# PROCEEDINGS

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### A simple graphic method of showing extensor tone in dial anæsthesia. By GRACE BRISCOE.

Since it has been found that the responses of peripheral nerve muscle preparations (with circulation intact) may vary with the general tonic condition of the intact limbs it is convenient to have a simple objective method of showing the presence of tone in the opposite innervated limb.

Exaggeration of extensor tone, resembling in outward appearance the extensor hyperactivity of a mild decerebrate rigidity, is found fairly frequently in dial anæsthesia. The quadriceps resists attempts to bend the knee, "shortening" and "lengthening" reactions and the "clasp knife" sensation may all be elicited. Sometimes the resistance to bending is more marked in the forelimbs than the hindlimbs.

The femoral and sciatic trunks are cut on one side, and then both legs are put up in a similar fashion, by a drill through the lower end of the femur. Movements of the legs are recorded by threads attached to the patellar tendons.

Both limbs are lifted into full extension by pressure under the heels and then the support is suddenly withdrawn. The denervated leg falls at once into a position of flexion. The intact leg falls as rapidly as the toneless side to about the half-way position and is then suddenly checked and remains in a partially extended position (1). A clonus sometimes develops in this position, but usually the leg sags down gradually to a more flexed position. If tone be well marked, this sagging may be slight, and considerable force will have to be used to bring the leg back to the fully flexed position. Once there it remains flexed, but repeating the passive extension and release will bring about another shortening reaction.

If tone be slight the leg may show a temporary check near the end of its fall, but even this forms a clear contrast with the unchecked fall of the denervated side (2*a*, 2*b*).

If ether be given until there is no feeling of resistance in the quadriceps, the records from the two sides are indistinguishable (2*a*, 2*e*).

The method may be used to illustrate the action of drugs (3*b*), and the result of decerebration (4*b*).
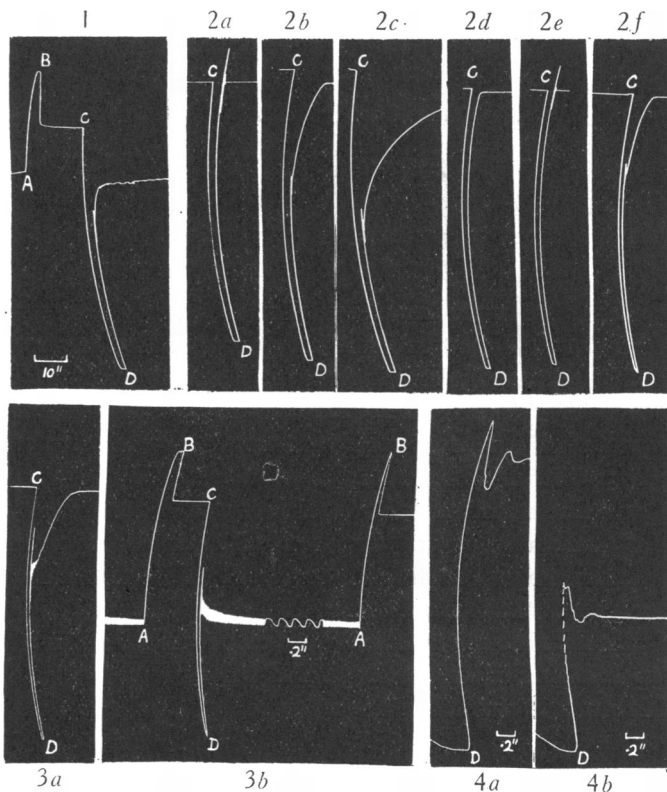
Fig. 1. Cat. Extension of knee is downwards.

Records on slow drum except 4 and small portion of 3 b.

1. Dial anæsthesia, well-marked tone. From *A* to *B* the knee (nerve supply intact) is forcibly bent and at *B* released. The limb remains nearly flexed. From *C* to *D* the leg is carried into full extension and released at *D*. Its fall is checked half-way and a slight clonus develops.

2. Dial-ether. Slight extensor tone. (*a*) Fall of denervated and (*b*) fall of intact leg. Ether given. Preliminary accentuation of tone (*c*) followed by diminution (*d*) and finally loss of tone (*e*). (*f*) Return of tone after removal of ether.

3. Dial-ether. (*a*) Before strychnine; slight tone and tendency to clonus. (*b*) After 0·1 mg. strychnine nitrate intravenously. Tone increased, lengthening and shortening reactions with well-marked clonus. In less than a minute clonus disappeared and the muscle lengthened. Clonus reappeared as soon as leg released again from extended position.

4. Decerebrate rigidity. No dial. Preliminary ether. (*a*) Fall of denervated leg. (*b*) Fall of intact leg; shows that first part of fall is as rapid as on atonic side.

**Direct chemical estimation of "carbamino-bound" CO₂ in hæmoglobin solution.** By J. K. W. FERGUSON and F. J. W. ROUGHTON. (*Physiological Laboratory, Cambridge.*)

Henriques in 1928 revived the idea that not all of the chemically-bound $CO_2$ of the blood was present as bicarbonate, but that a part was also combined directly to the hæmoglobin, possibly in the form of a carbamino compound. The idea of a direct compound has since been supported by Margaria, and also by Meldrum and Roughton, who in addition brought forward definite evidence in favour of the carbamino hypothesis.

If the carbamino hypothesis is true, it might be possible to separate the carbamino-hæmoglobin compound from the bicarbonate of the blood by precipitating the latter with excess $BaCl_2$ at alkaline $pH$, as has been done so successfully by Faurholt in the case of the simpler carbamino compounds of ammonia, glycine, etc.

The difficulties met with in applying such a method to blood were:

(i) Formation of hæmatin at alkaline $pH$. This was avoided by using a very resistant species of blood, *i.e.* ox, and by cooling to 0° C. as soon as possible after the various reagents had been mixed.

(ii) Decomposition of the $CO_2$-Hb compound during the actual mixing of the blood with alkali, such decomposition being favoured by the large amount of carbonic anhydraxe present. This was avoided by mixing the alkali with the blood completely in less than 0·01 sec.

(iii) Slow decomposition of carbamino compounds might even occur after the mixing with alkali was complete. This was reduced to an average value of 7 p.c. by the same means as in (i).

(iv) The proteins of the blood might inhibit the complete precipitation of $BaCO_3$ by acting as protective colloids. This effect was found to be considerable in laked blood, but to be negligible in purified hæmoglobin solutions.

(v) The dissolved $CO_2$ of the blood might, when the blood is made alkaline, combine with the proteins of the blood to form carbamino compounds, and hence be estimated as such. This effect turned out to be quite appreciable, but could be allowed for by blank experiments.

In the final arrangement, three 5 c.c. syringes were filled respectively with (*a*) hæmoglobin solution equilibrated with various pressures of $CO_2$, (*b*) 0·05–0·20$N$ NaOH (according to the amount of $CO_2$ in (*a*)), (*c*) 15 p.c. barium chloride. The solutions were driven from the three syringes into a mixing chamber of the Hartridge-Roughton type (kindly loaned by

G. E. Millikan): the mixed fluid from the chamber was collected under paraffin in a centrifuge tube and promptly centrifuged for 15 min. at 0–4° C. A 5 c.c. sample of the supernatant fluid was then analysed for "unprecipitated" $CO_2$ in the van Slyke-Neill manometric apparatus.

With this method, and the controls already mentioned, small but appreciable amounts of unprecipitated $CO_2$ (and therefore presumably of carbamino $CO_2$) were found both at room temperature and at 38° C. Under comparable circumstances reduced hæmoglobin solutions invariably contained much more than oxyhæmoglobin solutions. This phenomenon was specially investigated in the physiological range, *i.e.* temp. 38° C., $CO_2$ pressures ranging from 35 to 65 mm. Hg., $pH$ 7·2–7·6 roughly. Here it was found that, of the extra total $CO_2$ taken up by reduced hæmoglobin over that taken up by oxyhæmoglobin at the same $CO_2$ pressure, 50 p.c. or more might be attributed to difference in the carbamino content of the two solutions. This would seem to establish the importance of carbamino compounds in physiological $CO_2$ transport, even though the absolute amounts of such compounds in the blood may be small, *i.e.* not more than 5 p.c. of the total $CO_2$.

**Pyruvic acid and the avitaminous brain.** By R. A. PETERS and R. H. S. THOMPSON. (*Biochemical Laboratory, Oxford.*)

Pyruvic acid is formed by avitaminous pigeon's brain (not by normal) during respiration *in vitro* in lactate solutions, and disappears largely upon addition of vitamin $B_1$. This is shown by nitroprusside reaction [Peters and Sinclair, 1934], and by present estimations of bisulphite binding substances [Clift and Cook, 1932] confirmed by a modified Neuberg-Case method. Added pyruvic acid behaves similarly. Increased respiration with pyruvate occurs regularly with added vitamin $B_1$ [contrast Meiklejohn *et al.*, 1932], in presence of pyrophosphate. These results are independent of added sulphite or fluoride and constitute a strong support to the view that pyruvic acid is a normal tissue metabolite [Meyerhof and Kiesling, 1933].

REFERENCES.

Clift, F. P. and Cook, R. P. (1932). *Biochem. J.* **26**, 1788.
Meiklejohn, A. P., Passmore, R. and Peters, R. A. (1932). *Ibid.* **26**, 1872.
Meyerhof, O. and Kiesling, W. (1933). *Biochem. Z.* **264**, 40.
Peters, R. A. and Sinclair, H. M. (1934). *Biochem. J.* **27**, 1910.

**Action potentials in the saccular nerve of the frog.** By D. W.
ASHCROFT[1] and C. S. HALLPIKE[2]. (*Ferens Institute of Otology, Middlesex
Hospital, London, W.* 1.)

Since the publication of Magnus's monograph [1924] the evidence
accumulated has failed to substantiate the view therein expressed that
the saccule is an organ of so-called static equilibrium supplying to the
higher centres information concerning the position of the head in space in
relation to gravitational lines of force. Prior to this date Parker [1909]
and Maxwell [1923] had failed to demonstrate any connection of the
saccular macula with equilibrium in the dog-fish. Subsequently, McNally
and Tait [1925] in the case of the frog and Versteegh [1927] in the case
of the rabbit succeeded in carrying out extirpation experiments upon the
saccular macula, employing operative methods of precision. In neither
case did any significant disturbance of equilibrium result.

The present position regarding the function of the saccule is one of
obscurity. In the investigation here recorded the problem has been
approached upon electro-physiological lines. Employing McNally's
technique for the micro-dissection of the frog's labyrinth it has been
found possible to isolate the saccular nerve in lightly anæsthetized frogs,
the cut end of the nerve being raised upon a micro-electrode of platinum
controlled by a Leitz micro-manipulator. The entire preparation is then
transferred *en bloc* to a platform upon which movements of rotation or
tilting (fast or slow) can be carried out, the preparation being kept under
observation through a binocular dissecting microscope. The active elec-
trode, together with an indifferent lead from the frog's skin, are connected
to the input of a high-gain resistance capacity coupled amplifier whose
output passes to a cathode ray oscillograph or loud-speaker; suitable
arrangements are made for the photographic recording of potential
changes displayed upon the face of the oscillograph tube.

*Results.* No response could be elicited from the nerve by rotation or
tilting movements. In response to vibration, however, marked reaction
has in most cases been observed. This type of stimulus may be applied
either in the form of light stamps upon the floor or the dropping of graded
weights. Alternatively the stem of a vibrating tuning fork may be applied
to the platform.

The response to the former variety of stimulus consists of a sharp
burst of impulses, initially monophasic, which die away rapidly in
frequency and amplitude.

The response to vibration from the stem of a tuning fork consists of a series of monophasic impulses in which stability of amplitude and frequency is quickly attained, the frequency corresponding exactly to that of the stimulating fork. With the 1024 ∼ fork the response is definite but dies away rapidly. Owing to the high rate of decrement of this fork it is not possible to draw any conclusions either as to the neural significance of this rapid extinction of the response or as to the reproduction of frequency during the period of response.

It is not thought that the electrical changes shown in these records can be regarded as other than true action potentials set up in the saccular nerve endings by the vibrational stimuli described. This view is based upon the following considerations:

1. The electrode holding system and amplifier have been found sufficiently non-microphonic as to give a steady base line in the presence of vibrational disturbance of the order employed.

2. The strict localization of the potential changes to the saccular nerve, and the high degree of sensitivity displayed by the preparation to

    (*a*) The short circuiting effect of excess of fluid in the operative field.

    (*b*) Lack of sufficient fluid in the field; this leaves the saccular otolith high and dry from its normal fluid surroundings.

    (*c*) Death of the animal.

The response under all three conditions outlined is speedily abolished.

3. Abolition of response by cocaine.

A drop of 2 p.c. cocaine is placed upon the operative field. Two minutes later this is sucked away by a micro-pipette, approximately optimal electrical conditions being restored. Under these circumstances a previously brisk response is found to be abolished.

### Conclusions.

The saccule in the frog contains end organs concerned with vibrational .sensibility. The propagated electrical changes reproduce accurately the frequency of the stimulus, at any frequency up to 512 ∼. At 1024 ∼ a response occurs, though evidence of frequency reproduction is unsatisfactory.

### References.

McNally, W. J. and Tait, John (1925). *Amer. J. Physiol.* **75**, 155.

Magnus, R. (1924). *Körperstellung*, 527. Springer, Berlin.

Maxwell, S. S. (1923). *Labyrinth and Equilibrium.* Philadelphia and London.

Parker, G. H. (1909). *Bull. Bureau of Fisheries*, **28**, 2, 1211.

Versteegh, C. (1927). *Acta Otolaryngol.* **11**, 393.

**The function of the tensor tympani muscle.** By C. S. HALLPIKE[1] and A. F. RAWDON SMITH[1]. (*Ferens Institute of Otology, Middlesex Hospital, London, W.* 1.)

The early theories as to the function of this muscle have been adequately summarized by Köhler [1909], Kato [1913], Schaffer and Giesswein [1926] and Mangold [1926]. Later investigators, especially Kobrak [1930] and de Nó [1933] are agreed that reflex contraction of the muscle appears in response to acoustical stimulation. Still other investigators [Hughson and Crowe, 1933] have also noted the contraction in response to mechanical stimulation of the pinna. In contradistinction to other work, that of de Nó [1933] shows that the response may be elicited in favourable preparations by sounds of relatively low intensity. (Some 80 decibels above the human threshold over the middle range of frequencies.) This communication records the results of observations upon the reflex activity of the tensor tympani in cats.

In these experiments, cats under light nembutal anæsthesia, or in many cases decerebrate preparations, were used. In the latter the reflex is particularly brisk.

In this connection it is worthy of note that Hughson and Crowe [1933], in a series of 200 cats, failed altogether to demonstrate the phenomenon. It is likely that this failure may be explained as being attributable to the employment of too deep a degree of anæsthesia.

In our experiments we have employed as a source of sound a loud-speaker, activated *via* an amplifier by a heterodyne oscillator, the output being suitably controllable for intensity and frequency. Considerable attention has been devoted to the effect upon the Wever and Bray phenomenon of the reflex contractions of the tensor tympani. In this part of the work a resistance-capacity coupled amplifier, of conventional design, has been employed in conjunction with a cathode ray oscillograph. Photographic records are made upon fast moving Cine bromide paper. Direct observations of the contractions of the muscle were made in all cases through a binocular dissecting microscope.

We have observed brisk contractions in response to pure tones of frequency 200–2000 cycles, of intensity some 80 decibels above the human threshold. The contractions are particularly well marked within the range 500–1000 cycles. As far as may be judged by eye, these appear to take the

form of an initial twitch, followed by a smaller contraction maintained so long as the sound is applied, and a terminal twitch when the stimulus is removed. The reflex is entirely abolished in deep anæsthesia, and is never present in the large proportion of cats (some 40 p.c.) in which the external meatus is found to be filled with wax (cf., however, de Nó [1933]). Further, we doubt the theory that this is a tendon reflex, owing to the persistent presence of reflex contractions observed in the muscle, following upon divisions of its tendon.

It is possible to summarize the effect of these contractions upon the Wever and Bray phenomenon, using frequencies of 500–1000 cycles, as follows:

(1) On switching on the speaker, the Wever and Bray response rapidly attains full amplitude. The shortest interval observed is approximately $2\sigma$.

(2) Following this, the response shows a gradual decrease, attaining a level some 5–6 decibels below the initial amplitude. It is noteworthy that the overtones, which are introduced by the vibrating components of the ear (these being absent in a microphone record), appear to suffer a greater relative decrement than does the fundamental.

(3) Following this period of decrement, the lowest level of which is attained some $30\sigma$ after its initiation, there follows a period approximately equal in time, during which the response rises. The initial value, however, is never regained. It may be seen, therefore, that these stages correspond in sequence to those of the contractions of the tensor tympani outlined above.

(4) On switching off the sound, the response disappears relatively slowly; that this is not altogether due to persistent vibration of the loud-speaker diaphragm has been demonstrated with the microphone.

On cutting the tensor tympani tendon, a fall in response of approximately 1–2 decibels takes place. This may possibly be due to the release of the tension normally exerted by the tone of the resting muscle and by its fascial sheath on the tympanic membrane, which, following upon this operation, immediately becomes somewhat flaccid. This operation removes all the effects detailed above under (2) and (3). The response is fully maintained, after its onset, but on switching off the response here shows a slower decrease in amplitude and considerable irregularity. This is indicative of the possible damping action of this muscle.

We conclude that reflex contractions of the tensor tympani exert a damping effect upon the amplitude of movement of the vibrating mechanism of the ear. The short duration and small degree of this

damping effect do not wholly favour the protective theory of the muscle's action. Overtones are relatively more affected by this damping action.

<div align="center">REFERENCES.</div>

Hughson, W. and Crowe, S. J. (1933). *Acta Otolaryngol.* **18**, 3, 291.

Kato, Toru (1913). *Pflügers Arch.* **150**, 569.

Kobrak, H. (1930). *Passow-Schäffer Beitr.* **28**, 138.

Köhler, W. (1909). *Z. Psychol.* **54**, 250.

Mangold, M. (1926). *Handb. norm. path. Physiol.* **12**, 421.

de Nó, L. (1933). *Laryngoscope*, **43**, 315.

Schaffer, K. L. and Giesswein, M. (1926). *Denker-Kahler's Handb. Hals., Nasen. u. Ohren.* **6**, 1, 389.

## The effect of ether on the action of adrenaline.

<div align="center">By HUGH A. DUNLOP. (<em>King's College, London.</em>)</div>

Since the work of McDonald, it is well recognized that ether anæsthesia is largely responsible for the depressor action of small doses of adrenaline. This action has now been studied by means of plethysmographic records of skinned and unskinned limbs in cats anæsthetized in the first instance with ether and subsequently with chloralose. In the skinned limb there is throughout an increase in volume with each dose of adrenaline, but before the ether has passed off only a slight diminution in the volume of the whole limb is obtained. Gradually, in the latter, the adrenaline constriction becomes greater and greater until when the same dose of adrenaline, which formerly caused a fall, now gives a rise of arterial blood-pressure, the constriction is very marked.

The results support the view previously put forward by Dunlop that whether adrenaline causes a rise or fall of blood-pressure depends chiefly on the amount of skin (and visceral) vaso-constriction which the drug is able to induce. This constriction tends to be abolished by ether.

## An apparatus for the study of whole isolated arteries.

<div align="center">By F. S. GORRILL. (<em>King's College, London.</em>)</div>

The apparatus consists essentially of a plethysmograph in which the artery may be placed and perfused with various solutions. The changes in volume show themselves in a horizontal capillary tube where the movements of the fluid may be photographed. The recording apparatus is made from an ordinary film camera driven by a gramophone motor. Results indicate that the artery can be caused to develop a tone which can be diminished or increased by appropriate treatment.

### A special purpose amplifier. By BRYAN H. C. MATTHEWS.
#### (*King's College, Cambridge.*)

The amplifier described below was designed for multiple recording, and has been in use for some months in a three-oscillograph recording system. It has been found to have several advantages over the conventional design of amplifier which make it specially suitable for use in the first stage of any amplifier for physiological purposes.
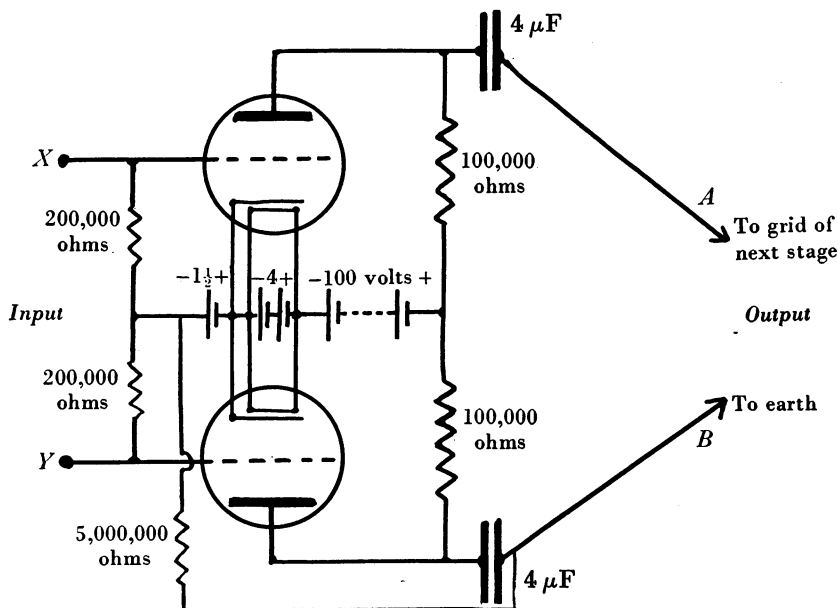


Fig. 1.

Its advantages are: (1) immunity from electrical feed back from later stages to the preparation; (2) great reduction in the stimulus escape when electrical stimulation is employed; (3) comparative freedom from external electrical interference; (4) no interconnection when several amplifiers are used simultaneously.

Fig. 1 shows the general arrangement of the first stage which may be followed by any conventional type of amplifier. At present Catkin valves (MH 4) are in use and are quite satisfactory, the pairs used must be roughly matched, the possibility of high frequency oscillation occurring can be eliminated by connecting a 0·001 $\mu$F condenser and a 5000 ohm resistance in series between each anode and cathode. As the batteries

are not at earth potential, the whole assembly including the batteries must be screened in an earthed case.

It will be seen that neither of the input leads goes to earth, so that the amplifier is only affected by potential changes between $X$ and $Y$, and if the potential of both $X$ and $Y$ changes equally relative to earth there is no resultant change between $A$ and $B$. This is the reason for (1) and (3) above, for most forms of electrical interference seem to result from electric fields fluctuating relative to earth potential; while such fields disturb an amplifier where the input goes to grid and earth by making the grid potential fluctuate, they affect both grids of this arrangement equally and so are not amplified.

If a pair of electrodes is placed on a frog's heart and a second pair on the liver, when these are connected to two recording systems having grid + earth input amplifiers, the pair on the liver yield an electrocardiogram only slightly smaller than that recorded from the pair on the heart, and of opposite sign; such potential changes in the liver being improbable, on further consideration it is clear that the effect is due to the interconnection of two of the electrodes *via* the common earth. Obviously very misleading results might follow from the simultaneous use of two grid + earth input amplifiers.

Using the amplifiers having grid + grid input circuits this anomaly disappears, the electrodes on the liver no longer show any substantial electrocardiogram, the deflections being reduced some thirty times from their former magnitude.

These amplifiers can thus give a true picture of changes between several points on the same preparation which is often unobtainable with grid + earth input amplifiers.

### Impulses leaving the spinal cord by dorsal nerve roots.

By BRYAN H. C. MATTHEWS. (*King's College, Cambridge.*)

In the course of observations on the impulses in spinal nerve roots I have found that there is considerable activity in the central ends of cut dorsal rootlets. Recent histological work [*e.g.* Kahr and Sheenan, 1933] has established the presence of a small number of fibres in dorsal roots which do not degenerate centrally from a section between the dorsal root ganglia and the cord. It is thought possible that the activity here reported is that of these fibres.

Cats have been anæsthetized, and after lumbar laminectomy decerebrated and the anæsthetic discontinued. The electrical activity of

the intradural nerve rootlets is recorded with an amplifier and moving iron oscillograph. As yet only the 5th, 6th and 7th lumbar roots have been examined.

Even the smallest rootlets of the dorsal roots show evidence of the activity of very large numbers of sensory fibres, and analysis of the ascending impulses is only possible when the majority of the fibres have been severed distal to the recording electrodes. The results of this analysis will be published later.

If the rootlets are cut and electrodes placed on their central ends there is still considerable activity due to impulses leaving the cord by them. It is estimated that roughly 5 p.c. of the fibres in the dorsal roots are still active after ascending impulses have been cut off by section peripheral to the recording electrodes.

By partial section central to the recording electrodes I have been able to record the impulses leaving the cord in preparations of a single active fibre. Three types of discharge are present: (*a*) those in small fibres evidenced by slow action potentials; (*b*) discharges of motor neurones (large fibres); (*c*) antidromic sensory discharges (large and medium fibres).

The activity of the small fibres (*a*) has not yet been analysed.

(*b*) These discharges are attributed to motor neurones for the following reasons. (1) The rate of rhythmic discharge is never above 70 per sec. (2) The stimulus which evokes or modifies them is movement of or pressure on the limbs. The receptive area is wide in contradistinction to (*c*). They are often slowed or stopped by like stimulation of the opposite limb. Often stimulation of a forelimb controls the discharge in 6th or 7th lumbar roots. (3) Cutting all the other sensory rootlets of the same and adjacent roots may reduce but seldom abolishes the discharge occurring when the appropriate region is stimulated. (4) The rate of the discharge is in nearly all cases greatly influenced by the position of the head, the discharge can often be started and stopped by raising and lowering or rotating the head. (5) The discharges are often accelerated in the early stages of chloroform and ether anæsthesia and are abolished in the deeper stages. (6) The fibres concerned do not appear to be derived by branching from motor fibres leaving the cord by the corresponding ventral roots as cutting and stimulating these with crystals of salt does not affect the discharge.

(*c*) Antidromic sensory discharges. (1) These can be evoked by appropriate stimulation of some sharply defined area, either by movement of hairs, pressure, stretch of a muscle, or joint movement. (2) They can

occur at high frequencies, an initial frequency of 650 per sec. has been recorded from a superficial receptor on sudden application of pressure. (3) The response in any single fibre preparation is abolished by section of some particular rootlet entering the cord from the same or another root. (4) They are not abolished by deep anæsthesia and are often increased in frequency (this may be due to the effects of failing circulation on the nerve endings from which they arise). (5) Prolonged discharges never occur, the impulses always appear in bursts at a frequency depending on the intensity of sensory stimulation, with pauses of 0·2–0·8 sec. between. The discharges are like intermittent samples of a sensory response which are managing to get across a region where conduction fails periodically. No correlation has yet been found between the frequency and number of impulses in each burst and the length of the pause which follows. These impulses thus appear to come from sensory receptors, to enter the cord and leave it again by a different rootlet after passing through some region with properties unlike those of a nerve fibre.

No activity of any sort has yet been detected in the peripheral ends of cut ventral rootlets.

REFERENCE.

Kahr and Sheenan (1933). *Brain,* **56,** 265.

## The effect of grafting or removing the posterior limb-rudiment on the spinal cord of Amphibian embryos [*Discoglossus pictus* (Otth.)]. By RAOUL M. MAY. (Introduced by J. NEEDHAM.) (*Collège de France and Institut Pasteur, Paris, France.*)

Braus [1906], Dürken [1916], as a result of ablations of the posterior limb-bud in Amphibian embryos, and Shorey [1909], after ablations of the wing-bud in the chick embryo, deduced that relations exist between the peripheral organs and the neuroblasts, both sensory and motor. Detwiler [1920, 1924], however, following his experiments of grafts and ablations of the forelimb rudiment in the Urodele *Amblystoma,* admitted only the sensory relations, and postulated that the motor neuroblasts were under the influence of internal factors of the neuraxis.

But I have shown [May, 1930], by means of grafts of the embryonic spinal cord, that the absence of the lumbo-sacral plexus, due either to the absence of a posterior limb whose place is occupied by a grafted cord, or to the fact that the limb is present but innervated by the implanted

cord, reverberates on the autochthonous spinal cord and its ganglia through a sensory and motor hypoplasia. It therefore follows that the hindlimb innervation plays a part in the development of both the sensory and motor neurones of the neuraxis. The present experiments confirm these results.

The rudiment of the right hindlimb was transplanted from an embryo of the Anuran, *Discoglossus pictus* Otth., at the tail-bud stage, on to the side of another embryo of the same egg lot, in the region immediately beneath the spinal cord. The grafted limb developed synchronously with the autochthonous hindlimbs, and contracted nervous relations with the cord.

The innervation of the grafted limb depended on the place of its implantation, and was not specific. In some cases it was innervated by nerves of the lumbo-sacral plexus, in others by nerves of the brachial plexus, and in others by nerves belonging to no plexus.

In all cases of nervous surcharge due to the innervation of a grafted limb, the cord, at the level of emission of the supplementary nerves, was asymmetric, larger on the side of this innervation, and hyperplastic in its motor region (11·7–33·7 p.c.).

The spinal enlargement and hyperplasia on the side and level of the supplementary innervation existed also in certain cases where the grafted limb was not innervated, but where the autochthonous right hindlimb was doubled in its distal part, beginning with the tibia, the two distal enantiomorphs being innervated.

When the total volume of the innervated limbs, autochthonous and grafted, on the operated side, was sensibly the same as that of the normal contralateral hindlimb, the cord was symmetrical, but hyperplastic (8 p.c.) locally, in its motor region, on the side of the graft, at the level of the latter's innervation.

The motor hyperplasia of the cord due to the emission of supplementary nerves was accompanied by an augmentation in weight (7·8–21·8 p.c.) of the same motor region.

A second series of operations consisted in ablations of the right hindlimb rudiment, at the tail-bud stage, and its replacement by the skin of a second embryo, so as to hinder the formation of the limb. In these cases the right hindlimb did not form, or else was very small and deformed. In all cases the lumbo-sacral plexus did not exist on the side of the ablation.

The spinal cord was then reduced and hypoplastic in its motor region (10·7–20·3 p.c.) on the side and level from which the lumbo-sacral plexus was absent, and there was no motor horn on that side and level. In some cases where the small and deformed limb was innervated, this feeble

innervation did not prevent the reduction of the cord, its motor hypo-plasia (8·6 p.c.), nor the absence of the motor horn on that side and level.

Thus, any peripheral surcharge, due either to the innervation of a supernumerary hindlimb or to the innervation of a double limb, gives rise to a motor hyperplasia and an enlargement of the cord on the affected side and level. On the other hand, any decrease in the innervation, due to the total absence of a plexus or to its strong diminution, reverberates on the affected side and level of the cord by a motor hypoplasia, the absence of the motor horn, and a reduction in size [May, 1933].

These results concerning the motor neurones, added to those observed by other authors and by ourselves [1932] in the case of the sensory neurones, lead to the statement of the following laws:

1. There is no normal development of the spinal cord, sensory or motor, without the action exerted by the stimulations of the peripheral organs.

2. In development, the bilateral equality of the sensory and motor neurones depends closely on the bilateral equality of innervation of the peripheral organs.

### REFERENCES.

Braus, H. (1906). *Morph. Jahrb.* **35**, 509.

Detwiler, S. R. (1920). *Proc. Nat. Acad. Sci. Wash.* **6**, 96.

Detwiler, S. R. (1924). *J. comp. Neurol.* **37**, 1.

Dürken, B. (1916). *Z. wiss. Zool.* **99**, 189.

May, R. M. (1930). *Bull. biol.* **64**, 355.

May, R. M. (1932). *La transplantation animale.* Paris: Gauthier-Villars. 352 pp. 170 figs.

May, R. M. (1933). *Bull. biol.* **67**, 327.

Shorey, M. L. (1909). *J. exp. Zool.* **7**, 25.