Local and general responses to injury in the newborn rabbit

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Summary

Some physiological responses to experimental bilateral hind limb ischaemia have been studied and compared in neonatal and young rabbits.

The fluid loss into the injured limbs after removal of the tourniquets has been expressed as a percentage of the plasma volume and of the total body water at the different ages.

The reactivity of skin blood vessels to a variety of known mediators of an inflammatory response has been investigated using labelling techniques.

THE EFFECTS of the environment on the responses to injury have been summarized by Dr Stoner at this symposium. As all this work has been done on the adult animal, the question arose: 'Does the age of the animal in any way modify the pattern of response to injury seen in the adult?' A MEDLARS computer literature search revealed a startling lack of information on the subject. The few relevant papers unearthed dealt with clinical observations, similar to those of Professor Wilkinson (1965) at the Institute of Child Health and Sukarochana (Sukarochana *et al.*, 1965) in Pittsburgh on babies and children after the trauma of surgery. This led us to further work by Wilkinson (1965) and by Sutherland & Batchelor (1965) on the responses of the human infant to burns. Although it is difficult to make generalizations, it did become apparent that the response of the neonate to injury, as measured by changes in urine and plasma levels of nitrogen and electrolytes does differ from that seen in the adult. We also became aware of a belief among surgeons that the newborn is more resistant to injury than the adult. There certainly seems to be very little experimental foundation for this belief and we decided to look at the problem rather more closely.

The experimental model we are using is bilateral hind limb ischaemia in the rabbit. The first part of the study was to determine the 48-hr survival of various age-groups of animals after varying periods of ischaemia. It is important to emphasize at this point that to prevent the rabbits becoming hypothermic while away from the nest, they were kept at their thermoneutral temperature (Hull, 1965). Bernard & Hull (1964) have shown that hypothermic

neonatal rabbits are unable to suckle and, as a result, will either die of starvation or be cannibalized by the doe. Complications such as these would obviously make the interpretation of survival experiments impossible.

The survival experiments showed that there was a fall in resistance (expressed as the LD₅₀ periods of ischaemia) from birth until the 11th day of life. Resistance then returned to the 1-day level by 20 days. Could these differences in resistance be explained on the basis that the percentage tissue rendered ischaemic varied with age? Differences such as this certainly did exist, but even when they were taken into account the overall pattern persisted.

During these early experiments, it was noted that the most obvious change after removal of the tourniquets was oedema formation in the injured hind limbs. This was very obvious in the youngest animals where the limbs were very distended and there was frequently oedema above the line of tourniquets, often extending, subcutaneously, into the lower abdominal regions. The oedema formation was very rapid and in the 1-day-old animal the limb doubled in size approximately 30 min after the end of a 4-hr period of ischaemia. This was, however, the extreme case; as one reduced the period of ischaemia the oedema formation also decreased, and as the age of the animal increased, the oedema formation, expressed as a percentage increase in limb volume, decreased.

As Courtice (1946) had shown that the local temperature can affect fluid loss in thermal burns, the greater oedema formation seen in the very young animal might have been a result of the higher environmental temperature during the experiment. To check this, the experiments on the 20-day-old animals were repeated at 35°C instead of 28°C. At the higher temperature there was only a slight increase in oedema formation—certainly not to the levels found in the 1–2-day-old animals.

From these results one does not know the amount of fluid lost into the hind limbs. To determine this: (a) wet/dry weight determinations in control limbs and in limbs taken at the time of maximum oedema, or (b) serial haematocrit determinations were performed. As expected the haematocrit-estimated

values were lower—reflecting the ability of the animals to compensate for the loss of fluid from the circulation by pulling in extravascular fluid from other parts of the body. Taking the wet and dry weight derived values as representing the true values, the LD_{50} fluid loss, expressed in ml/100 g body weight could readily be estimated. These results showed that the younger the animal, the greater the LD_{50} fluid loss into the hind limbs. At this point differences in fluid physiology between the neonate and the adult had to be taken into account—for example, the water content of the body (Spray & Widdowson, 1950) and the blood volume (Mott, 1965) are greater in the newborn rabbit than in the adult. Perhaps then, although the neonate was losing more fluid/100 g body weight than the adult, the loss expressed as a percentage of body fluid was no different with age. When the LD_{50} fluid loss was expressed as a percentage of plasma volume or as a percentage of total body water, it was still apparent that the younger the animal, the greater the LD_{50} fluid loss.

A clue as to how the newborn rabbit is able to tolerate the greater fluid loss came to light when we were measuring plasma volumes with labelled albumin. In the 1–2-day-old rabbit the measured plasma volumes were very high and after a more detailed examination we concluded that a proportion of the injected albumin was passing rapidly out of the intravascular albumin volume and equilibrating with an extravascular albumin volume. The presence, during the first 48 hr of life, of this extravascular albumin volume and the ease with which it mixes with the plasma could well help the very young rabbit tolerate a large loss of intravascular plasma.

One must also try to explain the greater oedema formation observed in the neonate. When considering the loss of protein-containing fluid from the circulation, the factors concerned are most conveniently divided into three categories. Firstly, those exerting their effects inside the vessel walls, secondly, those operating outside the walls and thirdly, the vessel walls themselves. The first group include the oncotic effects of the plasma proteins, the plasma electrolyte levels and the blood pressure. Preliminary experiments showed that from the 10th to the 40th day of life the plasma sodium levels were significantly lower and the plasma potassium levels significantly higher than at any other age. The osmolality of the plasma showed a significant fall from birth until 12 days after birth—it then rose to the young adult value which was not significantly different from the value in the 1-day-old rabbit. These changes in osmolality were, however, rather irrelevant as it is the protein content of the plasma that controls blood volume homeostasis. The plasma proteins do not behave as ideal solutions and small changes in the

protein concentration result in disproportionately large changes in the osmotic pressure (Landis & Pappenheimer, 1963). We found that the plasma protein concentration was low in the newborn rabbit and that it slowly increased with age—this is in agreement with the results of Clark & Holling (1931) in the puppy. With regard to the blood pressure Mott (1965) has shown that it is low at birth and increases steadily during the first weeks of life until it reaches the adult level.

Considering the factors operating outside the vessel walls, probably the most important is that in the neonate the skin on the limb is much looser fitting than that in the adult and the tissues in the leg are only loosely held together. These two factors would permit a greater oedema formation in the limb of the neonate before the skin became fully distended and acted as a physical barrier to further swelling. Majno (1964) has shown the importance of these factors in oedema formation using the bat's wing. In the wing the skin is very firmly bound to the underlying tissues and inflammatory oedema does not occur. As another example of the influence of the distensibility of tissues on oedema formation Burch (1940) noted that inflammatory oedema readily occurs in the eyelid but only under extreme conditions does it occur over the shin.

Finally the blood vessel walls themselves might well have certain properties which vary with age. The loss of protein-containing fluid from the circulation into the hind-limbs after ischaemic injury must be due to an increase in the permeability of the blood vessel walls. Tissue constituents such as histamine and bradykinin induce changes in the permeability of post-capillary venules and with this in mind a series of intradermal injections of histamine were given to rabbits of different ages. To make any responses easier to follow, pontamine blue was given intravenously immediately before the histamine. In the adult animal the characteristic blue patches at the site of injection were seen, indicating a loss of proteinaceous fluid from the circulation. However, no blueing was found in any animal younger than 28 days.

To check these observations the experiments were repeated using a suspension of colloidal carbon instead of pontamine blue, the basis of the technique being that the leaking vessels are labelled by the deposition of carbon on the basement membrane at the intercellular junctions (Cotran, Suter & Majno, 1967). In the adult rabbit the classical light microscope picture of venular carbon labelling described by Majno was seen; however, no labelling was found in animals less than 1 month old. Majno (1964) and Rocha e Silva (1940) have commented on this peculiarity of the newborn and Matheson, Nierenberg & Greengard (1952) reported that in the newborn

human baby, histamine elicited a flare but no weal when scratched into the skin. In other words in the neonate there is vasodilation but no evidence of any increase in vascular permeability induced by histamine.

In the tourniquet-injured rabbit patchy blueing and signs of carbon-labelling in the hind-limbs could be found at all ages.

The pattern of labelling was what one would expect from indirect or mediator induced leakage, the deposition of carbon being confined to the venular elements of the vascular tree. The vessels appeared to be heavily labelled but there were not many of them in the injured limb. This is very surprising considering the large amount of fluid passing from the circulation. Signs of direct damage to the blood vessels were often seen under the line of the tourniquets. The carbon appeared to be in most of the vessels and gave the appearance of blocking them rather than of being deposited on the basement membrane.

We have, then, a picture of oedema and carbon-labelling in the hind-limb after ischaemic injury at all ages. A mediator producing an increase in vascular permeability is indicated but it is clear that histamine is not the mediator in the newborn. Three other endogenous mediators of increased venular permeability have been used. Bradykinin was disappointing, giving only a very weak response in the newborn although by a fortnight the response was as marked as that seen in the adult. The lymph node permeability factor (Willoughby, Boughton & Schild, 1963) and the rabbit globulin permeability factor (Wilhelm *et al.*, 1958) both gave well defined venular labelling in the 1-day-old animal and merit consideration as mediators of oedema formation in the neonate.

Conclusions

Differences have been found between the adult and the newborn rabbit in both the local and general responses to injury. The oedema formation after a period of bilateral hind limb ischaemia is greater in the neonate than in the adult. This may be because of differences with age in the response to a permeability factor, structural differences in the blood vessel walls, differences in blood and extravascular fluid chemistry or more probably because there is more room for distension in the neonatal limb due to looseness of the skin. Secondly, the neonatal rabbit is able to withstand a greater percentage plasma loss than the older animal. This may be related to the presence of the extravascular albumin pool men-

tioned earlier and to the very large extracellular fluid volume of the skeletal musculature of the newborn mammal reported by Kerpel-Fronius, Nagy & Magyarka (1964). It is also of interest to note the results of Mott (1965) who has shown that rabbits 0-7 days old needed a greater reduction in blood volume to reduce the arterial pressure to 95% of its initial level than did older animals.

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