The Role of the Developing Kidney in the Maintenance of Internal Stability

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Within recent years we have begun to realise three things. First, the stability of the internal environment is as important before birth as after it. Second, the fetus does a lot to control its own internal environment. Third, the kidney contributes more in some ways and less in others to internal stability in the newborn period than was at one time supposed.

WATER AND ELECTROLYTES

The allantoic and amniotic fluids must be considered part of the fetus for, although they are outside its body, they are inside its membranes. In the sheep and pig the allantoic sac is at first much larger than the amniotic sac and highly functional in both species. The products of the fetal kidney, mesonephros and then metanephros, pass first into the allantoic sac through the urachus, and later into the amniotic sac through the ureter. Nevertheless, the genesis of these fluids, their composition and function in various species, is difficult to investigate, and, although the two fluids have now been studied serially over long periods of time in conscious ewes, together with the fetal and maternal plasmas (Mellor and Slater, 1971), most of these problems remain unsolved. In man, the allantoic sac is vestigial and unimportant.

Investigation of fetal renal function is difficult, but studies have been made in rabbits, guinea-pigs, pigs and sheep. The urines that premature and full-term human infants pass at birth have also been carefully investigated. The volumes of urines passed in mid-gestation are large by adult standards (Perry and Stanier, 1962). The subject has been reviewed by Alexander and Nixon (1961), McCance (1964), and others. In the sheep, Alexander and her colleagues found the glomerular filtration rates (GFR) at 61 days gestation to exceed those of adults per unit of body weight, and only 66 per cent of the filtered fluid was reabsorbed. Such filtration rates and volumes of urine are more than enough to account for the amount of the allantoic fluid and there is no doubt that in sheep and pig the kidney contributes largely, but not entirely, to its volume and composition (McCance and Dickerson, 1957). The mechanisms that control its volume are unknown. Fetal urine is charac-

teristically hypotonic, and, at this stage, may be extremely so, and contains no glucose (Alexander and Nixon, 1963), progressively less sodium, and sometimes only traces of phosphates. Some of the sodium may be reabsorbed from the renal pelvis (Stanier, 1971).

The glomerular filtration rates and the volumes of urine passed by the fetal kidney into the amniotic sac decrease greatly as term approaches and the nitrogenous excreta raise the osmolality of the urine, particularly in some species. The volume of the fluid in this sac is important for the welfare of the human fetus and it appears to be regulated by an interesting biological cycle. The volume of urine passed into the sac is balanced by a corresponding amount swallowed by the fetus, absorbed from the alimentary tract and so returned to the fetal circulation for redistribution to the placenta and kidney. The volume of fluid circulating in this way may well exceed one litre a day, and much higher estimates have been made, but what the feedback mechanism between these two functions is, and how the volumes passed by the kidney and imbibed by the fetus are regulated is quite unknown.

If the kidneys do not function for any reason there is little or no amniotic fluid surrounding the fetus and the child is born with anatomical deformities caused by pressure of the uterine wall (Potter, 1961), and if the fetus cannot swallow due, say, to atresia of the oesophagus, polyhydramnios results.

No two species are born at the same stage of development. This makes a considerable difference to the composition of the body at birth and to the anatomical and physiological maturity of the kidney. These matters have been investigated and reviewed in several species (McCance, 1950; McCance and Widdowson, 1952, 1954, 1960; Vesterdal, 1961). The percentage of water and the percentage of the extracellular fluids in the body of the fetus fall throughout gestation in all species. The place of extracellular fluid is taken by the developing cells and the proteins and minerals that fill them or that they shed into the extracellular space, and also by calcifying material in the bones. In no species are these processes complete at the time of birth, but they are much more nearly so in the guinea-pig than they are in the rat, and the human baby stands in an intermediate position. In a baby, the serum at birth has the same osmolar concentration as that of an adult and it contains the same percentage of sodium. The total CO_2 is lower and the chloride correspondingly higher, indicating that a mild metabolic acidosis is the normal steady state at this age. The percentages of calcium and magnesium may be the same as the mother's or slightly higher, as they have been in fetal life. The percentage of potassium tends to be higher, possibly due to anoxia during birth, and the percentage of phosphorus consistently higher. It is not known to what extent the kidney has been regulating the composition and internal

stability of the body fluids before birth for, in the functional absence of the kidneys, both are usually normal when the baby is born.

If the newborn baby is reared on its mother's milk, only minor changes take place in the composition of the serum after birth. The percentage of urea may rise for a few days, but the electrolytes remain very stable.

We know from recent work (Widdowson *et al.*, 1972) that the human kidney at birth contains only some 13 per cent as many cells as it will in adult life and these cells are still only about 70 per cent of their ultimate size. Physiologically the organ is characterised by—

1. A lower, and a more labile GFR than that of an adult, whether the basis of comparison is body weight or surface area, and a considerably smaller capacity to excrete p-amino hippuric acid (PAH). The latter may be due to incomplete extraction of the PAH from the blood passing the tubules or to a small supply of blood. Both the GFR and the excretion of PAH improve during the first few weeks and months after birth, but may not be mature till near the end of the first year.

2. A low osmolar concentration in the urine of the baby in spite of its normally small fluid intake during the first days of life. This may be due to the anatomical immaturity of the medulla, and the osmolar gradient from cortex to medulla is certainly small (Fleischaker *et al.*, 1960; Strauss, 1960; Yaffe and Anders, 1960; Trimble, 1970), but by making the concentration test in a way likely to increase the gradient (Pratt *et al.*, 1948; Edelmann *et al.*, 1960) this function can be shown to be mature even in premature babies about a month after birth. Till then one would expect an osmotic diuresis to be produced by a relatively small solute load of a sodium salt; but this is not so.

3. A very limited capacity to excrete water administered in excess (Ames, 1953); hence the well-known risk of water intoxication developing during treatment. The ability to lower the osmolality of the urine appears relatively soon but, probably owing to the low GFR and the small amount of solutes being excreted, the capacity to excrete water in large volumes does not 'mature' until later (Barnett *et al.*, 1952; Theodenius *et al.*, 1971).

4. A tendency to reabsorb nearly all the sodium filtered off in the glomerulus and to absorb too much of it even when sodium salts have been administered and the concentration in the serum is rising (Theodenius *et al.*, 1971). This peculiarity may be part of the mechanism for maintaining the large volume of the extracellular fluids, but, if not understood, the administration of sodium can easily be overdone (DeGenaro and Nyham, 1971). The kidney can be regarded as though it were under the influence of too much aldosterone.

5. A very poor response to the administration of substances that would provoke a violent osmotic diuresis in an adult.

6. A tendency to reabsorb nearly all the phosphates from the glomerular filtrate as it passes down the tubules. This may be thought of as glomerulo-tubular imbalance due to a deficiency of, or a failure of, the kidney to respond to parathormone. This is the immaturity that may lead to neonatal tetany if the intake of phosphate is set too high (Craig and Buchanan, 1958; Dundon *et al.*, 1967; Dundon *et al.*, 1968; Watney *et al.*, 1971), but the causation may be much more complicated (McCrory *et al.*, 1952; Anast, 1969; Chiswick, 1971; Orme *et al.*, 1971).

7. A poor excretion of H ions in spite of the low total CO_2 in the plasma and other signs of a mild non-respiratory acidosis (Hatemi and McCance, 1961; Albert and Winters, 1966; Edelmann *et al.*, 1967; Kildeberg, 1968; Kerpel-Fronius *et al.*, 1970; Dicker and Shirley, 1970, 1971). This may be thought of as a low H ion clearance (Elkinton *et al.*, 1960) and is due to:

(*a*) a failure to lower the pH of the urine, and

(*b*) the scarcity of phosphates and other buffer substances in the urine preventing normal amounts of H ions being excreted at a relatively high pH.

In short, the kidney of the newly born must be regarded as an inefficient organ by adult standards. Its ability to excrete many substances is small owing to its low GFR, and its other functions are too fixed to allow it to respond in a sufficiently flexible way to the demands that would be made upon it were it an adult.

How then is the stability of the body fluids maintained? The answer is by the integration of three functions of which the composition of the food provided by nature and the voracious demands of the growing tissues for the greater part of it are quite as important as the kidney and may be more important in some animals that are very immature at birth (McCance and Widdowson, 1957). This integration is usually so perfect that only very small residues are left for the kidney to excrete. The incorporation of calcium into bone is an exception to this, for it involves the liberation of H ions which have to be excreted by the kidney (Stalder and Egli, 1964; Kildeberg, 1968). It follows from the generalisation that any departure from the food provided by nature should probably be avoided, if possible, and certainly never be made without due care—provided the situation is a normal one. This is not necessarily so, however, and a very premature infant is not in a normal situation and may require food that cannot be provided by its mother's milk so well as it would have been by the placenta. This is a challenge I hope we can meet, if we cannot prevent.

RENIN-ANGIOTENSIN-ALDOSTERONE

Apart from its classical function of excreting the soluble waste products of the body and by so doing helping to regulate the composition of the body fluids it has gradually become apparent that the kidney has other functions. One is the production of renin in the juxtaglomerular apparatus (Cook, 1963) and its release in response to a fall in the filling pressure or volume of the arterial side of the circulation (Tobian, 1967; Vander, 1967) or a change in the concentration of sodium in the tubules themselves (Thurau *et al.*, 1967). Renin is a protein, an enzyme, which splits a decapeptide off one of the plasma proteins, with the formation of angiotensin 1, and the removal of a histidine-leucine dipeptide from one end of this chain produces an octapeptide with similar properties (angiotensin 2). These peptides act on various sites, among them the adrenal, where they promote the liberation of aldosterone (Skeggs *et al.*, 1967; Balint, 1969). The effects vary to some extent with the species (Peart, 1967) but the general function of the peptides and/or aldosterone is to raise the peripheral resistance, promote the reabsorption of sodium from the tubules of the kidney and the lumen of the gut (Munday *et al.*, 1969; Edmonds and Marriott, 1970) and probably to encourage the intake of fluid by mouth (Gutman and Benzakein, 1969; Epstein *et al.*, 1970; Andersson and Eriksson, 1971). Aldosterone co-operates with the anti-diuretic hormone in raising the osmolar concentration of the urine during dehydration (Crabbé, 1962) and the release of ADH is linked by feedback mechanisms with the renin-angiotensin system (Bonjour and Malvin, 1970; Tagawa *et al.*, 1971).

Renin, or what is probably renin, can be recognised by its staining reactions, and granules of it are usually present in the juxtaglomerular and macula densa cells. Granules, presumably functional, have been seen in the juxtaglomerular cells of the human fetus from the 17th week of gestation, and in younger fetuses less characteristic cells containing similar granules can be found scattered through the renal cortex (Ljungqvist *et al.*, 1966). Much the same holds for the fetal pig (Bing and Kazimierczak, 1963, 1964; Sutherland and Hartroft, 1964) and it appears that renin may be found even in the pig's mesonephros and without visible granulation of the cells. Rats and mice are born in a much less mature state than the pig or man, and in rats Alexander and Grimason (1967) found no granules earlier than 12–14 days after birth and confirmed this with functional tests, but Albrecht (1968) found granules scattered diffusely through the kidney at birth and studied the effect of adding sodium or potassium to the maternal diet during pregnancy. In mice, granules have been found before birth (Kaylor and Carter, 1967).

One may suppose, therefore, that this renin-angiotensin-aldosterone mechanism operates before birth and in man is partly responsible for the

autonomy of the fetus and the characteristics of its urine and fluids. There must be some vicarious system of control, as it is possible for a fetus to be born without kidneys and still have a relatively normal serum chemistry. It is certain, however, that the system must be fully operative at birth or become so very soon afterwards in man, for, if enzyme defects prevent the normal production of aldosterone, the kidney of the infant fails after a few days to reabsorb the sodium required to maintain the concentration, volume and stability of the body fluids (Serini *et al.*, 1962; Visser *et al.*, 1964, 1966 a,b). Furthermore, the fact that premature infants can be reared shows that the enzyme system must almost certainly have been completed and be ready to become operative months before the normal age at which a baby is born.

ERYTHROPOIETIN

The stability of the levels of circulating haemoglobin and erythrocytes and their increase in response to anoxia or high altitudes has been recognised for years. It was originally supposed that the anoxia was acting as a direct stimulus to the bone marrow. In 1906, however, Carnot and Deflandre suggested that the marrow was likely to be under the remote control of some other organ which liberated a hormone or other humoral agent. It was only in 1957 that the kidney was recognised as being concerned (Jacobson *et al.*, 1957; Thorling, 1969; Krantz and Jacobson, 1970).

The kidney, it seems, responds to hypoxia by secreting a substance, erythropoietin (Kuratowska *et al.*, 1961; Naets, 1969; Krantz, 1970), and, so far as adults are concerned, it may be said that in rodents and dogs the kidney is probably the only site of its production. It is also by far the most important site in man, but the possibility of a small alternative site, or another stimulating agent (Shaldon *et al.*, 1971), has not been definitely excluded. This minor source, if it exists, may become more active after nephrectomy (Gordon *et al.*, 1967). The main sensing cells and site of production within the kidney are likely to lie in the juxtaglomerular apparatus (Hirashima and Takaku, 1962; Jepson and McGarry, 1968; Hartroft *et al.*, 1969), but the falling gradient of oxygen from the cortex to the medulla makes it worth considering the cells in the medulla as likely to be those most sensitive to the normal fluctuations of circulating oxygen and possibly a site of erythropoietin formation.

Erythropoietin is a glycoprotein with a molecular weight of about $\pm$ 65,000 and may be an enzyme on the lines of renin. It is excreted in the urine and attempts to isolate it have shown that minute amounts may be intensely active (Gordon *et al.*, 1967).

The position in the fetus and new born is interesting and varies with the maturity of the species of animal at birth. In the developing fetus erythrocytes

are first produced in the yolk sac and it is generally conceded that this process proceeds at its maximum rate without the stimulus of erythropoietin. The next sites of red cell formation are the liver and spleen. In rodents this phase lasts till birth, and in mice it is stimulated by erythropoietin (Rifkind *et al.*, 1969) on the 11th to the 14th days of gestation, but seemingly not later (Cole and Paul, 1966). It cannot be assumed at present that this erythropoietin is being produced by the kidney, and in rats this phase is said to be independent of it (Lucarelli *et al.*, 1968). The production of red cells is becoming active in the marrow about the time of birth in this species, and removal of the kidneys five days later affects it less than on the 15th day after birth (Stohlman, 1967; Carmena *et al.*, 1968). This argues that the kidney is only acquiring control of the process about this time whereas it becomes complete later in the life of rats. Guinea-pigs are one of the most matrue animals at birth and in them the adult situation has been reached by the time birth takes place (Lucarelli *et al.*, 1968).

In man, erythropoietin has been identified in cord blood from the 30th week of gestation onwards (Halvorsen and Finne, 1967). It has also been found in amniotic fluid, as one might expect, and its concentration in both these fluids increases in the presence of poor tissue oxygenation whatever the cause (Finne, 1968; Krantz, 1970). Its activity disappears for a time from the body fluids of a normal infant soon after birth when the formation of red cells normally falls, but this may be the work of an inhibitor (Lewis *et al.*, 1969; Lindemann, 1970; Jonxis, 1971; Skjaelaaen and Halvorsen, 1971), and absence of the normal tissue and plasma inhibitor has been invoked as the cause of polycythemia rubra vera (Krantz, 1968).

The production of erythropoietin by the human fetus appears to be regulated autonomously, regardless of the O_2 tension of the mother, and this has been demonstrated experimentally in the fetal lamb (Zanjani *et al.*, 1969). There is only one difficulty about this. I am not aware that profound anaemia is a feature of renal agenesis in man and there may be a site of erythropoietin other than the kidney before birth which can deputise more completely for it than it can do later in life.

THE METABOLISM OF VITAMIN D

Recently, the kidney has been found to be the only organ in the body of an adult capable of completing the conversion of cholecalciferol (vitamin D^3) to its active metabolite, 1, 25 dihydroxy-cholecalciferol (Fraser and Kodicek, 1970; Lawson *et al.*, 1971). Rickets is common enough in early life and has been demonstrated in fetal life, but this is due to a deficiency of cholecalciferol itself since its administration will cure the rickets, and we have no evidence

at present that the kidney, even of a small premature infant, is not fully mature in its ability to affect this conversion. The placenta has not been tested. It should be relatively easy to determine when this enzyme system appears in the developing kidney.

GENERAL CONCLUSIONS

1. The vital functions of the kidney change at birth and with the maturity of the developing organism.
2. Many of its functions after birth may be more or less dormant till birth.
3. The kidney is able to take over all the functions required of it after birth even if the latter is very premature.
4. The functional efficiency of the kidney depends upon the time since birth rather than the maturity of the baby at birth.
5. In spite of great progress in the study of renin and erythropoietin, the last twenty years has seen little advance in our knowledge of what sets the pattern for the function of the kidney as an organ of excretion before birth and what brings about its rapid development as an organ of excretion after birth.

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