successful pregnancy for the females). In all 20 couples were tested: 1 (couple) × 5 (positions) × 4 (arms of the centrifuge). After a spinning period of 4 weeks, the fertility of both males and females was checked anew in stationary cages for 4 weeks.

Results. Results on rat parturition and newborn longevity under persitent centrifugation are summarized in the table. As can be seen from the table, females in cages (positions) 4 and 5 of the centrifuge showed a tendency to abort. This tendency was enhanced in females which were spun at the beginning of their pregnancy. The life of the newborns in these 2 cages was significantly shorter than in the others ($p < 0.005$). In contrast, the life span of the adult males and females was apparently not affected by spinning. The females in BC's 4 and 5 showed poor mammary development, but otherwise displayed normal maternal behaviour. Of the 12 couples placed along 2 arms of the centrifuge (10 tests and 2 rotating controls), only one couple (in cage 3) did mate successfully. The couples were removed from the centrifuge after 3 weeks of spinning; of the spun males, 6 were fertile immediately after removal, 3 were not able to fertilize any female for at least 3 months, and 3 remained unfertile for one month but were then able to impregnate females which gave birth to normal youngs. All the spun females proved fertile after removal from the centrifuge, giving birth to normal youngs with no delay.

Figure 2 shows the uterus of a female which did not give birth in time and was necropsized. The right horn was swollen and the left one was empty (a); after opening the uterus (b) one dead embryo was found in the right horn, while blood clots were found in the left horn. Most females which did not deliver at the right time presented a similar pathological picture.

Discussion. This paper deals with the effects on rats of persistent centrifugation at a relatively low additional gravitational force of 1–1.47 × g. Under these conditions, longevity of adult rats was not apparently affected, but that of the newborns was significantly shortened at a resultant gravitational force exceeding 1.41 × g.

It seems that with a g-force higher than this critical one (in cages 4 and 5) the success of pregnancy depends on the time at which the pregnant rats start spinning: the earlier it has started the less successful the pregnancy. In cases where delivery has been delayed beyond the calculated time, necropsy shows various degrees of hemorrhage and damage to the fetus. All such fetuses are dead, in some cases their sizes indicating that development has stopped at mid-term, and in others that they have reached full-term, but for some inknown reason delivery has not taken place. In general there is no correlation between the spinning time of the pregnant rat and the time of abortion; in most cases the only macroscopical findings are blood clots in the lumen of the uterus and contraction of the myometrium. In cases of abortion, the ovaries contain corpora lutea at various stages of regression. However, no pathological changes have been observed in brain, muscles and viscera, as reported earlier to occur under persistent centrifugation[1–6]. It is thus possible that the reason is the relatively low additional gravity force used in this study.

We do not know whether the failure to deliver is in any way related to the feeding state of the animals. During the brief intervals in which the centrifuge was stopped, all the rats seemed quite normal and ate and drank as usual, but whether they did so during the spinning could not be determined. As mentioned earlier, the mammary glands and teats of the nursing females were poorly developed and their short-lived offsprings appeared starved and emaciated. At this stage of study it is difficult to say whether this mammary insufficient development was due to a) undernourishment of the female rat, b) stress of centrifugation on the female, c) failure of the offsprings to suckle due to the physical stress of centrifugation, i.e. inability of the neonates to fasten on to and to hold the mother's teat under the added gravity. (Such failure to suckle would rapidly provoke cessation of lactation in the mother.) It is of course possible that several of the abovementioned reasons combine to contribute to the early death of the offspring in cages 4 and 5. In contrast, the neonates in cages 1–3 and in the rotating control cages develop quite normally, although they were slightly underweight compared to neonates in the stationary control cages.

Effects of monovalent cations on the responses of motoneurones to different groups of amino acid excitants in frog and rat spinal cord

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Summary. Classification of excitatory amino acids into different groups, of possible value for transmitter identification, can be made on the basis of the differential effects of altered external [Na^+] and [K^+] on motoneurone depolarization in frog and immature rat spinal cord.

It is generally considered that the depolarization of mammalian central neurones by L-glutamate and related amino acid excitants involves an increase in the permeability of the neuronal membrane to Na^+ ions[2–6]. However, the structures of excitatory amino acids show considerable diversity[7,11], and it is not certain that the mechanism of their actions is similar in all cases. Indeed, Engberg et al.[8] have recently demonstrated that the excitatory action of DL-homocysteate on spinal motoneurones is associated with a decrease rather than an increase in membrane conductance. We now describe experiments conducted in vitro on the spinal cord of the frog and immature rat in which amino acid-induced depolarization of motoneurones, recorded from ventral roots, was studied in superfusion media of varying ionic composition. It was found that the responses of different amino acids were affected differently by the ionic changes studied, and that amino acids could be classified within distinct groups on the basis of the results obtained. Some of the observations suggest the possibility of more than

Effects of lowered [K^+] and [Na^+] on ventral root depolarization induced by amino acid excitants in frog spinal cord

Group	Amino acid	Percent control K^+-free	Percent control 10%-[Na^+]
I	L-Aspartate	320 ± 86 (4)	46 ± 4 (7)
	D-Aspartate	279 ± 30 (3)	84 ± 7 (3)
	L-Cysteate	272 ± 78 (4)	45 ± 3 (3)
	L-Cysteine sulphinate	223, 158	24
	L-Glutamate	231 ± 28 (7)	76 ± 2 (8)
II	Quisqualate	89 ± 3 (3)	53, 47
	Kainate	93, 103	24, 27
	N-Methyl-D-aspartate	100, 111	52, 42
	D-Homocysteate	115 ± 5 (5)	48, 34
III	L-Homocysteate	116 ± 6 (10)	140 ± 8 (9)
	L-Homocysteine sulphinate	111, 152	152
	D-Glutamate	157 ± 19 (4)	146 ± 15 (3)

The experiments were conducted in frog Ringers medium containing 1 mM procaine. Amino acids were tested in groups of 3 or 4 and the concentration of each was adjusted so that all gave equal depolarizations of between 1 and 3 mV under control conditions. The period of contact of the amino acid-containing solutions with the spinal cord was 40 sec. For K^+-free Ringers solution, the normal KCl component was omitted. For 10%-[Na], 90% of the normal NaCl component was replaced by choline chloride. Responses in media of altered ionic composition were measured 20–30 min after the changeover, and are expressed as per cent of the control responses measured in Ringers solution of normal ionic composition immediately prior to changeover. Figures are means ± S. E. (n) except where n is less than 3, where individual figures are given.

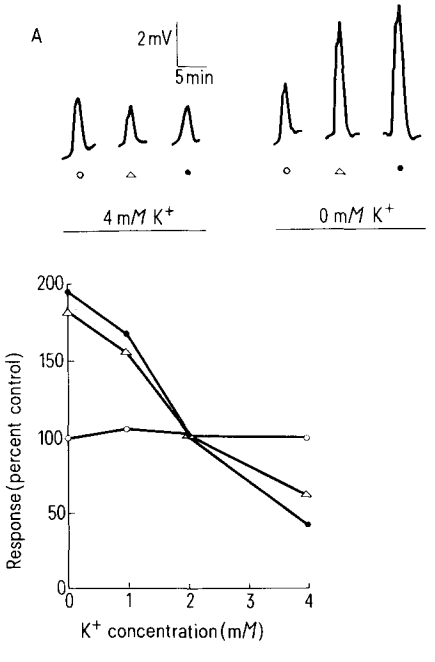

Fig. 1. Effect of changes in [K^+] on ventral root depolarizations induced by L-glutamate, L-aspartate and L-homocysteate on the same frog spinal cord. All Ringer solutions contained 1 mM procaine. Responses were measured 20–30 min after changing superfusion solution. The period of contact of the amino acid-containing solution with the preparation was 75 sec. *A* Examples of the VR depolarizations obtained in superfusion media containing either 4 mM or no KCl. ○, 80 µM L-homocysteate; △, 0.5 mM L-glutamate; □, 1.0 mM L-aspartate. *B* Plot of response (per cent of the control response measured in superfusion medium containing 2 mM KCl) against the K^+ concentration in the medium. Symbols as in *A*.

a single mechanism for excitatory amino acid action, while others are compatible with differential effects of the ionic changes on rates of amino acid uptake.

Recordings were made mainly from ventral roots of the spinal cords of Rana pipiens or Rana temporaria as previously described[9,11]. In order to abolish indirect effects of amino acids, procaine (1 mM) was usually included in the standard perfusion medium, which otherwise contained: (mM: NaCl, 111; KCl, 2; $CaCl_2$, 2; glucose, 12; tris base, 10; the pH was adjusted to 7.5 with 11.3 N HCl). The temperature of the perfusion medium was 12 ± 0.5 °C, and the flow rate was 1.5 ml per min. Several experiments were also carried out in the presence of tetrodotoxin (10^{-6} M) instead of procaine, or in the absence of any blocking agent. Additionally, a restricted series of experiments was conducted on the isolated hemisected spinal cord of the immature rat[10] (4–8 days old). The composition of the medium for these experiments was (mM): NaCl, 118; KCl, 2; $NaHCO_3$, 24; $CaCl_2$, 2.5; glucose, 12. This medium was gassed with 95% O_2, 5% CO_2 and the temperature was maintained at 20 ± 0.5 °C; the flow rate was 0.8 ml per min.

Ventral root recordings in media of varying K^+ concentration revealed marked differences between the responses to different amino acids. For example, figure 1 shows that the ventral root depolarizations produced on the procaine-blocked frog spinal cord by L-glutamate and L-aspartate were reduced in high (4 mM) and enhanced in low (1 mM and 0) K^+, compared with those observed in normal (2 mM) K^+ concentration. On the other hand, the responses to L-homocysteate were not greatly affected by changes in the medium K^+ concentration.

Figure 2 shows ventral root responses to 4 amino acids obtained in standard medium and in a medium in which 90% of the usual sodium chloride was replaced by choline chloride. It can be seen that the depolarizations produced by L-aspartate, L-glutamate and quisqualate were reduced in the 10%-[Na^+] medium, compared with the control responses, whereas the responses to L-homocysteate were markedly enhanced.

Similar results were obtained in experiments with low-[Na^+] media when LiCl or sucrose were used instead of choline chloride as osmotic substitutes for NaCl, and also when the experiments with either low-[Na^+] or K^+-free media were conducted on unblocked spinal cords or on preparations which were blocked with tetrodotoxin (10^{-6} M) instead of procaine.

Experiments conducted on the isolated spinal cord of the immature rat gave comparable results to those obtained on the frog. Thus, the responses to L-glutamate and L-homocysteate were depressed and potentiated respectively in low-[Na^+] media, and enhanced and unaffected respectively in K^+-free media. These observations are in

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accord with the fact that amino acids tested on both frog and rat spinal neurones show parallel structure-activity relations[7,11], suggesting that the mechanisms underlying their actions in the 2 species are similar.

The table classifies a series of amino acids into 3 main groups according to the effects of changes in the ionic composition of the perfusion medium. The responses of group I amino acids showed marked enhancement in K^+-free media and depression to varying degrees in low-[Na^+] media. The responses of group II amino acids were not markedly affected in K^+-free solutions, and were strongly depressed in low-[Na^+] media. The responses of group III amino acids were enhanced in low-[Na^+] media, and were either unaffected or showed slight to moderate enhancement in K^+-free media. It is noteworthy that the D- and L-forms of glutamate fall into different classes (I and III) as do those of homocysteate (II and III), and that the most potent excitants quisqualate, kainate, N-methyl-D-aspartate and D-homocysteate[11,12] can all be classified within the same group (II).

On the basis of the Nernst equation, sodium dependent depolarizations would be expected to be proportional to the logarithm Na^+ concentration assuming no redistribution of intracellular ions following changes in the composition of the medium. This relationship will need to be investigated under more rigorously controlled conditions. In general, however, the approximately 50% reduction in the responses seen with most of the group I and group II amino acids in 10%-[Na^+] is in conformity with this expectation. On the other hand, the opposite effect of low-[Na^+] seen with group III amino acids indicates that some other mechanism is predominant in these cases. The results may thus be related to the different changes in ionic conductance induced in cat motoneurones by L-glutamate and DL-homocysteate[8]. It will be important to carry our further such membrane conductance measurements using a series of amino acids selected on the basis of the above classification.

However, differential effects on uptake of the amino acids under the different conditions studied may also be involved in the observed ionic dependencies of the responses. Thus, group I amino acids are all known or thought likely to be rapidly taken up into central nervous tissue by both low and high affinity processes[13]. Low affinity uptake, which would predominate at the concentrations used in the present study, is both Na^+- and K^+-dependent[14–16]. Lowering the concentration of either Na^+ or K^+ in the superfusion medium would therefore be expected to diminish the rate of uptake of these amino acids and thus to potentiate the ventral root responses. However, this effect would be masked in the case of low-[Na^+] medium by the decrease in depolarization caused by the (presumed) decrease in E_{Na} across the motoneurone membranes. Group II amino acids, on the other hand, are not thought to be actively accumulated by central nervous tissue[13,17] (Cox and Watkins, unpublished). Hence the response to these amino acids would be expected to be influenced mainly by changes in the electrochemical gradient for Na^+ across the motoneuronal membranes, in conformity with our observations. Whether the uptake processes known to exist for group III amino acids[14–16,18] have special characteristics which contribute to the differences in the observed responses of this group of amino acids, compared with group I amino acids, requires further investigation.

In the absence of specific antagonists for either synaptically or amino acid-evoked excitation of central neurones, other methods for comparing such responses might facilitate transmitter identification. The observed dependence of amino acid responses on the ionic environment, and differentiation of amino acids on the basis of this dependence, may be of value in this respect.

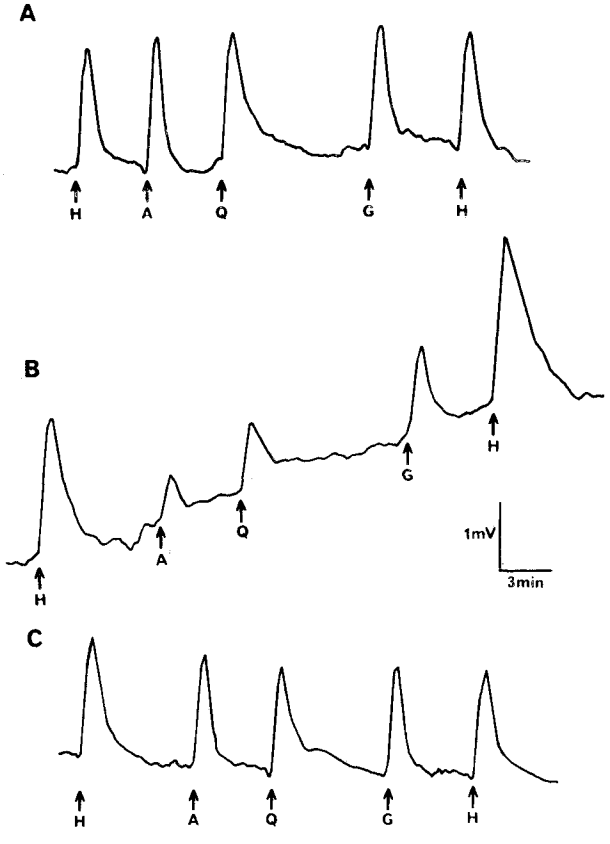

Fig. 2. Effect of change in [Na^+] on ventral root depolarization induced by L-homocysteate, L-aspartate, quisqualate and L-glutamate on the same frog spinal cord. The superfusion media contained 1 mM procaine. The period of contact of the amino acid-containing solutions with the spinal cord was 40 sec. *A* Control responses: VR depolarizations measured in medium of normal (111 mM) Na^+ concentration. *B* Responses measured between 6 and 36 min after replacement of the normal medium with 10%-[Na^+] medium (NaCl replaced by choline chloride). *C* Responses recorded between 17 and 42 min after return to normal medium. H, L-homocysteate, 120 μM; A, L-aspartate, 1.7 mM; Q, quisqualate, 4.7 μM; G, L-glutamate, 0.8 mM.

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