THE PHYSIOLOGICAL SIGNIFICANCE OF UREA

I. THE ELASMOBRANCH HEART

BY W. W. SIMPSON AND ERIC OGDEN.

(From the Division of Physiology, University of California Medical School, Berkeley, and from the Pacific Biological Station, Nanaimo, B.C.)

(Received 18th January, 1931.)

INASMUCH as they are the end products of katabolism, the carbon dioxide and urea in the blood have been regarded merely as waste products on their way to the excretory organs until the former was shown to play a very important rôle as the regulating stimulus to the respiratory apparatus. Urea, on the other hand, has not up to the present time been shown to subserve any useful function in the animal body. Nevertheless it is well known that the kidney maintains the concentration of urea in the blood at a remarkably constant level and that only very slight variations of this level are found in health.

For the preliminary investigation of the possibility that the maintenance of a constant percentage of urea in the blood may have some more definite significance than has hitherto been ascribed to it, the elasmobranchs were chosen. In the first place, the unusually high blood-urea level (2000 mg. per 100 c.c.) in these fishes suggests that they may provide a more easily followed clue than other species, and in the second place, the observation of Mines (1912) that the elasmobranch heart would beat only when surrounded by a medium rich in urea, seemed to provide a point from which the problem might be profitably attacked.

A few experiments made at the Marine Biological Station, Plymouth, England, in which the heart of the ray was caused to circulate about 15 ml. of the fluid advised by Mines showed that the substitution of iso-osmotic quantities of sucrose for the urea in this fluid was followed by stoppage of the heart within a few minutes. That this change was reversible was demonstrated by the recovery which followed the replacement of the Mines fluid, just before the arrest was complete and also after complete arrest, but only if the circulation of Mines fluid was forced either by massage or by increased venous pressure. Similar experiments in which the urea was replaced by thio-urea produced more rapid stoppage from which no recovery was obtained.

An attempt to continue this series of experiments at the Pacific Biological Station, Nanaimo, B.C., was frustrated by the difficulty of obtaining rays. Squalus

1 NaCl—M/2, 440 ml.; KCl—M/2, 14 ml.; CaCl₂—M/2, 8 ml.; MgCl₂—M/2, 10 ml.; Urea—10 per cent., 200 ml. Water to one litre.
sucklii, the most easily obtainable elasmobranch in this locality, is unsuitable for perfusion by reason of the difficulty of avoiding leakage through the hepatic veins and of avoiding laceration of the sinus venosus.

Accordingly, the following procedure was adopted. Large female¹ dogfish were pithed, immediately opened and the hearts excised and placed in chilled Mines fluid with the magnesium omitted (we omitted the magnesium as it did not appear to be essential for the maintenance of a regular heart beat of constant amplitude). The heart was then divided at the atrioventricular junction and the sinus and auricles attached to a glass rod and a light lever by silk threads passed through opposite corners of the auricles. No pins or hooks were used. The auricles were immersed in a measured quantity, usually 100 ml., of fluid through which passed a stream of oxygen bubbles. The lever was suitably weighted to produce a convenient record.

GENERAL OBSERVATIONS.

It was found that the auricle continued to beat throughout the operative procedure, but usually some 15 minutes elapsed before the rhythm and amplitude of the beats were constant. It was possible to maintain a regular beat for upwards of 8 hours provided that oxygen was supplied and that the temperature was not allowed to vary more than a few degrees either way.

Oxygen.

Stoppage of the oxygen bubbling produced a very rapid diminution in the amplitude of the beat, but the rhythm was unaffected until the oxygen lack was extreme. Renewal of the oxygen supply brought about recovery if the lack had not been prolonged beyond the point of cessation of contraction. Since the preparation was found to be so sensitive to oxygen supply, care was taken in subsequent experiments to insure a steady stream of gas from the oxygen cylinder.

Temperature.

Chilling the tissue to 0°C stopped the beat, and as the temperature was slowly raised the beats usually reappeared at about 4°C. From this temperature up to about 12°C. there was a steady increase in amplitude, after which the beats began to diminish, falling off very markedly above 20°C. though they did not entirely disappear until 25°C. Provided that the high temperature was not maintained too long, it was possible to produce converse changes by cooling. These observations may be correlated with the fact that when the surface temperature of the sea rose above 18°C. we had great difficulty in keeping dogfish alive in sea crates at the Station float. When the crates were submerged to a depth where the temperature of the water was approximately 12°C. the fish remained alive.

The rate of beat throughout the whole temperature range increased with increase of temperature. The average rate at 12°C. (at which temperature most of the experiments were made) was 15 beats per minute.

¹ Males were very difficult to obtain at this time of year (July and August).
The Physiological Significance of Urea

pH.

The most constant results were obtained with a perfusion fluid of pH 7.6. As made up with the laboratory distilled water the solution was usually about pH 7.4 and had to be carefully adjusted with dilute sodium bicarbonate. A perfusion fluid more acid than pH 7.4 immediately stopped all beats. On the other hand, the auricles continued to beat fairly well in a solution as alkaline as pH 8.0. Beyond that, however, the preparation stopped in mild rigor.

IMPORTANCE OF UREA IN THE FLUID.

Determinations of the urea content of the auricles while they were under the influence of fluids containing various urea substitutes were made as follows: The preparation was set up as usual in Mines fluid and when beating at a constant amplitude and rate the fluid was exchanged for one in which the urea was entirely replaced by either sucrose or thio-urea. As soon as the first irregularities of rhythm began to appear the auricles were taken down, pressed between dry filter paper, weighed, and cut up in 5 per cent. trichloracetic acid. Urea determinations were then made by the urease and aeration method. The whole procedure gave an accuracy of about ± 5 per cent.

The results of these analyses are given in Table I.

Table I.

<table>
<thead>
<tr>
<th>Fish No.</th>
<th>Urea %</th>
<th>Fluid</th>
<th>Average</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>1970</td>
<td>Urea</td>
<td>1980</td>
<td>Normal</td>
</tr>
<tr>
<td>35</td>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>2010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>1930</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>1220</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>1010</td>
<td>Sucrose</td>
<td>1170*</td>
<td>59 %</td>
</tr>
<tr>
<td>38</td>
<td>1550</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>520*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>1700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1640</td>
<td>Thio-urea</td>
<td>1640</td>
<td>82 %</td>
</tr>
<tr>
<td>42</td>
<td>1540</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>1550</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>1740</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>1680</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The last analysis of this group has been omitted from the average, as it is so low as to suggest some large loss of nitrogen during the analysis.

Substitution of sucrose for urea. An attempt to lower the urea content of the fluid with the least possible alteration in the physical properties was made by preparing fluid of similar salt content with an equimolecular quantity of sucrose instead of the urea and using this to dilute the original fluid in the desired proportions. In a
typical curve obtained by halving the urea content of the fluid, the first point noted is an immediate slight increase in amplitude (amounting to about 10 per cent. and lasting for about 25 beats). This occurs in every case and is reported by Mines, but we have no explanation to offer for it. This increase is followed by a progressive decrease culminating in the development of irregularities of rhythm and stoppage. In the case of sucrose substitution these irregularities usually take the form of missed beats. Analyses (see Table I) show that these irregularities arise when the urea content of the muscle is diminished to about 59 per cent. of the normal. In every case replacement of the normal fluid after the stoppage produced a return to the normal rhythm and a recovery of amplitude to about 90 per cent. of the original.

**Sodium sulphate.** If 50 per cent. of the urea be replaced by an iso-osmotic concentration of sodium sulphate, the primary increase is absent, and the diminution in amplitude is immediate and at first rapid, slowing off so as to produce a somewhat hyperbolic curve, while the rhythm is unaffected. The recovery which does not seem to be so complete as that after sucrose, is also rapid at first and falls off asymptotically.

**Thio-urea.** In the case of substitution of urea by thio-urea, although there is no primary increase in amplitude the diminution closely resembles that in the case of sucrose, but the curve differs in that the onset or irregularity is much earlier. Frequently there occurred an alternation of large and small beats. Analyses made at this stage (see Table I) show a diminution in the urea content of the muscle of only 18 per cent. In only about half of such cases were we able to restore the rhythm and in these the amplitude never recovered to more than 70 per cent. of its original level.

The close chemical connection between urea and ammonium carbonate suggested to us that this latter might make an interesting substitute, but attempts in this direction were abandoned owing to the difficulty of adequately buffering so strong an alkali without disturbing the osmotic equilibrium.

**DISCUSSION.**

Since in every case a diminution in the urea content was followed by diminution in amplitude from which, generally speaking, recovery could be produced by replacing the urea, it seems likely that this decreased beat is the result of the decreased urea rather than of the presence of the substitutes. The similar effect produced by three widely different substitutes is strong evidence against the obvious possibility that the urea is necessary solely for its osmotic effect, but we do not consider that the evidence we have gives any further indications as to the nature of the process involved.

The primary increase in amplitude which we observed only with sucrose is particularly interesting in view of the relatively inert character of this sugar.

In the case of the thio-urea we are probably dealing with a duplex phenomenon, the diminution of amplitude due to urea lack being superimposed upon a positive
toxic action of thio-urea upon the rhythm. We have described this latter effect as toxic, since it is effective in fairly small concentrations and its effect tends to be permanent. We feel that this action is probably principally on the pacemaker in the sinus rather than on cardiac muscle.

This research is the result of work on upwards of sixty fish, and was conducted at the Pacific Biological Station at Nanaimo, B.C., to the director of which, Dr W. A. Clemens, we wish to offer our thanks for the facilities he placed at our disposal. We also wish to thank Dr Allen of the Marine Biological Station at Plymouth, England, for the facilities extended to one of us (E. O.) during that part of the work which was carried out on rays.

SUMMARY.

The optimum conditions for the beating of the excised dogfish auricle have been investigated.

The substitution of urea in the surrounding medium by sucrose, thio-urea, or sodium sulphate in iso-osmotic concentrations will not maintain the beat.

Thio-urea has a toxic effect on the rhythm.

REFERENCE.