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The total depolarisation of crustacean nerve.

By K. FURUSAWA.

In 1926, shortly before his sudden and untimely death, Levin described the remarkable behaviour of crustacean nerve in response to tetanic stimulation. The action potential appears to persist for a considerable period after the stimulus. This phenomenon, which he termed the "retention of action current," is very striking yet easily observable with crustacean nerve. Levin found also a "ceiling effect," which signifies that under favourable conditions the maximal galvanometer deflection caused by a group of tetanic stimuli reaches a constant level; and he recognised the profound local fatigue which arises near the stimulating electrodes.

The first clue to an explanation of this peculiar phenomenon was provided by a number of observations on the injury current and the maximal galvanometer deflection, employing a single pair of stimulating electrodes. Frequently these two quantities were connected by a linear relation or by a simple parabolic proportionality. The injury current falls off gradually after dissection and the maximal galvanometer deflection falls also. There is, moreover, a strong indication that injury and action potentials vanish together. This latter observation suggests that the two quantities are not independent but related to one another.

The "ceiling effect" provides a second clue. Two tetanic stimuli are applied to a nerve. As soon as the first one gives a maximal deflection, D_1, the stimulus is stopped and the potential divider which supplies the current to balance the injury potential is adjusted to the value (P_2) required to bring the spot of light to zero. The second stimulus is then applied, the result being a deflection, D_2. The ratio $\frac{D_1 - D_2}{P_1 - P_2}$, where P_1 denotes the initial injury potential, gives the decrease of deflection (say in mm.) accompanying a drop of one unit (say 1 millivolt) in the injury potential. This value was compared with one similarly obtained by the

external application of one unit of potential difference when the nerve was in a resting condition. The result showed a remarkable agreement between the two sets of observations.

A third step in the analysis was then taken. A wax chamber with three pairs of filter paper stimulating electrodes was made. These electrodes are to be used in succession: thus as soon as the first pair of electrodes gives a maximal deflection the stimulating current is switched over to the second pair, and so on. Three pairs were found sufficient for the present purpose. The injury potential was not balanced, so that the galvanometer, suitably shunted, registered a deflection due to the injury current. Under favourable conditions (*i.e.* if the nerve had the same potential along its length and if the injury at the end were complete) the spot of light from the galvanometer came back exactly to its zero as the result of stimulation. This means that the injury potential is abolished quantitatively by the action potential. It has often been observed that an injury potential as high as 10 millivolts or more can be abolished completely in this way. The maximal effect is reached about 5 seconds from the beginning of stimulation. If we assume that in the injury potential we are dealing with a polarisation potential across the surface of the nerve fibre (*i.e.* that a galvanometer lead at the injured end is connected essentially to the inside charge, and the lead on the intact nerve to the outside charge, of a certain membrane at which the potential difference resides), then the result obtained is nothing but a total depolarisation of the nerve fibre. In other words the nerve fibre is completely discharged by a tetanic stimulus, and the gradual return of the injury potential (described by Levin as an abolition of the "retention of action current") is really a re-charging of the surface of the nerve fibres.

In nitrogen the injury potential falls off quickly and reaches a low level from which it can be restored to its original value by supplying oxygen. Under such conditions the action potential also falls. In nitrogen there is little indication, when the injury potential has been diminished by stimulation, of any return to its pre-stimulation value.

We may conclude therefore that the injury potential and the action potential are the same thing looked at from different points of view, and that the injury potential represents a boundary potential in the surface of the nerve fibre maintained by some oxidative process.

The antirachitic effect of ergot. By E. MELLANBY, E. M. SURIE AND D. C. HARRISON.

In the course of feeding experiments in which ergot of rye was added to the diet, it was noticed, although the experiments were carried out for quite another object, that this substance had a definite, and, in the case of some specimens, a powerful antirachitic action. It is difficult to get young dogs to eat more than 2 to 4 grm. of ergot daily because of its general toxic effect, but even in these quantities its power to promote calcification is striking. The general trend of recent work on the subject of the calcification of bones has been to emphasise the fact that calcification, whether specifically induced by food or by ergosterol after irradiation by ultra-violet rays, is due to one factor, the antirachitic vitamin first described by one of us in 1918 (1). It seemed most probable, therefore, that the antirachitic action of ergot was due to the presence in it of vitamin D. This was of particular interest because it will be remembered that ergot was the substance from which ergosterol was first isolated by Tanret (2) in 1889. It is possible that Tanret actually obtained vitamin D with the ergosterol.

Up to the present time, however, it has not been possible to prove definitely that the antirachitic substance in ergot is vitamin D. Like vitamin D it is soluble in alcohol and can be removed by it from ergot. On evaporating off the alcohol the active substance can be dissolved in ether, leaving an insoluble resin, free from calcifying influence. If, after removal of the ether, the fatty residue be saponified by alcoholic potash and extracted with petrol ether, then the active substance, if it is vitamin D, ought to be in the extract together with the other unsaponifiable substances. Ergosterol is present, but so far most of the calcifying factor has been left in the soap, traces only being associated with the inactive ergosterol. It is hoped by using other solvents to separate this factor entirely from the soap.

If the calcifying action of ergot is due, as seems most probable, to vitamin D the question must be faced as to its mode of production. It is possible that it may have its origin in the action of sunlight on the ergosterol of the developing ergot. On the other hand, ergot has a bluish-black covering which is probably impenetrable to radiations, and this suggests that the active factor may be produced directly from ergosterol in ordinary growth independently of sunlight.

1. Mellanby, E. This Journ. Proc. 52. pp. xi and liii. 1918.
2. Tanret. Compt. rend. Acad. Sci. 108. 98. 1889.

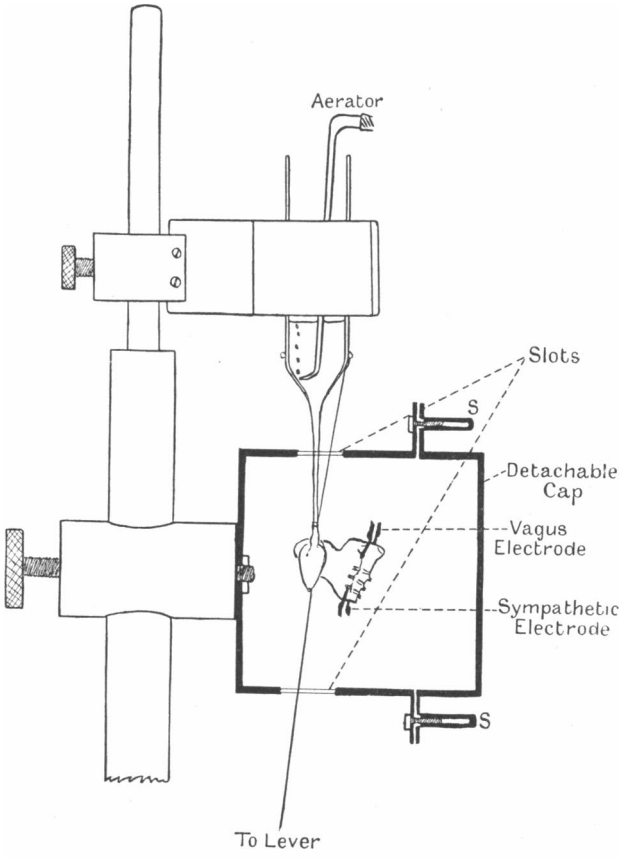
Preparation for the measurement of the excitability of the cardiac nerves of the frog. By B. FINKLEMAN (Manchester).

The preparation is a modification of the Loewi isolated heart-vagus preparation of the frog. It is designed to give separable vagus and sympathetic effects. A decerebrated frog is pinned out and a cut is made down the left side of its spinal column, which is then cut across at the level of the fourth spinal vertebra. The heart is next exposed, the sternum and both fore limbs being removed. The right aorta and the sinus are then ligatured and a fine-pointed glass cannula containing Ringer's solution is passed down the left aorta into the ventricle; the aorta is then tied to the cannula. The heart is now dissected out, together with a strip of tissue containing the right vago-sympathetic trunk, attached to which is the previously freed upper part of the spinal column.

The heart is now transferred to the damp chamber as in the figure, and the attached portion of spinal column is mounted on the supporting electrodes. One pair of electrodes, which are made of stout D.C.C. copper wire with platinum ends, is placed at the level of the third spinal root, one electrode being inside the spinal cord and the other resting on the outside of the vertebræ. On stimulation with a weak induction current pure sympathetic effects are obtained.

The other pair of electrodes is placed at the level of the medulla, one electrode being within the spinal canal and the other resting on the levator anguli scapulæ muscle. Stimulation of this end gives pure vagus effects if the current is weak enough to prevent spread.

To secure the survival of the nerves it has been found desirable to lead a stream of pure oxygen through the damp chamber, the atmosphere of which is kept saturated with water vapour by moist blotting paper fixed to the walls. The 1 c.c. of Ringer in the cannula is kept well stirred up by bubbling air through it. Under these conditions the heart will maintain a steady beat for many hours, the factor governing the useful life of the preparation being the time of survival of the nerves. This varies from a half to four hours. Frequently the effects of vagus and sympathetic stimulation are not lost simultaneously.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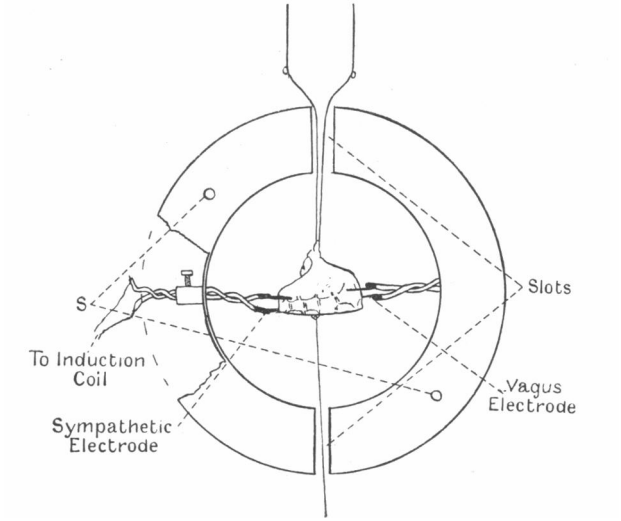
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The effect of morphia on the adrenaline content of the suprarenal glands. By G. P. CROWDEN and M. G. PEARSON.

Owing to the lack of uniformity in the reaction to morphia in cats a series of experiments was conducted in which the conditions under which the cats were kept were very carefully controlled, particularly in regard to temperature and quiet.

The following table shows that following an injection of 20 mg. of morphia there is no depletion of the innervated gland in relation to the denervated gland provided the animal is quiet, undisturbed and kept warm, whereas if the animal is loose in the laboratory in strange surroundings and thereby excited, considerable depletion of the innervated gland occurs.

Furthermore, if the animal is kept in cold air subsequent to the injection of morphia and is not disturbed, then again a depletion of the innervated gland occurs.

It would appear therefore that morphia alone does not cause exhaustion of the adrenaline content of the gland, but it certainly renders the animal more excitable, morphia + excitement causing a definite depletion of the gland. Again, morphia without excitement but with cold, causing a definite drop in body temperature, brings about a depletion of the innervated gland. The experiments were performed on male cats.

Exp.	Operative procedure	Conditions	Relative content of glands		Fall in body temperature (rectal)
			Denervated	Innervated	
1.	Left splanchnic nerves and nerves to left semilunar ganglion cut. 9 days	Injection 20 mg. morphia. Cage 8 hr. Quiet	100	96	—
2.	Ditto. 7 days	20 mg. morphia. Cage 8 hr. Quiet	100	104	—
3.	Ditto. 14 days	20 mg. morphia. Loose in laboratory for 7 hr. Excited	100	66·5	— —
4.	Ditto. 7 days	30 mg. morphia. Loose in laboratory for 5 hr. Excited	100	68·5	—
5.	Ditto. 18 days	20 mg. morphia. Cold air. Cage 3 hr. Quiet	100	74·5	38–32° C. =6° C.
6.	Left nerves severed by ligature immediately prior to injection	30 mg. morphia. Cold air. Cage 3 hr. Quiet	100	54	38–34° C. =4° C.

The expenses of this research have been defrayed by grants from the British Medical Association and the Medical Research Council.

The biological action of iodides. By JOHN FREUD.

In order to ascertain whether the action of thyroxin might be due to its slow conversion into iodides, the effect on the basal metabolic rate of minute amounts of iodine ion was studied. Slow intravenous injections of a 0·0006 p.c. solution of KI in Ringer's solution were made into chloralosed dogs in which the gaseous metabolism was measured. The amounts of iodide found to be efficient was 40γ (1/25 mg.), injected during half an hour, at the end of which time the metabolism of gases was once more measured. The determinations of the gaseous metabolism were carried out in three different ways. In a preliminary series[1] the tracheal cannula was temporarily connected (5–10 min.) with a large glass vessel of some 13 litres and the change of the composition of air in it was determined (Table I, Exps. 1–6, and *a–c*).

In the second series the inspired air was measured and samples of the expired air taken from a 10-litre Douglas bag were analysed (Table I, Exps. I–VIII, and *A–B*).

In the third series (Table II) the O_2 consumption was graphically registered by means of a Krogh's spirometer.

The three methods gave similar results, which are comparable with certain reservations. O_2 measure is the more reliable index of the change. The R.Q. is variable. In 20 out of 22 experiments the dogs reacted to the injection of KI into the jugular vein by an increase of the respiratory exchange within half an hour from the beginning of injection. Less or no response occurred to saphenous injection. The body temperature was carefully observed and kept as constant as possible; even when it fell slightly the increase of metabolism was still observed. In several cases trembling occurred. Of 8 thyroidectomised dogs 3 reacted to large doses.

Injection into the left jugular vein gives an average increase of O_2 consumption of 31·5 p.c. (Exps. 1–6) and 30·2 p.c. (Exps. I–VIII). In Exps. VI–VIII an increase of 52·6 p.c. occurs when KI is used, while equivalent quantities of Ringer's fluid alone have no appreciable effect. Injection into the saphenous vein on the day before or the day after the jugular injection, causes in Exps. 3–6 an average decrease of 3·7 p.c., in II–VIII (small dogs) an average increase of only 15·5 p.c. Ringer's fluid in V, VII, VIII gave a decrease of 1·74 p.c., *i.e.* negligible change. In Table II, when the jugular was used (5 dogs), the average increase was 40·1 p.c. and when the saphenous was used (as in β, γ, δ, ζ) only 3 p.c. Injection of KI into the jugular immediately after the saphenous

[1] Done at the Collège de France with collaboration of E. Czarnecki.

injection gave an increase of 37·4 p.c. (β, γ, δ, ζ), thus showing the importance of the way of administration. Thyroidectomised dogs *a*, *c*, *A*, *B*, show a decreasing or stable tendency (Table I) and *b* with a comparatively large dose an increase of 14 p.c. In Table II c_1 did not react, to 40γ b_1 reacted with 3·5 p.c. increase, to 80γ a_1 with 13 p.c. and b_1 with a slow increase from 14–31·5 p.c.

Table I.

Oxygen consumption in c.c. per minute and kilo.

			Injection into						
			Jugular			Saphenous			
Exp.		Dog	Before injection	Of Ringer	Of KI	Before injection	Of Ringer	Of KI	Remarks
1	♂	22·3 K	3·9	—	5·7	—	—	—	—
2	♂	13 „	6·5	—	9·6	—	—	—	—
3	♂♂	17 „	4·7	—	6·9	5·1	—	3·6	—
4	♂♀	20 „	5·6	—	6·5	4·9	—	4·9	—
5	♀♂	10·5 „	6·8	—	7·7	7·5	—	8·5	NaI used
6	♂	19·9 „	5	—	6·2	5	—	4·6	—
I	♂	9 „	6·7	—	5·1	—	—	—	—
II	♂♀	10 „	5·8	—	6·4	5·4	—	5·2	—
IV	♂♂	7·5 „	6·7	—	7·5	7·4	—	7·5	—
V	♂♀	7·5 „	—	—	—	7·2	7·4	8	—
VI	♀♀	7·5 „	10·3	9·1	18·2	8·3	—	11·6	—
VII	♀♀	7·5 „	8·1	9·5	12·3	9·9	10·3	13	—
VIII	♀	6·5 „	9·7	9·3	12·1	11·3	11·9	10·1	—
a	♀	15·3 „	6·7	—	6	—	—	—	65 γ
b	♂	12·7 „	7·2	—	8·2	—	—	—	70 γ
c	♂	25·2 „	4·6	—	4·4	—	—	—	79 γ
A	♀	9 „	14·1	—	12·1	—	—	—	Tetany
B	♀	10·8 „	5·8	—	5·9	—	—	—	—

Table II.

Percentage difference in O_2 consumption as found with Krogh's Spirometer.

			Injection into					
			Jugular			Saphenous		
Exp.		Dog	Of Ringer	1st KI	2nd KI	Of Ringer	KI	Jugular after saphenous
a	♀♀	15·5 K	0	28	—	—	—	—
β	♀♀	8·5 „	0	62	—	0	0	20
γ	♀♀	9·2 „	−6·6	6·6	33·3	—	4·3	17·4
δ	♀♂	12 „	—	37·5	—	—	7·6	41·6
ϵ	♂♂	11·5 „	—	66·6	—	—	—	—
ζ	♂♀	14·2 „	—	—	—	—	−2·9	70·6
η	♀	9·5 „	—	—	—	—	−2·2	−27·2
a_1	♀	9·5 „	—	13	—	—	—	80 γ
b_1	♀	29·5 „	—	3·5	14	—	—	15 min. later 31·5
c_1	♂	16 „	—	0	0	—	—	2nd injection 80 γ

A smooth muscle vagus nerve preparation.

By M. RABINOVICH.

Experiments were made with smooth muscle nerve preparations, the smooth muscle strip being taken from the fundus and body of the stomach or the œsophagus with the vagus nerve attached. Under ether anæsthesia the stomach and œsophagus were removed. The vagus was identified at the upper part of the œsophagus and dissected down. The strip of muscle to which the nerve was traced was separated from the mucous membrane and its ends fixed by means of Michel clips. The preparation was left in Tyrode solution at 37° C. for half an hour and then placed in a muscle chamber as previously described (1). The chamber was filled with Tyrode solution maintained at 37° C. and at *p*H 7·5 by means of a thermostat and a constant current of oxygen and carbon dioxide bubbled through it. The muscle was attached to an isometric lever fitted with a small galvanometer mirror and optical records were taken on stimulation of the

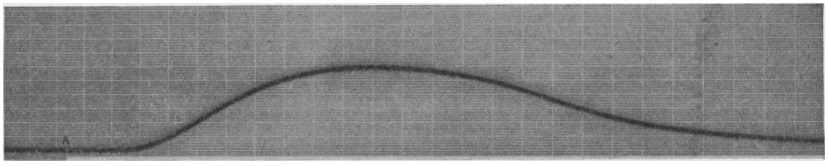


Fig. 1. Response of circular strip of smooth muscle of cat's œsophagus to single induction shock. Time intervals = 0·2 sec. *A* = point of stimulation.

nerve fibre. A time marker recorded 0·2 sec. intervals and a signal marker was in series with the stimulating current. The nerve was stimulated by means of platinum electrodes kept outside the solution.

The make or break of a constant current has so far not proved capable of causing stimulation. A single induction shock causes a contraction in a minority of cases. Faradic stimulation causes a contraction in practically all the preparations made. The latent periods are given in Table I. They are fairly constant for a given preparation but vary over a fairly wide range for different strips of muscle. The shortest interval recorded was 0·38 sec.; the longest latent periods were observed when only a small response was obtained. With a single induction shock a smooth curve is obtained; contraction lasts 1·5–2·5 sec. and is fairly constant for a given strip. Relaxation is slower and takes 5 or more seconds to complete, though the first part is more rapid. Half relaxation occurs within 1·5 sec. Faradic stimulation of a circular preparation gives

a response similar to that obtained with a single shock. The contraction lasts 1·5–3 sec. Relaxation is again much slower. Continuation of the stimulation appears to delay the relaxation. The longitudinal muscle gives a more sustained contraction with faradic stimulation; the contraction seems to consist of a series of waves superimposed upon one another, lasting for 30 sec. or more when relaxation slowly occurs.

A preparation is very quickly fatigued and even after a short period of stimulation it is necessary to allow a few minutes rest, otherwise a smaller response with a longer latent period is obtained. Repeated stimulation rapidly abolishes the response.

Preparations of the striped muscle of the upper end of the cat's œsophagus with the recurrent laryngeal nerve give a typical striped muscle response. Strips from intermediate regions give a mixed response in which the striped and unstriped elements can be clearly discriminated.

Table I

Preparation	Latent period sec.	Number of observations	Type of stimulation
Circular œsophagus	Average: 0·55 Longest: 0·75 Shortest: 0·38	33	Faradic
Circular œsophagus	Average: 0·52 Longest: 0·7 Shortest: 0·4	8	Single induction shock
Longitudinal œsophagus	Average: 0·47 Longest: 0·39 Shortest: 0·52	7	Faradic
Stomach	Average: 0·7 Longest: 0·65 Shortest: 0·75	6	Faradic

1. McSwiney and Newton. This Journ. 63. p. 51. 1927.

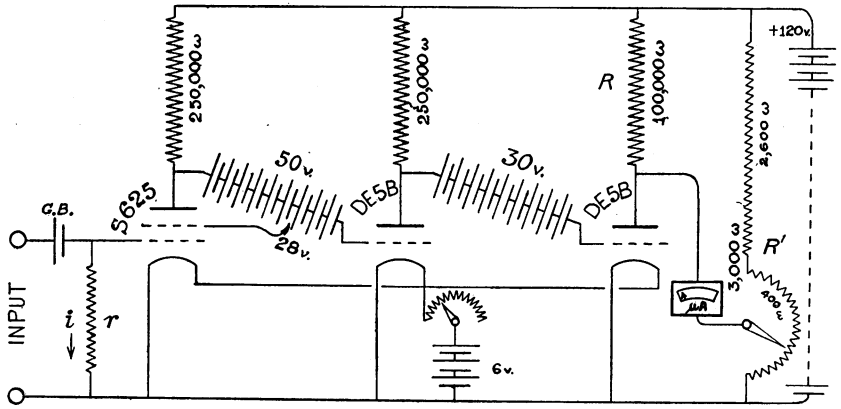
Three-stage amplifier for bioelectric currents and potential differences. By D. T. Harris.

This assembly was demonstrated at the annual meeting of the Society when the variations in P.D. of an amphibian heart were shown on a portable galvanometer giving full scale deflexion when used as a micro-ammeter.

The general design of the amplifier follows standard practice (1) and the magnitudes of the various electrical quantities have been estimated with a view to stability and ease of working. When applied to biological tissues no screening or earthing will be found necessary on account of the heavy damping thus introduced into the grid circuit of the first valve;

wire-wound anode resistances with low distributed capacity should be chosen and accumulators, in virtue of their low internal resistance and their steady E.M.F., are advisable—the large WH (Exide) type for the main H.T. battery and the small WJ for the coupling batteries. Under these circumstances oscillation will be avoided and the zero will be found remarkably steady—no change being seen on a C.S.I. pointer galvanometer reading 1 division per $\frac{1}{2}$ micro-amp. The amplifier has not been tested on a more sensitive instrument since its sole object is to render the minute electrical changes of living tissues visible on a handy type of robust recording instrument.

When used for an A.C. input the mode of working is apparent. On the application of a direct current i across the grid leak r (2 megohms is



a suitable value) there is a voltage drop at the control grid of magnitude ir which alters the plate current of the first valve and a similar enhanced change occurs in the subsequent stages, part of the change in plate current of the last valve being indicated by the micro-ammeter μA. By making R large compared with R' the bulk of the plate current change is diverted through the galvanometer.

If a high magnification is required, the more usual DE5B valve (Marconi) in the first stage may be replaced, as shown in the figure, by an S625 valve (2), the screen being led off to the + 28 v. tapping on the coupling battery; if still more magnification is required, a similar replacement may be effected in the second stage also, or another method may be tried, namely, that of giving the first valve an independent large H.T. battery and coupling its anode resistance at the anode end only, by means of the coupling battery; by this latter method the valve can be made to operate under its optimum conditions.

The inclusion of a potentiometer in the grid circuit of the first valve is a useful means of setting the grid bias G.B. (usually about 0 to $-\frac{1}{2}$ volt) which is rather critical; the permissible range is not a large one, and this is not essential since only small variations can be applied to so powerful an amplifier. The potentiometer serves the further useful purpose of a quick and ready means of calibrating the amplifier. It is advisable, as with other amplifiers, to keep the grid leads as short as possible and to place the valve with the largest emission in the last stage. The inclusion of a micro-ammeter in the grid lead solves any difficulty in adjusting the voltage of the coupling battery.

For D.C. inputs and A.C. of frequencies up to half a million per sec. this amplifier is practically distortionless; for extremely high frequencies the resistances and valve inter-electrode capacities will constitute sources of distortion.

1. Bovie, Chaffee and Hampson. Journ. Optical Soc. Amer. 7. p. 1. 1923.
Adrian, E. D. This Journ. 62, p. 49. 1926. Ergeb. d. Physiol. 27. p. 501. 1928.
2. Round, H. J. The Shielded Four-electrode Valve. (Cassell & Co., 1927.)