends support to the suggestion.

The relatively high content of ammonia in the bloods examined
agrees with the observations of Denis on marine fish. We have
not obtained, however, figures approaching the maximum values
which she reported. The rapidity with which ammonia de-
velops in blood on standing, together with the fact that several
hours are unavoidably required to collect sufficient material for
the experiments, would indicate that the minimal values prob-
ably represent more exactly the actual amounts of ammonia in
the circulating blood.

The content of creatinine in whole blood and plasma was simi-
lar to that in mammalian blood. Creatine was present in some-
what larger quantities than has been found in the blood of higher
animals by a similar method. The preponderance of creatine

8 Our figures range even higher than those reported by J. C. Bock,
(J. Biol. Chem., 1917, xxix, 191) for the amino nitrogen in the blood of birds.
12 Costantino, A., Biochem. Z., 1918, lv, 402.
over creatinine in the plasma of fish corresponds to the relationship of the two compounds in the urine and emphasizes the relative importance of the former in the creatine-creatinine metabolism of these animals at least in so far as concerns excretion.

The variations in concentrations of materials between plasma and corpuscles have been demonstrated in many ways, especially in connection with inorganic constituents and lipid materials. The importance of such study in connection with the nitrogenous substances has not been sufficiently emphasized. The known compounds making up the non-protein nitrogenous fraction of the blood are for the most part materials of interest chiefly as end-products ready for elimination from the body. The study of the distribution of these substances between corpuscles and plasma as well as between plasma and tissues may assist in solving some of the intricacies of the intermediary metabolism with which the various compounds are concerned.

**SUMMARY.**

The following constituents were determined in the whole blood and in the plasma of several species of fresh water fish including ganoids and teleosts: total non-protein nitrogen, urea, ammonia, amino nitrogen, creatinine, and creatine.

The urea content of most of the bloods was unusually low. The concentration in the plasma was less than that in the corpuscles.

The amino nitrogen constituted the major part of the total non-protein nitrogen of the blood. The corpuscles contained considerably more than the plasma.

Creatine was present in larger amounts in the plasma than in the corpuscles and was unusually high in the plasma.
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