The Passage of Urea and Creatinine across the Placenta of the Pig

by E. Zweymüller,1 E. M. Widdowson,2 and R. A. McCance2

From the Medical Research Council Department of Experimental Medicine,
University of Cambridge

WITH ONE PLATE

It is generally considered that urea diffuses freely across the placental barrier and that creatinine does so too (Needham, 1931), and that the concentration of both compounds is the same in foetal and maternal plasma. Alexander, Nixon, Widdas, & Wohlzogen (1958), however, found that the concentration of creatinine was higher in the serum of the foetal lamb than it was in the maternal serum.

TABLE 1

<table>
<thead>
<tr>
<th>Sow 1</th>
<th>Serum creatinine (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglet a</td>
<td>2-35</td>
</tr>
<tr>
<td></td>
<td>4-60</td>
</tr>
<tr>
<td></td>
<td>7-85</td>
</tr>
<tr>
<td>Piglet b</td>
<td>3-72</td>
</tr>
<tr>
<td></td>
<td>3-90</td>
</tr>
<tr>
<td>Piglet c</td>
<td>5-50</td>
</tr>
<tr>
<td>Piglet d</td>
<td></td>
</tr>
<tr>
<td>Piglet e</td>
<td></td>
</tr>
</tbody>
</table>

The authors made no specific comment upon this. McCance & Widdowson (1953) discussed the difficulties which would arise if all the chromogens reacting with alkaline picrate in human cord serum were accepted as creatinine, and in work still to be published these two authors found that the concentration of creatinine 'chromogens' in the serum of the foetal pig at term was always higher than in the maternal serum, although the concentrations of urea were the same.

1 Travelling Fellow of the Bundesministerium für Unterricht, Österreich. Present address: Universitäts-Kinderklinik, Vienna, Austria.
2 Address: Medical Research Council Department of Experimental Medicine, University of Cambridge, Tennis Court Rd., Cambridge, U.K.

Needham (1931) gave no information about this species in his review. McCance & Widdowson further observed that, while the concentration of urea in the serum of the suckled new-born piglet rose in the first few days of life, the concentration of creatinine chromogens fell. The nature and extent of these changes are illustrated in Tables 1 and 2. The figures in Table 2 are the averages for two piglets in each litter. A rise in serum urea is normal at this time of life in many species, including man (McCance & Widdowson, 1947; McCance & Otley, 1951; Boylan, Colbourn, & McCance, 1958; Joppich & Wolf, 1958), but the creatinine chromogens have not been studied to the same extent. Nevertheless, Boylan, Colbourn, & McCance (1958) found relationships at and after birth in the guinea-pig similar to those shown for the pig in Table 2. They did not, however, find concentrations of creatinine in the sera of foetal guinea-pigs to be higher than in those of the mother.

These observations led to the present study in pigs of the chromogens in the foetal and maternal plasma which react with picrate in alkaline solution. The need for such an investigation was suggested also by the finding of Boylan et al. (1958) that the concentration of creatinine chromogens in the urine of the foetal guinea-pig near term was sometimes so high that it exceeded the concentration of urea.

**METHODS**

Most of the animals were reared on the laboratory farm and have been described before (McCance & Widdowson, 1956), but two litters of pure bred ‘Large Whites’ were investigated through the co-operation of the staff at the Agricultural Research Council’s farm at Babraham. Blood was removed from the piglets at birth by heart puncture or by snipping the umbilical cord; the serum was separated within 1–2 hours and stored at $-20^\circ$C. The sow was bled from the ear during the early stages of labour or while farrowing and her blood treated in the same way. The figures given in this paper refer only to blood removed during farrowing, since there appears to be a small rise in the concen-
tration both of creatinine and urea between the onset of labour and farrowing.

The serum was prepared for chromatography by passing it through an ultrafiltration shell (Membranfiltergesellschaft-Sartorius A.G., Göttingen) using a suction pressure of about 600 mm. Hg., and the salts were removed from the ultrafiltrate by the technique described by Dent (1951). 0.5 ml. of the product were submitted to one-dimensional descending chromatography in n-butanol-acetic acid-water (60:15:25) for 22 hours at 19 ± 1° C. The papers (Whatman No. 1) were dried at room temperature and sprayed with a mixture of 10 per cent. NaOH in water (1 part) and saturated picric acid (5 parts).

The concentrations of creatinine in serum were determined by the method described by Hawk, Oser, & Summerson (1954). Other substances chemically determined have been urea (Lee & Widdowson, 1937), fructose (Bacon & Bell, 1948), and pyruvic acid (Friedemann & Haugen, 1943).

RESULTS

Chromatographic

A few minutes after spraying the papers an orange spot with an Rf value of 0.51 began to develop. This was often visibly more intense in the serum of the piglet than in that of the sow (Plate, fig. A 2, 3). It was identified as creatinine and did not fade for many days in the absence of light. An hour or so after spraying a browner spot with an Rf value of 0.26 slowly began to develop in piglet sera. This continued to become more intense for at least a week and was identified as fructose by its Rf value and its specific colour reactions on other papers. By this time a weaker spot with a similar colour and an Rf value of 0.21 had appeared in the maternal and foetal sera and was found to be glucose. Photographs of these spots 3 days and one week after spraying are shown in figs. A 2, 3 and B 2, 3 of the Plate. The colours produced by 15 μg. of creatinine, 180 μg. of fructose, and 120 μg. of glucose are also shown in figs. A 4 and B 4, and figs. A 5 and B 5 give the reactions of a human cord serum to which 120 μg. of fructose had been added. Fructose was not detected in the serum of the sow. No chromogens other than creatinine and fructose were found in piglet serum treated in the way described. It is not easy to separate glycocyanidine from creatinine in secondary butanol solvents (Maw, 1948), but this substance is not generally considered to be a biological precursor of creatinine and it has an Rf value sufficiently different from that of creatinine to make a band of colour rather than a spot when present in the same solution. It also reacts more slowly. It is, therefore, not considered likely to have been present in appreciable amounts.

Pyruvic acid was not detected in either foetal or maternal sera although it could be determined chemically in both (see below). This was presumably because it was oxidized on the paper, or during the deproteinization and desalting of the sera in preparation for chromatography. Plate, figs. A 1 and B 1 show the spot produced by 25 μg. of Na pyruvate in water. Koštíř & Rábek (1950) obtained
a spot on their chromatograms by the addition of 6 \( \mu \text{g.} \), but Maw (1948) found it necessary to add 100 \( \mu \text{g.} \).

**Chemical**

Table 3 shows the concentrations of creatinine chromogens, fructose, and pyruvic acid in the serum of four piglets, two from each of two litters, within a few moments of birth and again when they were 24 hours old. The serum of both sows contained approximately 2.4 mg. creatinine chromogens, 2 mg. fructose, and 2 mg. pyruvic acid per 100 ml. The piglet's serum, therefore, had a considerably higher concentration of creatinine chromogens than that of the sow, but within 24 hours the figures were at the adult level. It is probable that some of the figures for serum creatinine in the new-born pig shown in Table 2 were already lower than they had been at the moment of birth, for some of the samples were collected by heart-puncture an hour or more after the animals were born. The

<table>
<thead>
<tr>
<th>Piglet</th>
<th>Creatinine (mg./100 ml.)</th>
<th>Fructose (mg./100 ml.)</th>
<th>Pyruvic acid (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth 1</td>
<td>4.02</td>
<td>49.3</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>3.35</td>
<td>34.2</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>7.42</td>
<td>40.2</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>3.38</td>
<td>49.8</td>
<td>2.7</td>
</tr>
<tr>
<td>24 hours old 1</td>
<td>1.63</td>
<td>3.3</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>2.13</td>
<td>2.5</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>2.20</td>
<td>2.7</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>2.54</td>
<td>2.1</td>
<td>4.2</td>
</tr>
</tbody>
</table>

**Table 4**

*The effect of pyruvic acid and fructose on the colour produced by alkaline picrate*

<table>
<thead>
<tr>
<th>Serum from day-old piglet alone</th>
<th>Apparent creatinine (mg. per 100 ml.)</th>
<th>Difference due to pyruvic acid or fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum + pyruvic acid 2 mg. per 100 ml.</td>
<td>1.80</td>
<td>—</td>
</tr>
<tr>
<td>Serum + fructose 50 mg. per 100 ml.</td>
<td>2.03</td>
<td>0.23</td>
</tr>
<tr>
<td>Serum + pyruvic acid 2 mg. + fructose 50 mg. per 100 ml.</td>
<td>1.94</td>
<td>0.14</td>
</tr>
<tr>
<td>Creatinine solution alone</td>
<td>2.14</td>
<td>0.34</td>
</tr>
<tr>
<td>Creatinine + pyruvic acid 2 mg. per 100 ml.</td>
<td>3.00</td>
<td>—</td>
</tr>
<tr>
<td>Creatinine + fructose 50 mg. per 100 ml.</td>
<td>3.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Creatinine + pyruvic acid 2 mg. + fructose 50 mg. per 100 ml.</td>
<td>3.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Creatinine + pyruvic acid 2 mg. + fructose 50 mg. per 100 ml.</td>
<td>3.33</td>
<td>0.33</td>
</tr>
</tbody>
</table>
amounts of pyruvic acid found in the serum of the piglet and sow were about the same, and the concentration of pyruvate tended to rise during the first 24 hours of life. The results for fructose confirm Goodwin’s (1957) observation that the concentration in serum is high at birth but falls rapidly afterwards.

Table 4 shows the effect of adding physiological concentrations of pyruvic acid and fructose on the colour produced by creatinine in alkaline sodium picrate. The effect of pyruvic acid was much smaller than that observed by Košťíř & Sonka (1952) and it is clear that neither substance could have accounted for the difference between the ‘creatinine’ values of piglet and sow sera, or for the fall in these values in piglet sera after birth. Glycocyamine gave no colour at all with alkaline sodium picrate.

DISCUSSION

The evidence presented above leads to the conclusion that there is normally a greater percentage of creatinine in the serum of the foetal pig at term than there is in the maternal serum. The same is probably true of the sheep (Alexander, Nixon, Widdas, & Wohlzogen, 1958). This animal is also an ungulate, but it has a very different placenta, and it looks as though the small molecule of creatinine must be added to others, such as fructose and some of the amino-acids, which are not equally distributed on either side of the placenta in all mammals. There is nothing new in the finding that some cells in the body may be much less ‘permeable’ to creatinine than to urea, for such cells must exist in the kidney, but the physico-chemical basis is unknown. One further point of possible significance has emerged from this investigation, and that is that, within one and the same litter, one piglet may have a creatinine value in its serum not much higher than that of the sow, whereas another may have a value over three times as great (Table 1). There may be other great differences between the foetal fluids and membranes of litter-mate piglets (Wislocki, 1935), but no explanation has been forthcoming.

SUMMARY

1. The concentration of ‘creatinine chromogens’ in the serum of the foetal pig at term may be over three times that in the maternal serum. The concentrations of urea were always about the same.

2. The values for these chromogens in piglet sera fell to the adult level in the day following birth.

3. Fructose was the only chromogen other than creatinine found in piglet serum by chromatography.

4. Pyruvic acid was not the cause of the difference.

5. It is concluded, therefore, that the chromogens were mostly true creatinine, and that the pig’s placenta is less ‘permeable’ to it than to urea.
ACKNOWLEDGEMENTS

The authors are very grateful to Professor C. E. Dent for his help over the chromatography, and to Dr. L. E. Hill for the determination of pyruvic acid. The co-operation of Drs. L. E. Mount and J. S. Perry over the supply of some of the material has been much appreciated.

REFERENCES


EXPLANATION OF PLATE

Key: c = creatinine; p = pyruvic acid; f = fructose; g = glucose

Fig. A. Descending paper chromatogram 3 days after spraying with alkaline picrate. A' is a drawing providing a key to the identification of spots.

2. Sow's serum (0.5 ml. desalted ultrafiltrate). Weak creatinine spot.
3. Piglet serum (0.5 ml. desalted ultrafiltrate). Distinct creatinine and fructose spots.
5. Human cord serum (0.5 ml. desalted ultrafiltrate) with added fructose (120 µg.). Weak creatinine spot, distinct fructose spot, and very weak glucose spot.
Fig. B. The same paper chromatogram as shown in fig. A, 7 days after spraying with alkaline picrate. B' is a drawing providing a key to identification of spots.

1. Pyruvate spot barely visible.
2. Distinct glucose spot.
3. Distinct fructose and weak glucose spots.
4. Distinct fructose and weak glucose spots.
5. Distinct fructose and glucose spots.

(Manuscript received 28: x: 58)