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BARORECEPTOR REFLEXES IN NEW-BORN RABBITS

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This paper describes an investigation of the baroreceptor reflexes from the carotid sinus and aortic arch in the new-born rabbit. Bauer (1939) concluded from measurement of the heart rate in rabbits that the depressor reflex was not activated during asphyxia until the 30th day from birth, and the carotid sinus reflex until the 40th day. He also stated that 'faradic stimulation of the depressor nerves and carotid sinus regions in rabbits produces a reflex inhibition of the heart as early as the 11th and 14th days, respectively'. Both Bauer (1939) and Barcroft (1946) inferred from these and other observations in Bauer's paper that the threshold pressures for the baroreceptor reflexes were high, 65 mm Hg for the depressor and 80 mm Hg for the carotid sinus reflex; the normal blood pressure of a new-born rabbit did not reach these levels until several weeks from birth. The implication is that in the new-born rabbit the cardiovascular system is not under the control of the baroreceptors unless the blood pressure rises above the threshold levels quoted above. The object of the experiments now to be described was to determine by more direct methods whether the baroreceptor reflexes are functioning in the rabbit during the first few days of life at the low arterial pressure which is then normally present.

METHODS

Observations were made on seventeen adult, nine young (3-weeks-old or more, 0·2–1·0 kg body weight) and seventy-five new-born (0–15 days) rabbits, anaesthetized with pentobarbitone and/or urethane. Of the new-born rabbits fifty-five were given pentobarbitone (10–15 mg/kg intraperitoneally, supplemented with additional pentobarbitone as necessary), and the remainder received urethane (1·2 g/kg intraperitoneally). Older rabbits were given pentobarbitone (20–25 mg/kg intravenously) followed by urethane (1·2 g/kg subcutaneously). The trachea was cannulated. In most experiments rectal temperature was recorded by a thermocouple, and was maintained at $38 \pm 1^\circ$ C by application of external heat as necessary. This was found to be particularly desirable in very young rabbits.

Pressure changes applied to the carotid sinus. In new-born rabbits the left carotid sinus was isolated from the rest of the circulation. The cardiac end of the right or left common carotid artery was cannulated to record systemic blood pressure. The cephalic end of the left common carotid artery was connected by a polyethylene tube to a pressure bottle filled with saline and to a manometer. The left external carotid was tied, together with all branches of the common carotid above the polyethylene tube and the internal carotid distal to the sinus. Static and pulsatile pressures were applied to the carotid sinus. Pulsations were generated by an armature, activated by an electromagnet, which compressed the soft plastic tube connecting the pressure bottle with the carotid sinus, at a frequency which was adjusted to about 180 cycles a minute.

The Valsalva manoeuvre. The endotracheal pressure was raised for short periods of time in the following way. The tracheal cannula was attached to a T-tube through which a constant stream of air was passed from a roller-pump freely into the atmosphere. At any moment this stream of air could be diverted through a glass tube immersed to a depth of 5, 10 or 15 cm in water.

Records of blood pressure and nerve action potentials. Single and multifibre preparations of the aortic depressor, carotid sinus and vagus nerves were made by conventional methods. The action potentials were amplified and displayed on a cathode-ray oscilloscope together with the arterial blood pressure, recorded from a polyethylene catheter in the cardiac end of the right carotid artery by a condenser manometer. When critically damped, the catheter and manometer assembly used for observations on new-born rabbits had a frequency response of 120 c/s. No great difficulty was found in cannulating the carotid arteries of new-born rabbits under a binocular microscope (× 16), with a cannula drawn out from a piece of polyethylene to have an external diameter at the tip of 0·78 mm and an internal diameter of 0·63 mm. Various nerves were stimulated with a constant-current rectangular pulse generator.

RESULTS

Blood pressure and heart rate of rabbits 0–15 *days old*

The mean blood pressure of eight rabbits which were about 12 hr old was 36 mm Hg (Fig. 1). During the next 2 weeks the blood pressure rose by about 3 mm Hg a day. The heart rate also rose during the first few days after birth, from about 250 beats a minute to 330 or more by the fifth day from birth. There was no significant difference between rabbits anaesthetized with urethane or with pentobarbitone.

Electrical stimulation of the depressor and carotid sinus nerves and of the peripheral end of the vagus

Maximal stimulation of the central end of the left depressor nerve of rabbits less than 6 days old, anaesthetized with pentobarbitone, caused a mean fall in blood pressure of 17 ± 2·5 (S.E.) % and in heart rate of 7 ± 1·7 %. An example is shown in Fig. 2. In rabbits of the same age anaesthetized with urethane the mean fall in blood pressure (0·6 ± 0·6 %) and heart rate (2 ± 1 %) were negligible. In rabbits 6–15 days old under urethane anaesthesia stimulation of the left depressor nerve caused a significant fall in blood pressure (17 ± 4 %) and in heart rate (8·5 ± 3 %). Younger rabbits therefore seem very susceptible to urethane anaesthesia.

Stimulation of the central end of the carotid sinus nerve of rabbits less than 6 days old caused two types of response similar to those described by Douglas & Schaumann (1956) in adult cats. With stimuli of 3–5 mA (at a frequency of 13/sec and duration 5·4 msec) there was an average rise of blood pressure of nearly 30 %, a large increase in respiratory rate and some slowing of the heart. When the current was increased to 7·5 mA there was sometimes a small fall in blood pressure and heart rate (7–10%), but respiratory rate still doubled. It was thus sometimes possible to convert a pressor to a depressor response by increasing the strength of the stimulus. In contrast to the effects of depressor nerve stimulation, the response was not affected by the use of urethane in place of pentobarbitone.

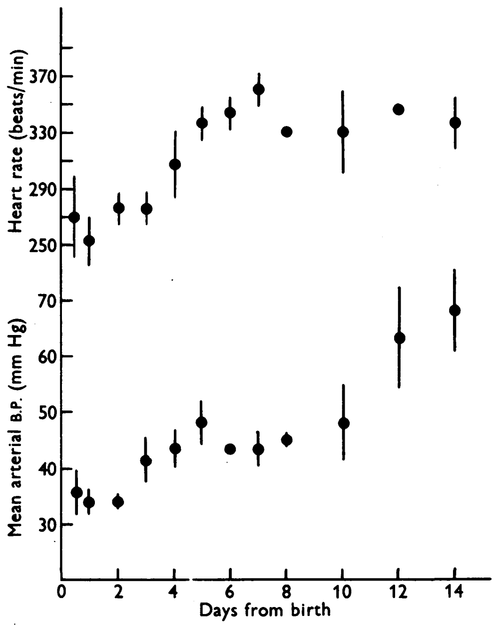

Fig. 1. Observations of heart rate (above) and mean arterial blood pressure (below) from sixty-two rabbits to show the changes during the first 2 weeks of life. The vertical lines represent the standard error of the mean.

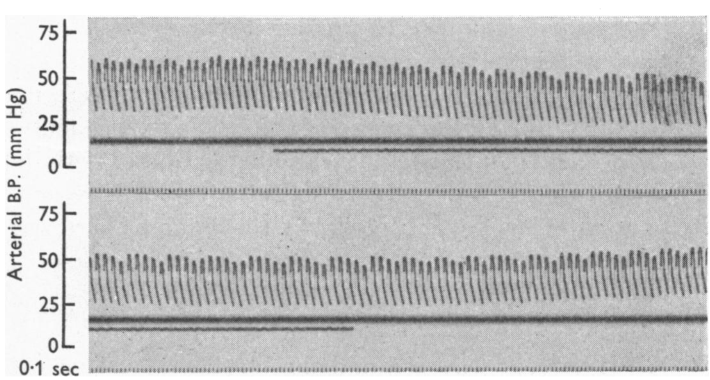

Fig. 2. Rabbit 55 g, 12 hr old, pentobarbitone anaesthesia. Stimulation of the central end of the left depressor nerve during signal mark (continuous record).

Stimulation of the peripheral end of one vagus nerve in fifteen new-born rabbits caused a mean fall in blood pressure of 45 % and in heart rate of 65 %. There was no difference whether pentobarbitone or urethane were used for anaesthesia.

Section of the depressor and carotid sinus nerves

In the following experiments blood pressure was recorded from the cardiac end of the right common carotid artery which was tied; the right carotid sinus nerve was intact. In rabbits less than 6 days old section of the right depressor nerve alone caused only a trivial rise in mean blood pressure. When the left depressor nerve was then cut there was a rapid

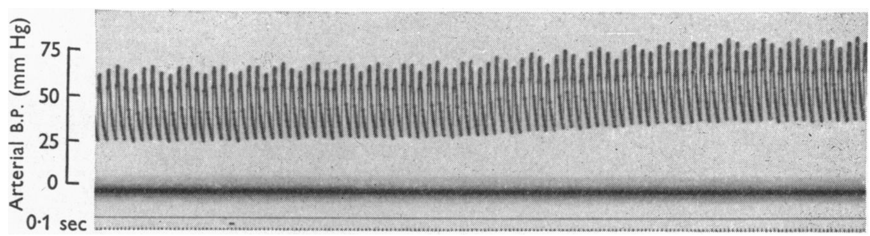

Fig. 3. Rabbit 78g, 3 days old, pentobarbitone anaesthesia. The left depressor nerve was cut at the signal mark (the right depressor had been cut previously).

rise of $13 \pm 2{\cdot}4$ % under pentobarbitone anaesthesia (Fig. 3), and of $7 \pm 4{\cdot}1$ % under urethane. But as in the adult this rise of pressure was not maintained; the pressure returned to its previous level during the next 5–15 min. When the left carotid sinus nerve was cut, after section of both depressor nerves and when the pressure had returned to its initial level, there was a rise of 12 ± 7 % under pentobarbitone anaesthesia. Once again, after an interval of some minutes, the pressure returned to its initial level. There was no change of respiratory rate. There was little immediate change in the heart rate during any one of these manœuvres, though it usually increased by 15–20 % during the course of the whole experiment.

In similar experiments on three adult rabbits, section of both depressor nerves and the left carotid sinus nerve caused a rise of blood pressure, which returned to its original level over a period of 15–20 min. There was only a small change in heart rate.

Occlusion of the carotid arteries

The right carotid artery was tied and the blood pressure was recorded from its cardiac end in rabbits less than 6 days old. Temporary occlusion of the left carotid caused a mean increase in arterial pressure of $19 \pm 4{\cdot}5$ %

under pentobarbitone, but of only $2{\cdot}7 \pm 2{\cdot}6\,\%$ under urethane. There was no significant change in heart rate. After cutting both depressor nerves in rabbits under pentobarbitone there was still a rise of $17 \pm 5{\cdot}9\,\%$ when the left carotid artery was occluded. But when the left carotid sinus nerve was also cut, the rise of pressure was reduced to $5 \pm 3{\cdot}5\,\%$. This demonstrates that the rise of pressure on occlusion of the left carotid artery was mainly due to a reflex arising in the left carotid sinus, rather than to a rise of systemic resistance due to the mechanical interruption of flow up the left carotid.

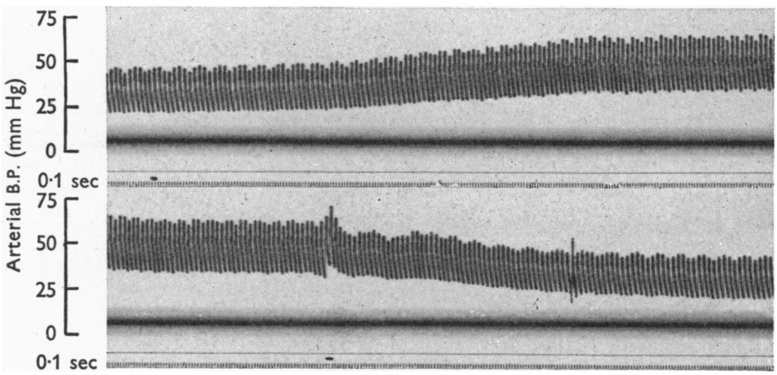

Fig. 4. Rabbit 78 g, 4 days old, pentobarbitone anaesthesia. The left common carotid artery was occluded at the first signal mark (the external, internal and muscular branches had been tied previously), and was released at the second signal mark (continuous record).

When the main carotid artery is occluded, the pressure in the carotid sinus does not fall to zero, because of anastomotic connexions with the rest of the circulation. In six rabbits less than 6 days old, under pentobarbitone anaesthesia, the left external and internal carotid arteries were tied, together with an occasional muscular branch. When the left common carotid artery was then occluded, the arterial blood pressure (recorded from the central end of the right carotid artery) rose by $23{\cdot}5 \pm 8{\cdot}5\,\%$ (Fig. 4). This evidence also supports the view that the rise of systemic pressure is due to a carotid sinus reflex rather than to an increase in systemic arterial resistance, However, under these conditions the possibility of the response being due in part to activity of the carotid body chemoreceptors (because of anoxia from lack of blood) cannot be excluded.

Raising carotid sinus pressure

In the following experiments blood pressure was recorded from the cardiac end of the left or right common carotid artery. The cephalic end of the left common carotid artery was connected to a manometer and pressure bottle filled with saline, so that the pressure in the left carotid sinus, isolated from the rest of the circulation (see Methods) could be varied. Nine experiments were carried out on rabbits from 12 hr to 9 days old. In every experiment raising the pressure in the left carotid sinus caused a significant fall in blood pressure; there was little change in heart rate (Fig. 5). When a static pressure of 30 or 40 mm Hg was applied to the

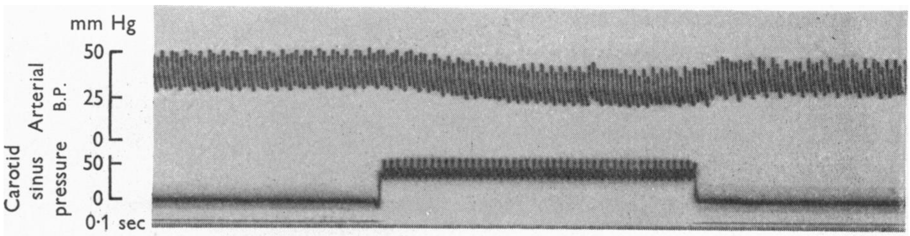

Fig. 5. Rabbit 62 g, 12 hr old, pentobarbitone anaesthesia. Above, arterial blood pressure; below, pressure in the vascularly isolated left carotid sinus.

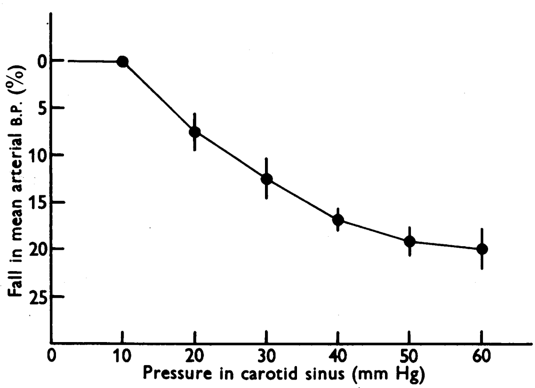

Fig. 6. Observations from nine new-born rabbits to show the fall in mean systemic blood pressure (%) at different pressures applied to the isolated left carotid sinus. The vertical lines represent the standard error of the mean.

carotid sinus, the fall in systemic arterial pressure was significantly less than when a pulsatile pressure of the same mean value was applied; at higher pressures no difference was observed. The rise in pressure required was not large. Thus in one 6-day-old rabbit, raising the pressure in the left carotid sinus from 0 to 41/19 mm Hg (pulsatile) caused the systemic arterial blood pressure to drop from 63/35 to 52/25 (fall in mean B.P. 23 %). When a static pressure of 30 mm Hg was applied to the sinus, arterial pressure only fell from 61/33 to 59/33. A positive correlation between the level of induced carotid sinus pressure and fall in systemic arterial pressure was observed (Fig. 6). While these changes are not large, it should be remembered that only *one* carotid sinus was being stimulated in these experiments. Denervation of the sinus in five animals completely abolished the response.

The Valsalva test

The characteristic cardiovascular response to a rapid elevation of endotracheal pressure (and hence intrapulmonary pressure) in the adult rabbit consists of a sharp fall in systemic arterial blood pressure followed by a gradual rise. When the endotracheal pressure is returned to atmospheric the blood pressure rapidly overshoots its initial level and thence slowly returns. In the adult rabbit the gradual rise in pressure during the Valsalva manoeuvre, and the overshoot, are dependent on the integrity of the baroreceptors (Joan C. Mott, unpublished). An example is shown in Fig. 7.

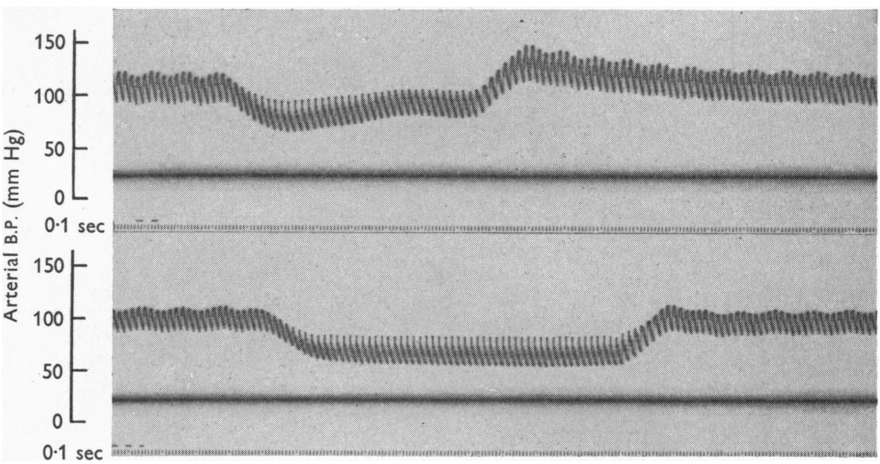

Fig. 7. Rabbit 625 g, 32 days old, urethane anaesthesia. The upper tracing shows the characteristic blood pressure response to rapid elevation of the endotracheal pressure to 15 cm H_2O (Valsalva manoeuvre). The lower tracing shows the response after cutting both depressor nerves.

In only eight of twenty-one rabbits less than 15 days of age was the same sequence of arterial pressure changes clearly seen when the endotracheal pressure was raised and lowered. In the others the responses were equivocal. There was no obvious relation between age and the unequivocal demonstration of these responses. In three out of six new-born rabbits which showed a positive response, cutting the depressor nerves greatly reduced or abolished both the rise in pressure during the Valsalva manoeuvre and the subsequent overshoot.

Baroreceptor nerve activity

Multiple and single fibre preparations were made from the depressor and carotid sinus nerves in adult, young and new-born rabbits. In every animal multiple nerve activity synchronous with the pulse wave was recorded. It was more difficult to obtain good single-fibre preparations in new-born rabbits, as the nerves are so much more fragile. Figure 8*a* shows the record from a whole depressor nerve in a one-hour-old rabbit, and it is evident that there are numerous discharges coincident with the systolic pressure wave; the mean pressure was 33 mm Hg. Figure 8*b* shows a typical multifibre preparation from the depressor nerve of a

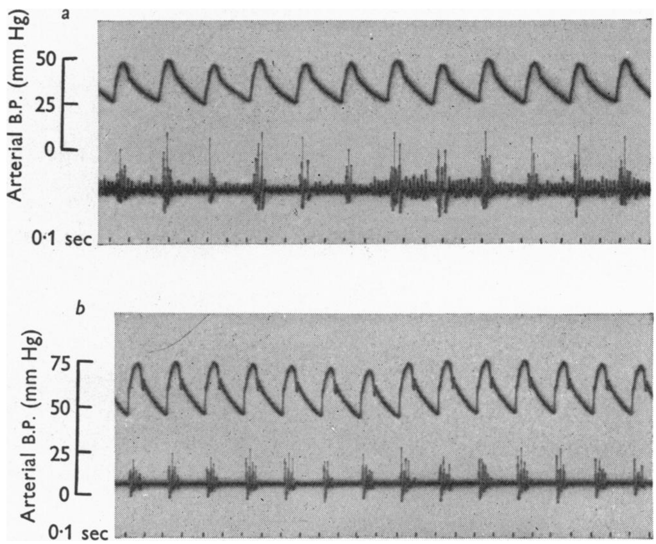

Fig. 8. The upper record (*a*) shows the arterial blood pressure, and action potentials from the entire left depressor nerve of a 38 g, one-hour-old rabbit. The lower record (*b*) shows a typical multifibre preparation from the depressor nerve of a 70 g, 4-day-old rabbit.

4-day-old rabbit; the mean pressure was 50 mm Hg. Numerous preparations of this type were obtained in rabbits 1 hr old and upwards. Figure 9 shows single fibre baroreceptor discharges from the left depressor nerve of a 2-day-old rabbit, at different arterial pressures. In adult rabbits the arterial pressure was altered by haemorrhage or by transfusion of heparinized blood; in the new-born rabbit it was raised by compression of the abdomen. In addition, new-born rabbits usually showed a variation in arterial pressure with breathing. In all instances the frequency of discharge increased with an increase in arterial pressure, and vice versa.

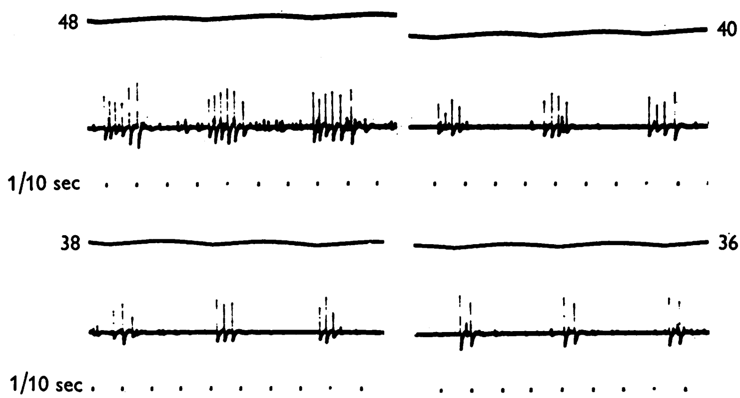

Fig. 9. Records of single baroreceptor discharges from the left depressor nerve of a 68 g, 2-day-old rabbit at four different blood pressures. In each record the arterial pulse is heavily damped and the mean pressure is indicated in mm Hg (upper trace); the middle trace shows the baroreceptor discharges, and the lower trace time.

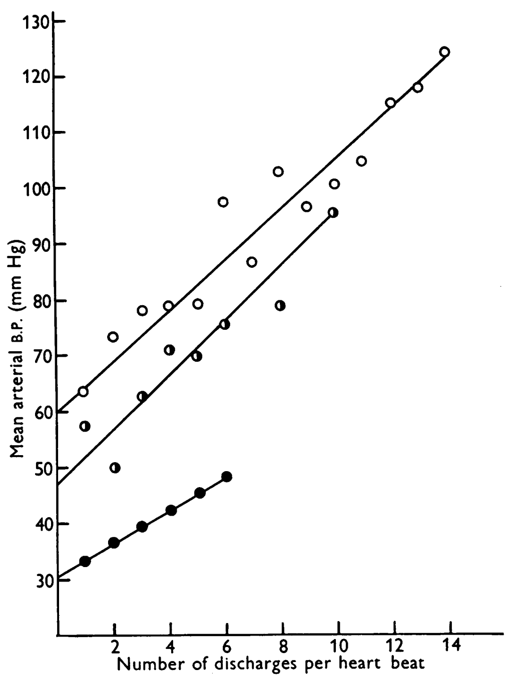

Fig. 10. Observations of single nerve fibre activity from the depressor nerve. The discharge frequency per heart beat of fourteen fibres from adult rabbits (○), eleven fibres from young rabbits, 200–1000 g (◑), and four fibres from new-born rabbits, < 2 weeks old (●), plotted against the mean arterial blood pressure.

Figure 10 shows the number of discharges in each heart beat plotted against *mean* blood pressure for a number of baroreceptor nerve fibre preparations in adult, young and new-born rabbits. It is evident that baroreceptor afferent nerve fibres can discharge at the low arterial pressure of a new-born rabbit. The observations also suggest that the threshold in older rabbits may be higher.

DISCUSSION

There is no doubt that the central and efferent mechanisms for actuation of the baroreceptor reflexes are present in the rabbit at birth. Electrical stimulation of the central ends of the depressor and carotid sinus nerves caused a fall of blood pressure and heart rate when pentobarbitone was used as the anaesthetic. The afferent side of the reflexes was also studied in an attempt to obtain a measure of the threshold pressures at which the reflexes were brought into operation. It proved more difficult to obtain baroreceptor single-fibre preparations from the depressor and carotid sinus nerve in new-born rabbits than in adults. However, the observations on multi-fibre preparations clearly indicated that many afferent nerve fibres were discharging synchronously with each heart beat as early as 1 hr. after birth.

When both depressor nerves were cut in the new-born rabbit there was a rise of blood pressure. When the left carotid sinus nerve was then cut there was also a rise of blood pressure. These observations suggest that there was considerable afferent activity in the nerves before section. Mechanical stimulation of the central end of the depressor nerve at the time of section would have caused a fall of blood pressure. Mechanical stimulation of the carotid sinus nerve might have caused a rise of blood pressure, as does electrical stimulation with a low current, but it is improbable that the rise would have lasted as long as was observed, and the increase in respiratory rate (usually seen during electrical stimulation of this nerve) was not present. There must, therefore, be tonic activity of the baroreceptor reflexes in the rabbit during the first few days after birth.

It will be recalled that Schmidt (1932), Gernandt (1946) and Neil, Redwood & Schweitzer (1949) all found that in rabbits the depressor nerve does not contain chemoreceptor afferent nerve fibres. In none of the experiments quoted above can the results of section of the depressor nerve be attributed to anything but interruption of baroreceptor afferent nerve fibres.

The effects of occlusion of the left carotid artery (the right having been tied previously) of the experiments on raising the pressure in the carotid sinus, and of the Valsalva manoeuvre all provide direct evidence that the threshold of the baroreceptors is within the normal pressure range of the arterial pulse in a new-born rabbit. Once again, however, the changes in heart rate were very small.

We now have to consider how it came about that Bauer (1939) and Barcroft (1946) came to the conclusion that the baroreceptor reflexes were not normally in action in the new-born rabbit. With the apparatus then available Bauer found it difficult to record the arterial pressure in new-born rabbits, and relied mainly upon the heart rate as an index of changes in the cardiovascular system. Barcroft wrote: 'If the depressor reflex is present, ligature of the depressor will quicken the heart'. This may be true of the adult, but not of the new-born rabbit, in which there is a significant rise in blood pressure on section of the depressor nerves, but a negligible change in heart rate. Bauer's paper shows falls of heart rate on stimulation of the depressor or carotid sinus nerve, of which the greatest is 33 %. This is just a little more than the most I saw (29 %) in forty rabbits 0–15 days old on maximal stimulation, but the *mean* fall in heart rate was only $9{\cdot}5 \pm 2{\cdot}7$ (S.E.) %. The change in blood pressure was considerably greater. All Bauer's observations were made under urethane anaesthesia, and, as compared with pentobarbitone, this anaesthetic very much reduced the size of the response in the first 5 days after birth. Neither of these considerations alter the fact that Bauer observed a difference as between rabbits 21 days and 34–42 days old when the depressor nerves were cut during asphyxia. In the older rabbits there was an immediate increase in heart rate, which did not occur in the younger. However, his other observations show that the rise in blood pressure during asphyxia was greater in older than in young rabbits, so that the depressor afferent activity would have been greater in the older. The fact that cutting the depressor nerves during asphyxia had no demonstrable action on the heart rate in the young rabbits does not necessarily mean that the depressor reflex was not in action either in asphyxia or in the normal animal.

Finally, there are other problems which will require further investigation. During the first 5 days of life in rabbits the heart rate increases (Fig. 1). After several weeks of life the heart rate begins to decrease according to Bauer (1939). Throughout the whole of this period of time blood pressure is gradually rising. Bauer attributed the secondary fall in heart rate to the rise in blood pressure above the threshold of the depressor and carotid sinus reflexes. If these reflexes are active at birth, this explanation is no longer satisfactory. Another question concerns the sensitivity of the baroreceptors to transmural pressure. The evidence at present available (as in Fig. 10) would be consistent with the view that, with increasing age, the threshold mean arterial pressure required to excite receptors increases. But there is no certainty that some unconscious selection was not at work (e.g. that those fibres which survived the process of dissection had different characteristics from the mean). The problem as to whether barosensory receptors change in sensitivity with increasing age, and so with increasing arterial pressure, may have to be examined in other ways.

SUMMARY

1. The aortic depressor and carotid sinus baroreceptor reflexes were studied in new-born rabbits.

2. Electrical stimulation of the central end of one depressor nerve caused a 17 % (mean) fall in blood pressure and a small reduction in heart rate. When one carotid sinus nerve was stimulated there was either a rise in blood pressure or a fall according to the strength of the stimulus. Stimulation of the distal vagus caused a slowing of the heart (65 % mean) and a reduction in blood pressure (45 % mean).

3. When the depressor and carotid sinus nerves were cut there was a rise of blood pressure but very little change in heart rate. Occlusion of the carotid arteries caused a 19 % (mean) increase in blood pressure. This was much reduced by cutting the carotid sinus nerve.

4. Raising the pressure in one carotid sinus isolated from the rest of the circulation caused a fall in systemic blood pressure. Pulsatile pressures with mean values of 30 or 40 mm Hg produced a significantly greater response than non-pulsatile pressures. There was a positive correlation between the rise in carotid-sinus pressure and the percentage fall in systemic arterial blood pressure.

5. As compared with pentobarbitone, urethane anaesthesia reduced or abolished baroreceptor reflex responses in rabbits less than 6 days old.

6. Single and multifibre preparations of the depressor and carotid sinus nerves demonstrated activity synchronous with the pulse wave during the first few days of life.

7. In the rabbit, during the first week after birth, the blood pressure is relatively low; nevertheless it was concluded that the baroreceptor reflexes are functional and maintain tonic activity.

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