II.—Studies on the Pharmacological Action of Tetra-Alkyl-Ammonium Compounds. By Professor C. R. Marshall.

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I. THE ACTION OF TETRA-METHYL-AMMONIUM CHLORIDE.

In a paper on "The Pharmacological Action of Protocatechyl-tropeine," communicated to the Society in 1909,* I drew attention to the fact that this substance, when injected intravenously in certain doses produces transient paralysis of the respiration; and I mentioned further that TAPPEINER† had described a similar temporary cessation of the respiration after the intravenous injection of certain quaternary isoxazol and pyrazol compounds, and of tetra-methyl-ammonium chloride, and POHL,‡ after the intravenous injection of some quaternary papaverine derivatives. TAPPEINER came to the conclusion that the effect was due to stimulation of the terminations of the fifth cranial nerve in the nose; that it was, in fact, of the nature of a Kratschmer-Hering reflex, since he was unable, in the case of methyl-phenyl-isoxazol-methochloride, to produce cessation of the respiration after anæsthetising the nasal mucous membrane with cocaine; and IODLBAUER,§ working in TAPPEINER'S laboratory, also found that anæsthetisation of the nasal mucous membrane prevented the cessation of the respiration produced by tetra-methyl-ammonium chloride. POHL, on the other hand, was able to produce this temporary paralysis of the respiration after section of the ophthalmic branches of both fifth nerves, and consequently he concluded that the effect was due to an action on the respiratory centre. I came to the same conclusion, since the effect was still obtained with protocatechyl-tropeine after section of both fifth nerves in the base of the skull and after section of both phrenic nerves, and was not synchronous with the effect on the circulation or with the paresis of the nerve-endings in the muscles of the hind limbs. Further work with tetra-methyl-ammonium chloride, however—my stock of protocatechyl-tropeine being exhausted,—showed that the effect was in large measure peripheral and due to a transient paresis of the nerve-endings in the respiratory muscles.

ACTION ON MOTOR NERVE-ENDINGS.

The effect of tetra-methyl-ammonium compounds on the motor nerves was first observed by CRUM BROWN and FRASER.|| They showed that the iodide when injected into frogs produced muscular paralysis due to an action on the myo-neural junctions;

* *Trans.*, xlvii., pt. ii. p. 273 [1909–10].

† *Arch. f. exp. Path. u. Pharm.*, xxxvii. p. 325 [1896].

‡ *Arch. Internat. de Pharmacod.*, xiii. p. 479 [1904].

§ *Arch. Internat. de Pharmacod.*, vii. p. 183 [1900].

|| *Proc. Roy. Soc. Edin.*, vi. p. 556 [1869].

and their observations were corroborated by RABUTEAU* and BRUNTON and CASH.† Later, DUFAUX,‡ SANTESSON and KORAEN,§ and IODLBAUER|| showed that the same effect was obtained with tetra-methyl-ammonium chloride.

So far as I am aware, no experiments demonstrating this action on mammals have been described. RABUTEAU,¶ in the two experiments he made with tetra-methyl-ammonium iodide on dogs, observed muscular paralysis as one of the symptoms; and BRUNTON and CASH,** after injecting 1 g. of the same compound into a rabbit, found the animal lying apparently paralysed two minutes after the administration. When half this dose was injected, they noticed the head falling to one side two and a half minutes afterwards, but one and a half minutes later violent movements of the limbs occurred. IODLBAUER,†† working with the chloride, had similar experiences. The animal usually fell on its side, and there were frequently convulsive movements and later fibrillary tremors. JACOB and HAGENBERG‡‡ also noticed muscular paralysis after tetra-methyl-ammonium tri-iodide. But none of these observers appears to have investigated the cause of the paralytic symptoms.

My own experiments have been made on rabbits and cats during a state of anaesthesia or after excision of the cerebral hemispheres. The rabbits were anaesthetised with ether, or, in the case of animals to be decerebrated, with a mixture of chloroform and ether; the cats with chloroform followed by ether. The blood-pressure and respiration were recorded, and one of the nerves supplying a fore or hind limb was isolated and stimulated continually during an injection or intermittently before and after an injection. The contraction of the limb was recorded by inserting a hook into the limb and connecting this by thread working over pulleys to a writing lever. This method of recording the contractions of the limb was found better for the purpose in view than that of recording the contractions of individual muscles, probably owing to less interference with the blood supply. The injection of the substance was made in the case of rabbits into the right anterior facial vein and in the case of cats into the right external jugular vein.

Both in rabbits and cats the intravenous injection of 0.5 to 1 mg. p. kg. body-weight diminishes the excitability and often paralyses the motor nerve-endings, the muscles themselves still retaining their irritability, over the whole of the body. Rabbits seem to be somewhat more susceptible than cats, but otherwise the effects on the two animals are identical. When the injections are made into the facial or external jugular vein, the muscles of the fore part of the body are earliest and most markedly affected. After a dose of 1 mg. p. kg. moderately quickly injected, the nerve endings in these muscles are more or less paralysed within ten seconds of the commencement of the injection, and in susceptible animals may remain paralysed for several minutes. Fairly quick recovery then occurs.

* *Compt. rend.*, lxxvi. p. 887 [1873].

† *Trans. Roy. Soc. Lond.*, clxxv. p. 215 [1884].

‡ *Dissert. Berlin.*, 1888, quoted by SANTESSON and KORAEN, p. 220.

§ *Skand. Arch. f. Physiol.*, x. p. 220 [1900].

|| *Loc. cit.*, p. 185.

¶ *Loc. cit.*

** *Loc. cit.*, p. 209.

†† *Loc. cit.*, p. 189.

‡‡ *Arch. f. exp. Path. u. Pharm.*, xlviii. p. 48 [1902].

FIG. 1.—Effect of tetra-methyl-ammonium chloride on the nerve-endings of right fore-limb muscles and diaphragm. Cat. 3000 g. Ether. L=contractions of right fore limb produced by stimulation of divided musculo-cutaneous nerve; R=respiration (upstroke=inspiration) showing effect of stimulating right phrenic nerve; P=blood-pressure in right carotid artery; H=Hurtle's feder-manometer; b=blood-pressure by Hurtle's feder-manometer; t=time in seconds. The line above t shows time of stimulation of nerves with secondary coil at 40 cm. Depression on line below t shows time of injection. At 3.32 artificial respiration was performed for two minutes. $\times \frac{1}{2}$.

The action on the fore limbs of a cat is shown in the uppermost tracings (L) of figs. 1 and 2. Both figures are taken from the same experiment. Previous to the injection shown in fig. 1 an injection of half the dose of tetra-methyl-ammonium chloride had been given. The first figure shows the results of intermittent stimulation of the musculo-cutaneous nerve with the secondary coil at 40 cm., the primary

FIG. 2.—Effect of tetra-methyl-ammonium chloride on contraction of fore limb produced by continuous stimulation of musculo-cutaneous nerve. Same animal as fig. 1. Letters as in previous figure. $\times \frac{1}{3}$.

current being obtained from an accumulator with an E.M.F. of two volts. The wavy appearance of the earlier part of the tracing is due to the presence of small spontaneous rhythmical contractions of the limb. Almost immediately after the cessation of the injection these spontaneous movements ceased and stimulation of the nerve a few seconds later produced a scarcely appreciable effect. In this case total paralysis to this strength of stimulus lasted six minutes, then recovery gradually occurred; but the nerve did not regain its former irritability, and the stimulus was consequently increased.

The effect of stimulation with the secondary coil at 30 cm. is shown in fig. 2, which also shows the action of the same dose of tetra-methyl-ammonium chloride during continuous stimulation of the nerve. The cessation of the contraction seven seconds after the commencement of the injection is striking. The method of continuous stimulation does not, however, show recovery from the effects of the drug owing to fatigue being so easily induced. For this purpose intermittent stimulation is necessary.

When strong or moderately strong intermittent stimuli are employed, complete paralysis of the nerve-endings may not be observed after the injection of 1 mg. p. kg. body-weight of tetra-methyl-ammonium chloride. Each stimulation after the admini-

FIG. 3.—Contractions of fore limb of rabbit produced by stimulation of musculo-cutaneous nerve during recovery from tetra-methyl-ammonium chloride. For description see text.

stration of the drug may produce a well-marked, although less than normal, contraction; but the contraction is not maintained, and notwithstanding the continuance of the stimulus, quickly falls to or near the abscissa (fig. 3). This contraction, indeed, may be so short as to resemble a simple twitch. This effect of apparent rapid exhaustion was first shown by BOEHM* in the case of curarin.

Occasionally another effect—a staircase effect—is obtained by stimulating a nerve during recovery from the paralysing action of tetra-methyl-ammonium chloride, and this, combined with the rapid exhaustion of the nerve-endings, produces a curious, humped-back form of curve. This is shown in fig. 3. The animal, a rabbit, was anaesthetised with ether; the brain was excised at 10.43, and the anaesthetic discontinued at 11.0. The musculo-cutaneous nerve was isolated and divided, and the

* *Arch. f. exp. Path. u. Pharm.*, xxxv. p. 16 [1894].

movements of the limb were registered as in the previous experiment. Stimulation with the secondary coil at 26 cm. produced a well-marked contraction, sometimes with a slight staircase phenomenon. An injection of 1 mg. tetra-methyl-ammonium chloride was made into the right facial vein at 12.3, and after preliminary convulsive contractions, produced paresis of the nerve-endings. Stimulation of the nerve at 12.7 with the secondary coil at 26 cm. produced a simple muscle twitch. At 12.11, with the coil at 22 cm., a slight sustained contraction was obtained, and at 12.19, with the coil at 20 cm., the divided humped-back contraction shown at (a). The effect is shown better at (b), the curve obtained by continuous stimulation of the nerve with the same strength of stimulus at 12.21.

The cause of the staircase phenomenon in tetra-methyl-ammonium chloride poisoning has not been further investigated. It differs from that produced under physiological conditions by adjusted repetitions of a stimulus in being preceded by a well-marked rapid contraction which, in some cases, exceeds in height the summit of the staircase, thus resembling in form the initial contraction and reflex rebound contraction observed on stimulating an uncut mixed nerve with a single induction shock,* and in the muscle requiring a much longer period of rest before it can be reinvoked. As is seen in (a), a repetition of the stimulus fifteen seconds after the summit of the staircase has been reached, is followed by no staircase phenomenon, and this is also true if the stimuli are repeated at half-minute intervals. In this case the only observable difference shown by each successive curve from that of the latter half of (a) is a more rapid exhaustion. It is not until an interval of one to one and a half minutes between the successive stimulations is allowed that the staircase effect begins to reappear.

In one experiment the spinal accessory was isolated and stimulated intermittently, along with the phrenic nerve, and the contractions of the sterno-mastoid muscle recorded before and after injection of tetra-methyl-ammonium chloride. The effects obtained were so similar to those observed in the case of the fore limb that further description is unnecessary.

In the case of a hind limb the effects observed after the injection of tetra-methyl-ammonium chloride from stimulation of the sciatic or crural nerve are similar to those occurring in a fore limb; but when the injections are made into the external jugular vein the effects are less marked, and are later in appearing. Thus in a rabbit anaesthetised with ether the injection of 1 mg. tetra-methyl-ammonium chloride into the external facial vein merely caused gradual diminution in the contractions of the hind limb, produced by stimulation of the sciatic nerve, for two minutes, at the end of which time only a slight twitch was obtained from continued stimulation. Recovery commenced one and a half minutes later, and was nearly complete six minutes after the injection. The respiration, on the other hand, rapidly diminished in extent, and had almost ceased eleven seconds after the commencement of the injection. It began to increase ten seconds later, and was normal a minute after the beginning of the injection.

* Cf. SHERRINGTON, *Journ. of Physiol.*, xxxvi. p. 202 [1907].

Similarly, when the injection of tetra-methyl-ammonium chloride was made during stimulation of the sciatic nerve, the fall of the muscle lever was gradual, and it did not reach the abscissa before the respiration had recovered from its paralysis. The same want of synchronism between the respiratory paralysis and the paralysis of the hind limbs occurred after the injection of protocatechyl-tropeine, and led me to minimise the peripheral origin of the respiratory paralysis.

The effect of tetra-methyl-ammonium chloride on the terminations of the phrenic nerve was determined in a number of instances, and found to be similar to that on the nerves of the fore limbs. It will be referred to in considering the action of this substance on the respiration.

Effect on Decerebrate Animals.—After division of the brain through the quadrate bodies and crura and delay for half to three-quarters of an hour after cessation of the anæsthetic to allow of its excretion, the intravenous injection of tetra-methyl-ammonium chloride produces in cats, less commonly in rabbits, immediate short clonic convulsions (fig. 4). These convulsions may terminate in death. If not, the convulsions cease or almost cease, and more or less paralysis of the motor nerve-endings may be demonstrated. Even if convulsions do not occur, increased excitability can be shown to be present for a few seconds after the injection by blowing on the animal. The reflex response to this form of excitation is markedly increased immediately after the injection, and is still present when the respiration has ceased (fig. 5). It quickly diminishes, however, and a few seconds later no response can be elicited. The slight normal response returns with or soon after the recommencement of the breathing, but no distinct increased excitability occurred in the experiments in which this form of stimulation was tried until another injection was made. Occasionally slight convulsive movements were noticed in anæsthetised animals after a second or later injection.

Most observers who have investigated the general effects of tetra-methyl-ammonium compounds on mammals mention convulsions as a prominent symptom. BRUNTON and CASH,* for example, state that after the injection hypodermically of 0.2 g. tetra-methyl-ammonium iodide to a rat, the animal was affected at once with powerful convulsions; and similar results with much smaller doses of the chloride on mice and guinea-pigs were observed by IODLBAUER.†

The seat of origin of the convulsions has not been determined. The simple response to external stimulation (fig. 5) points to the spinal cord being involved; but as the convulsions exhibit a similar form to those produced by drugs acting on the basal ganglia, it is probable that these structures are also affected, and as such drugs also act on the motor areas of the cerebrum, these centres may be influenced as well.

Effect on Frogs.—In the frog convulsions are not produced by the injection of tetra-methyl-ammonium chloride into a lymph sac. The predominant symptom in these animals is muscular paresis or paralysis. After a minimal paralytic dose the respiratory movements cease within two minutes, and a minute later the animal is

* *Loc. cit.*, p. 207.

† *Loc. cit.*, p. 190.

unable to turn over when laid upon its back. The effect is upon the nerve-endings mainly as may be shown by electrical stimulation of the nerves and muscles after the intoxication.

According to IODLBAUER* the minimal paralytic dose for the edible frog is 0.005 mg.

FIG. 4.—Clonic spasms of fore limb of decerebrate cat produced by 2 mg. tetra-methyl-ammonium chloride intravenously forty-five minutes after excision of cerebrum and cessation of anaesthetic. Injection shown at X. Time in seconds.

FIG. 5.—Reflex excitability during paralysis of respiration in decerebrate rabbit after intravenous injection of 2 mg. tetra-methyl-ammonium chloride. Rabbit. 2200 g. Cerebrum excised 2.50. Both vagi cut. Atropine 5 mg. injected at 4.4. At points marked X animal was sharply blown upon. Letters as in previous figures.

p. g. body-weight; according to SANTESSON and KORAEN† 0.01 mg. p. g. body-weight. The latter observers give as the minimal paralytic dose for the grass frog 0.12 mg. p. g. body-weight. My own experiments with smaller frogs indicate that it is 0.08–0.09 mg. p. g. body-weight.

DUFAUX‡ and IODLBAUER§ describe fibrillary contractions as occurring in frogs

* *Loc. cit.*, p. 186.

† *Loc. cit.*, pp. 221, 222.

‡ *Loc. cit.*

§ *Loc. cit.*, p. 188.

after tetra-methyl-ammonium chloride. They do not appear to have been noticed by SANTESSON and KORAEN,* and they were not observed in my experiments with winter frogs. On the contrary, I found that this substance would inhibit or prevent the fibrillary contractions produced by tetra-methyl-ammonium chloride.

They occurred, however, to a slight extent when an isolated muscle was steeped in certain concentrations of tetra-methyl-ammonium chloride, and I have frequently seen fibrillary twitchings in rabbits occurring both spontaneously and as the result of stimulating a nerve. To this point I shall return in a later communication.

FIG. 6.—Effect of 1 mg. tetra-methyl-ammonium chloride intravenously on respiration and blood-pressure of etherised rabbit. Weight 1650 g. Letters as in previous figures.

EFFECT ON RESPIRATION.

In anæsthetised animals the most interesting effect on the respiration is the temporary paralysis which follows the intravenous injection of certain doses of this drug. The effect is most typically seen in rabbits, and is shown in fig. 6. The effect of a larger dose on a cat is seen in fig. 1. As will be observed, the respiration ceases or almost ceases a few seconds after the injection of the substance, and some seconds later recommences, at first slightly and somewhat less frequently than normally, and then with increasing depth and frequency until the normal is reached. The dose required to produce the effect is 0.5 to 1 mg. p. kg. body-weight intravenously, and it is necessary to make the injection moderately quickly (in about five seconds) in order to obtain for a sufficiently brief interval the proper concentration of the substance in the

* *Loc. cit.*, p. 220.

blood. If the injection is made much more slowly, greater or less retardation and shallowness, but not actual stoppage, of the respiration may be produced. If larger doses are employed and rapidly injected, the respiration is quickly and completely paralysed, and it usually fails to restart spontaneously; or, if it does commence again, it remains inefficient. In such cases artificial respiration is generally able to reinstate the respiration unless the dose administered has been excessive.

Effect on Decerebrate Animals.—On decerebrate animals, if time has been allowed for the influence of the anæsthetic to pass away, the effect of tetra-methyl-ammonium chloride on the respiration is, in some respects, different from that described as occurring in anæsthetised animals. In the latter no preliminary stimulation of the respiration, beyond a slight increase in depth of one or two respirations, occurs with any dose of the drug; in the former this effect is a common feature of its action. And not only may there be the appearance of stimulation before the paralysis occurs, but also the recovery from the paralysis is quicker and is often accompanied by greater respiratory activity. Further, in some decerebrate animals the quantity of tetra-methyl-ammonium chloride necessary to cause respiratory paralysis has been several times that required for anæsthetised animals; and in these animals especially the stimulant effect on the respiration has been very marked. If, however, the decerebrate animal is lightly anæsthetised, the effects are similar to those seen in anæsthetised but otherwise normal animals.

The stimulating effect on the respiration of a decerebrate animal of a dose of tetra-methyl-ammonium chloride usually sufficient to paralyse the respiration of an anæsthetised animal, and the paralysing effect of a large dose, are shown in fig. 7. The animal, a rabbit (2450 g.), was anæsthetised with ether containing a little chloroform. At 11.3 the brain above the corpora quadrigemina was removed, and between 11.10 and 11.20 the fifth cranial nerves were cut proximal to the Gasserian ganglia. The anæsthetic was then stopped. At 11.36, 0.6 mg. tetra-methyl-ammonium chloride was injected into the right facial vein, with much the same result as is shown in the first part of the figure. At 11.44½, 1 mg. was injected, and, as is seen, the respiration was powerfully stimulated. No depression or paralysis occurred. At 11.58 the same dose was injected more slowly, and a similar but less marked effect resulted. Before the effects of this dose had passed away, 2 mg. was injected, and further transient stimulation followed by temporary paralysis obtained. Subsequently 5 mg. was injected, with the result shown in the second part of the figure. Occasional shallow respiratory movements occurred later, but the respiration failed to re-establish itself and the animal succumbed.

As the effects obtained on decerebrate animals were slightly variable, and as the matter is of some importance for the purposes of this paper, a summary of twelve experiments made on rabbits is given. The animals were anæsthetised with ether, and the anaesthesia maintained with a mixture of ether and chloroform until the mid-brain was divided and the cerebrum removed. In some experiments the fifth cranial nerves

were divided close to the Gasserian ganglia; in one experiment the ganglia were removed. The anæsthetic was then stopped. Cannulæ were introduced into the right common carotid artery and the right anterior facial vein, for the purposes of recording the blood-pressure and injecting the substance respectively. The respiration was recorded by attaching a phrenograph or one limb of the tracheal cannula to a recording tambour. The time occupied by the injections, unless otherwise stated, was about five seconds.

FIG. 7.—Effect of tetra-methyl-ammonium chloride on respiration and circulation of decerebrate rabbit. Both fifth cranial nerves cut. Letters as in fig. 1. $\times \frac{1}{2}$.

Experiment I.—Rabbit, decerebrate. Weight, 2300 g. Anæsthetic stopped, 10.45.

11.14. 2 mg. tetra-methyl-ammonium chloride injected. Almost complete and immediate cessation of respiration for three seconds, followed by increased depth and frequency for ten seconds, and succeeded by a second almost complete paralysis lasting twenty-five seconds, then quick and good recovery.

The blood-pressure (62 mm. Hg) showed a transient rise to 78 mm. Hg, followed by a moderately quick fall to 36 mm. Hg, with slowing of the frequency of the heart, and then a moderately quick rise to 111 mm. Hg. This rise, in turn, was followed by a gradual fall to 38 mm. Hg (at 11.18), and the fall by a gradual return to the normal height.

- 11.24. 2 mg. again injected. Respiration quickly depressed and paralysed for three seconds, followed by quick recovery and subsequently increased frequency and depth.

The blood-pressure (70 mm. Hg) showed a very slight preliminary rise, followed by a fall to 39 mm. Hg and a secondary rise to 66 mm. Hg, and subsequent fall to 34 mm. Hg at 11.26.

Experiment II.—Rabbit, decerebrate. Weight, 2100 g. Anæsthetic stopped, 11.30.

Very low initial blood-pressure (26 mm. Hg).

- 11.44. 1 mg. tetra-methyl-ammonium chloride into right external jugular vein. Slight gradual depression of respiration and then (thirty-five seconds after the injection) rapid paralysis. The blood-pressure gradually fell to 11 mm. Hg. Artificial respiration was performed for three minutes. The respiration was re-established, and the blood-pressure rose to 104 mm. Hg.
- 11.52½. 1 mg. again injected. Rapid and almost complete paralysis of respiration six seconds after commencement of injection with rapid recovery eleven seconds later. The blood-pressure fell from 106 mm. Hg to 95 mm. Hg, and, after a transient rise to 102 mm. Hg, fell to 91 mm. Hg.

Experiment III.—Rabbit, decerebrate. Weight, 2025 g. Anæsthetic stopped, 3.5.

- 3.31. 1 mg. tetra-methyl-ammonium chloride injected. Rapid, shallow, and irregular respirations almost immediately followed. Sixteen seconds later deep convulsive respirations occurred and were replaced, after thirty-five seconds' interval, by deep, regular respiration.

The blood-pressure (80 mm. Hg) fell at first to 56 mm. Hg, and the heart-beats diminished to less than half their previous frequency. Five seconds before the commencement of deep breathing, the blood-pressure commenced to rise, and rose to 123 mm. Hg. With the onset of good regular breathing it gradually fell to 89 mm. Hg.

- 3.37. 1 mg. again injected. Caused almost immediate paralysis of the respiration followed by gradual recovery.

The blood-pressure fell from 84 mm. Hg to 58 mm. Hg, and the heart-beats from 13 to 5 in four seconds. The blood-pressure then rose to 103 mm. Hg and the heart-beats to $6\frac{1}{4}$ in four seconds. At 3.39 the blood-pressure was 79 mm. Hg and the heart's frequency $9\frac{1}{2}$ in four seconds.

- 3.45. A third similar injection produced the same effects, but these were accompanied by marked muscular tremors.

Experiment IV.—Rabbit, decerebrate. Weight, 1850 g. Fifth cranial nerves cut distally to Gasserian ganglia. Anæsthetic stopped, 2.54. Respiration irregular in rhythm.

- 3.18. 0.5 mg. tetra-methyl-ammonium chloride injected. Transient stoppage of respiration followed by deeper, regular, and more rapid respiration. The blood-pressure fell from 80 mm. Hg to 68 mm. Hg, and with the onset of respiration rose to 108 mm. Hg. It then gradually fell to 72 mm. Hg (at 3.19½).
- 3.20. 0.5 mg. again injected. Produced only slight depression of respiration. The blood-pressure was influenced as by previous injection, but to a somewhat less extent.
- 3.21. 1 mg. injected. Produced paralysis of respiration for twenty seconds although blood-pressure was less influenced than by previous injections.

Experiment V.—Rabbit, decerebrate. Weight, 2000 g. Both fifth cranial nerves cut. Anæsthetic stopped, 11.0.

- 11.26. 2 mg. tetra-methyl-ammonium chloride injected. Respiration almost ceased for two seconds immediately after injection, then increased in depth and frequency for one minute, and subsequently returned gradually to normal.
The blood-pressure (82 mm. Hg) rose slightly during the injection, and then, with marked slowing of the heart's frequency, fell rapidly to 49 mm. Hg for a few seconds. A gradual rise to 71 mm. Hg followed, succeeded by a gradual fall to 51 mm. Hg and a gradual return to normal at 11.30.
- 11.36. 1 mg. atropine injected. Slight transient stimulation of respiration and slight increase in frequency of heart.
- 11.37. 2 mg. tetra-methyl-ammonium chloride injected. Respiration almost ceased for one second, then increased in frequency and gradually in depth for one minute, after which it gradually returned to normal.
The blood-pressure showed successively a transient rise to 85 mm. Hg, a fall to 74 mm. Hg, a rise to 108 mm. Hg (at 11.38), a gradual fall to 67 mm. Hg (at 11.39).
- 11.40. 1.5 mg. tetra-methyl-ammonium chloride injected. Effects similar to previous injection, but somewhat greater diminution of frequency of heart.

Experiment VI.—Rabbit, decerebrate. Weight, 2250 g. Both fifth cranial nerves cut. Anæsthetic stopped, 12.0.

- 12.21. 1 mg. atropine injected slowly.
- 12.25. 2 mg. tetra-methyl-ammonium chloride injected. Twelve seconds after commencement of injection respiration increased markedly in depth and some-

what in frequency. There was a simultaneous rise (occupying six seconds) of blood-pressure from 114 mm. Hg to 150 mm. Hg, and after maintaining this level for twenty seconds it gradually fell. The effect on the respiration continued somewhat longer than that on the blood-pressure. The frequency of the heart was not affected.

12.27. 2 mg. again injected. Respiration affected as after previous injection, but increase in depth somewhat greater. The blood-pressure fell from 124 mm. Hg to 102 mm. Hg for a few seconds, then rose to 156 mm. Hg, which was maintained for twelve seconds. It then fell, at first quickly, afterwards slowly, to 102 mm. Hg at 12.28½.

12.29. 10 mg. tetra-methyl-ammonium chloride injected. Respiration completely paralysed seven and a half seconds after commencing the injection. No recovery occurred.

Experiment VII.—Rabbit, decerebrate. Weight, 1850 g. Both fifth cranial nerves cut, the right not completely, proximal to the Gasserian ganglia. Anæsthetic stopped 10.50.

11.21. 2 mg. tetra-methyl-ammonium chloride injected. Marked increase in depth of respiration commencing six seconds after beginning of injection and lasting four seconds and passing on to complete paralysis five seconds later. Spontaneous recovery did not occur, and artificial respiration only established infrequent respirations.

The blood-pressure (96 mm. Hg) rose slightly and then fell rapidly to 56 mm. Hg. Afterwards it rose to 98 mm. Hg and then fell to near the base line.

Experiment VIII.—Rabbit, decerebrate. Weight, 1775 g. Left fifth cranial nerve cut. Anæsthetic stopped, 11.30.

11.59. 1 mg. tetra-methyl-ammonium chloride injected. The respirations trebled in frequency a few seconds after the commencement of the injection, but rapidly diminished in depth and ceased ten seconds after beginning the injection. The paralysis lasted eleven seconds. After recommencing, the respiration quickly became efficient and remained somewhat more rapid although less deep for two minutes before gradually returning to normal.

The blood-pressure (95 mm. Hg) showed a transient rise, then a quick fall to 76 mm. Hg, which was succeeded by a slight rise and then a gradual fall, concurrent with the recommencement of the breathing, to 56 mm. Hg. Recovery to the normal took several minutes.

12.4½. Injection repeated with similar result.

12.29. 1 mg. again injected. A few very powerful respirations almost immediately followed, but the respirations quickly diminished in depth and ceased

twelve seconds after the commencement of the injection. The paralysis continued for nine seconds, and then quickly became approximately normal for about six seconds, after which it became somewhat convulsive in type.

The blood-pressure (104 mm. Hg) exhibited a transient rise, then a fall to 77 mm. Hg. With the recommencement of the respiration a slight transient rise occurred, followed by a fall to 39 mm. Hg, which was maintained.

Experiment IX.—Rabbit, decerebrate. Weight, 1800 g. Both fifth cranial nerves cut, but left not completely. Anæsthetic stopped, 11.0.

11.39. 1 mg. tetra-methyl-ammonium chloride injected. Respiration increased in frequency three seconds after the beginning of the injection, but diminished in extent, and four seconds later ceased. The paralysis lasted nine seconds, then respiration recommenced and rapidly became efficient, the depth being somewhat less than, but the frequency double, that before the injection. The increased frequency continued for two minutes, then gradually passed away.

The blood-pressure (73 mm. Hg) fell to 56 mm. Hg, and then commenced to rise with the complete cessation of the respiration and continued until it reached 104 mm. Hg, just at the time when the respiration had become well re-established. It then fell gradually to 70 mm. Hg at 11.41.

Experiment X.—Rabbit, decerebrate. Weight, 1900 g. Both fifth cranial nerves cut. Anæsthetic stopped, 10.40.

11.4. 1 mg. tetra-methyl-ammonium chloride injected. Respiration diminished in extent five seconds after commencement of injection and was quickly followed by increasing frequency and gradually increasing depth for eight seconds. Then it diminished in extent and later increased until, forty-five seconds after the injection, it was 30 per cent. more rapid and the excursion of the lever was double that of the normal. Gradual return to normal then occurred.

The blood-pressure (77 mm. Hg) showed a preliminary rise and then a prolonged fall, being 54 mm. Hg eleven seconds, and 40 mm. Hg two minutes, after the commencement of the injection. At three and a quarter minutes it was 49 mm. Hg, at six minutes 58 mm. Hg, and at thirteen minutes after the injection 77 mm. Hg.

11.20. 2 mg. injected. Increased rapidity of respiration commenced four seconds after beginning of injection, but the curve gradually diminished in extent until twenty seconds later it was only one-seventh its previous height. The respiration then increased in extent, but diminished in frequency,

and twenty seconds later was almost normal. Afterwards it gradually became slower and finally ceased five minutes after the injection.

The blood-pressure (77 mm. Hg) showed a slight preliminary rise and then a fall to 58 mm. Hg at ten seconds, and 49 mm. Hg at twenty seconds after the injection. No recovery occurred.

Experiment XI.—Rabbit, decerebrate. Weight, 2450 g. Both fifth cranial nerves cut proximal to Gasserian ganglia. Anæsthetic stopped, 11.20.

- 11.36. 0.6 mg. tetra-methyl-ammonium chloride injected. Six seconds after commencement of injection the respiration increased suddenly to three times its previous frequency and about 20 per cent. in depth. This effect was maintained half a minute and then commenced to pass away, but the respiration had not reached its normal rate eight and a half minutes after the injection.
- 11.44½. 1 mg. injected. The earlier part of the effects of this injection is shown in fig. 7.
- 11.58. 1 mg. injected (took twelve seconds). Thirteen seconds after the commencement of the injection there occurred a marked increase in the frequency and a transient increase in the depth of the respirations, which soon commenced to pass away.
- 11.59½. 2 mg. injected (took eleven seconds) while respirations still rapid. Increased frequency and increased depth of respiration commenced three seconds after the beginning of the injection and was replaced by slowing and diminished depth five seconds later. After a further interval of five seconds the respiration ceased. The paralysis lasted for thirteen seconds. When the respiration recommenced, it was shallow and rapid, but it quickly reached its normal form. There was marked slowing of the heart and a fall of blood-pressure after the previous injection and a further slight slowing of the heart and slight fall of blood-pressure after this injection.
- 12.5½. 5 mg. injected. The effect is shown in fig. 7. Occasional shallow respirations occurred afterwards.

Experiment XII.—Rabbit, decerebrate. Weight, 2200 g. Both Gasserian ganglia excised. Anæsthetic stopped, 3.38.

- 3.53. 0.5 mg. tetra-methyl-ammonium chloride injected. Gradual diminution in depth of respiration commencing four seconds after beginning of injection and reaching maximal diminution, which was maintained six seconds, ten seconds later. Recovery gradually occurred during the next fifteen seconds.

The blood-pressure (65 mm. Hg) rose slightly and then fell to 45 mm. Hg nine seconds after the beginning of the injection, and to

33 mm. Hg one minute after the injection. It showed practically no recovery.

4.0½. 1 mg. injected. A rapid increase in depth and frequency occurred seven seconds after the beginning of the injection. Seven seconds later the frequency, and ten seconds later the depths of the respiration, began to diminish, and forty-five seconds from the commencement of the injection respiration ceased. After twenty seconds a few shallow respirations occurred at intervals during the next half-minute. The blood-pressure very gradually fell to 15 mm. Hg.

Cause of the Respiratory Paralysis.—As previously stated, IODLBAUER* ascribes the temporary paralysis of the respiration to stimulation of the terminations of the fifth cranial nerves in the nose. He came to this conclusion because (1) he observed closure of the glottis—an associated act of the Kratschmer-Hering reflex—during the respiratory standstill; (2) he was unable to produce respiratory paralysis after anæsthetising the nasal mucous membrane; (3) he did not observe the characteristic effect in cats, animals in which the typical Kratschmer-Hering reflex is absent. In these animals he obtained transient powerful stimulation of the respiration followed by some depression and rapid return to the normal.

It is difficult to understand why IODLBAUER failed to obtain the respiratory paralysis in cats. It is possible that the animal used for the experiment in which efficient doses were given may have been relatively insusceptible to tetra-methyl-ammonium chloride, since variations in the reaction to this substance occur in these animals and also in rabbits. In the few experiments I have made on cats, respiratory paralysis has been as easily induced as in rabbits, although generally somewhat larger relative doses were necessary for the purpose (see fig. 1).

Nor have I been able to corroborate IODLBAUER's statement that anæsthetising the nasal mucous membrane of rabbits prevents the appearance of the respiratory paralysis. Even after the careful injection of large quantities of a 5 per cent. solution of cocaine hydrochloride so as to reach the whole mucous membrane, the intravenous injection of 1 mg. tetra-methyl-ammonium chloride produced the same effect as before the application of the cocaine.

It would seem further that if this action is due to stimulation of the trigeminal nerve-endings in the nose, the local application of such a diffusible substance as tetra-methyl-ammonium chloride would induce it. As I shall show in a later communication, the application of 1 in 5000 solutions of this substance to frogs' muscles produces a maximal effect within a few seconds, and it is inconceivable that the nasal mucous membrane should act as a semi-permeable membrane to it. Yet the injection of 1 to 2 per cent. solutions into the nasal cavities of anæsthetised animals, the respiration of which was paralysed by intravenous injections of 1 mg., produced no effect whatever. IODLBAUER apparently used this method with equivocal results, which he attributes to

* *Loc. cit.*

the difficulty of avoiding mechanical stimulation of the mucous membrane by the instillation.

Moreover, as is evident from the experiments already summarised (Exps. IV., V., VI., X., XI., XII.), the effect is still obtained after section of both fifth cranial nerves in the base of the skull. And in one experiment in which these nerves were divided in the interval between two injections the effect produced by the injections was similar in both cases (fig. 8).

It would therefore seem that the cause of the temporary paralysis of the respiration is not to be found in stimulation of the nerve-endings in the nose. As previously stated, it is in all probability due mainly to transient depression or paralysis of the myo-neural junctions in the respiratory muscles. It has already been shown that

FIG. 8.—Effect of tetra-methyl-ammonium chloride before and after division of both fifth cranial nerves in decerebrate rabbit. 12.12, right fifth nerve cut; 12.13 left nerve cut. Letters as in previous figures. $\times \frac{1}{2}$.

greater or less paresis of the motor nerve-endings to the sterno-mastoid and the muscles of the fore limbs occurs a few seconds after the injection of a dose of tetra-methyl-ammonium chloride sufficient to paralyse the respiration. An examination of the various experiments in which the effect on the respiration and the excitability of the fore-limb muscles was observed shows that the paresis of the fore-limb muscles is synchronous with the paralysis of the respiration. This is seen in fig. 1 in the cessation of the spontaneous muscular movements, and also in fig. 2, although the respiration in the latter case was not so quickly paralysed. It was further found that the duration of the paralysis in each case was similar. After some injections the limb muscles seemed to be more influenced than the respiration—a weak stimulus which had previously produced a good contraction inducing for a short time no reaction after a dose sufficient to depress, but insufficient to paralyse, the respiration: or, as shown in fig. 1, failing to react to the same degree as before, after the respiration

had completely recovered from the paralysis; but even in these cases the depression or paralysis of the motor nerve-endings and of the respiration was shown to run a parallel course. When the influence of tetra-methyl-ammonium chloride on the terminations of the phrenic nerves was investigated, complete agreement was found to exist between their irritability and the effect on the respiration. In these experiments the phrenic nerves were isolated in the base of the neck and one or other, usually the right, stimulated in continuity. The respiration was recorded by means of a phrenograph connected to a tambour recorder. A considerable number of such experiments were made, but as they all conform to that shown in fig. 1, it is only necessary to refer to this. In this experiment the right musculo-cutaneous and right phrenic nerves were stimulated simultaneously. As will be seen from the tracing (line R), the effect on the respiration and the effect of stimulating the phrenic are completely concordant.

It would seem therefore that the respiratory paralysis produced by tetra-methyl-ammonium chloride is largely, if not solely, due to paralysis of the nerve endings in the respiratory muscles. This being the case, we might expect a comparable effect to be given by curare. This substance, however, is somewhat slower in its action and much more prolonged in its effects than tetra-methyl-ammonium chloride, and after the injection of doses sufficient to paralyse the respiration spontaneous recommencement of the breathing cannot be obtained without artificial respiration. According to TILLIE,* the smallest dose of curarin, administered intravenously, which will completely paralyse a rabbit is 0.2 mg. p. kg. body-weight; 0.3 mg. to 0.5 mg. p. kg. cause loss of reflexes and spontaneous breathing for ten to fifteen minutes. Consequently in an experiment on a cat—animals which, according to BOEHM,† are somewhat less sensitive to curarin than rabbits—carried out in a similar manner to those referred to on p. 18, anæsthesia being maintained throughout the experiment, a dose of 0.16 mg. p. kg. body-weight was decided on. The intravenous injection of this quantity of curarin caused gradual failure of the respiration commencing twenty-one seconds after the beginning of the injection and going on to complete paralysis two minutes later. Electrical stimulation of one of the cords of the brachial plexus and of the right phrenic nerve showed gradually diminishing contraction of the fore limb and the diaphragm respectively *pari passu* with the failure of the respiration. With complete cessation of the respiration stimulation of the phrenic nerve caused no contraction of the diaphragm, but stimulation of the brachial cord produced a just appreciable movement of the limb.‡ Artificial respiration was performed, and five minutes later spontaneous breathing recommenced, but even with the aid of short periods of artificial respira-

* *Arch. f. exp. Path. u. Pharm.*, xxvii. p. 22 [1890].

† *Festschrift zu C. Ludwig's 70. Geburtstag*, 1886, quoted by TILLIE, *loc. cit.*, p. 4.

‡ This result although slight is probably unusual. By careful dosage of curarin TILLIE was able to keep an animal paralytic without stopping the respiration; and A. MOSSO (*Memoria della R. Acad. delle Scienze di Torino*, ser. ii. vol. liii. p. 397, 1903) and M. CHIO (*Arch. di Farmacol. speriment. e Scienz. affin.*, xii. p. 533, 1912), by comparing the respiratory movements of the thorax and abdomen, have shown that the muscles of the thorax are paralysed before those of the diaphragm.

tion nearly twenty minutes elapsed after the paralysis of the respiration before practically normal respiration was established, and it was some minutes later before the diaphragm reacted to stimulation of the phrenic to the extent it did previous to the injection. After the injection of double this dose, paralysis of the muscles and respiration appears much more quickly, but three to four times the dose mentioned is necessary to produce that rapid paralysis of the respiration which is seen after tetra-methyl-ammonium chloride. Owing to the prolonged action of curarin on the myo-neural junctions, a strict comparison with the paralysing action of tetra-methyl-ammonium chloride on the respiration is not possible; but it is interesting to note that the type of the paralysis is the same in both cases and that after large doses of the two substances the paralysing effect on the respiration is practically identical. In view of the convulsant action of tetra-methyl-ammonium chloride, it is further of interest to note that TILLIE* observed weak convulsions, apparently on non-anæsthetised animals, after the injection of 1 mg. to 2 mg. curarin prior to the appearance of complete paralysis, and these were more marked when artificial respiration was performed throughout the experiment.

There still remains the question whether the cause of the respiratory paralysis is wholly due to the paresis of the nerve endings in the respiratory muscles. As previously mentioned, POHL,† working with some quaternary papaverine derivatives, observed this form of temporary expiratory paralysis with spontaneous recovery after intravenous injections into rabbits. And yet he found that these substances had no paralysing action on the motor nerve-endings of frogs. He came to the conclusion that the effect on the respiration was due to an action on the respiratory centre, and he explained the effect of tetra-methyl-ammonium chloride in the same way. But the only experiment he appears to have made was one to controvert the view of TAPPEINER, and in this he divided the ophthalmic branches of the fifth cranial nerves, and still found that he obtained the characteristic paralysis of the respiration after the intravenous injection of this substance. Its action was not further investigated.

In order to determine whether the paralysing action on the respiration produced by tetra-methyl-ammonium chloride is partly central, two experiments in which the injections were made alternately into the carotid artery and the facial vein were performed on anæsthetised rabbits. Although the concentration of tetra-methyl-ammonium chloride reaching the respiratory centre after injection into the artery must have been considerably greater than after injection into the vein, a larger dose was necessary to induce respiratory paralysis. Thus the injection of 1 mg. into the carotid artery produced in both animals merely slight gradual diminution of depth and slowing of the respiration, whereas this dose, when injected into the facial vein, caused almost complete temporary paralysis. When double the dose injected into the vein was injected into the artery, almost identical effects on the respiration were obtained from the two injections. Some difference was noted in the initial effects on the blood-pressure, the

* *Loc. cit.*, p. 22.

† *Loc. cit.*

fall being more gradual and the slowing of the heart's frequency much less marked after the intra-arterial than after the intravenous injection.

The paralysing action of this substance on the respiration would therefore seem not to be due to an action on the respiratory centre. Nor is it due to an action on the circulation or on the peripheral nervous mechanism within the lungs, since, as will be shown later, it is much more transient than the circulatory action, and it is obtained after section of both vagi or after the injection of sufficient atropine to paralyse the vagal terminations.

That tetra-methyl-ammonium chloride is not without action on the respiratory centre is evident from the experiments on decerebrate animals. But instead of being depressant to this centre, it is powerfully stimulant. And that the paralysis is not due to exhaustion following stimulation is obvious from the fact that stimulation of the respiration is usually present in decerebrate animals after the paralytic stage has passed away. The larger doses of tetra-methyl-ammonium chloride which are sometimes necessary to paralyse the respiration of decerebrate animals, and the more rapid recovery from the paralysis, also point to this conclusion. It has been mentioned that strong or moderately strong electrical stimuli applied to nerves may still produce contraction of the innervated muscles after doses of tetra-methyl-ammonium chloride sufficient to paralyse the respiration, although moderately weak stimuli may no longer do so, and there can be little doubt that a powerfully stimulated respiratory centre acts in a similar manner, only more efficiently. In other words, the impulses from a respiratory centre in this condition are able to overcome a greater degree of depression in the peripheral structures, and hence the depression must be made more profound by the injection of larger doses in order to obtain paralysis; and as the stimulation continues for a much longer time than the paralysis, the more rapid recovery may be similarly explained. It is probable that the convulsant action noticed after toxic doses to normal animals has a similar explanation. That the stimulation of the respiratory centre does not occur in anæsthetised animals when the substance is administered intravenously is due to the well-known fact that it is more difficult to stimulate this centre in anæsthetised than in normal animals, and any slight stimulation which may be induced would, in this case, be checked by the simultaneous paralysing action on the myo-neural junctions. By the injection of small doses into the carotid artery of anæsthetised animals, slight stimulation of the respiration, both increase in depth and in frequency, can be demonstrated.

Influence of the Vagus on the Respiratory Paralysis.—Anæsthetised animals in which both vagi have been divided are more sensitive to the action of tetra-methyl-ammonium chloride than animals with the vagi intact. Thus a dose which may produce in an ordinary anæsthetised animal slight temporary depression without cessation of the respiration will, after section of both vagi, cause complete temporary paralysis; or a dose sufficient to paralyse for a few seconds the respiration of an animal with undivided vagi, may, after the vagi have been cut, produce permanent respiratory

paralysis. If not, the period of paralysis is longer than before the vagi were severed and the period of recovery of the respiration is prolonged. Decerebrate animals in which the section was made above the anterior quadrate bodies also proved more sensitive to the paralysing action of tetra-methyl-ammonium chloride after the vagi were cut than before. When the section through the mid-brain was made behind these bodies, division of the vagi was followed by irregular, spasmodic, and inefficient breathing, which gradually failed. All that could be deduced from the injections of tetra-methyl-ammonium chloride in these vagotomised animals was that they produced no stimulation of the respiration. This apparently increased sensitiveness of vagotomised animals to the influence of tetra-methyl-ammonium chloride is doubtless due to the fact that after division of the vagi the centripetal impulses produced by inspiration and expiration, which normally play an important part in the regulation of the respiration, are cut off, and the respiratory centre is thus deprived of one of its most efficient forms of stimulation to activity. After doses of atropine sufficient to paralyse the pulmonary vagus, no increased sensitiveness to tetra-methyl-ammonium chloride was observed, which is probably due, as some of my experiments seem to suggest, to a limited antagonism existing between these substances.

The experiments which have been described prove that the action of tetra-methyl-ammonium chloride in paralysing the respiration is fundamentally due to a depressant action on the myo-neural junctions of the respiratory muscles. It will be shown in subsequent communications that the paralysing effect on the respiration of other tetra-alkyl-ammonium compounds is proportional to their power of causing muscular paresis.

EFFECT ON THE CIRCULATION.

In anæsthetised animals the intravenous injection of tetra-methyl-ammonium chloride invariably causes a fall of blood-pressure and almost invariably a decided diminution in the frequency of the heart-beats (fig. 6). The fall of blood-pressure, occasionally after an insignificant rise, begins a few seconds after the commencement of the injection and usually about two seconds before the respiration is influenced. At first it is generally rapid, and later more gradual. After a dose of 1 mg. the fall may occupy twenty seconds and the blood-pressure may then be less than half its original height. If the respiration is inefficient, a slight asphyxial rise may occur, but commonly this is absent and the low blood-pressure continues, notwithstanding an efficient respiration, for one to three minutes and then gradually returns to normal. In a few cases after the administration of this dose the blood-pressure has fallen to a low level and no recovery has occurred.

The fall in the frequency of the heart-beats runs a similar course, but is of somewhat shorter duration. It commences with the fall of blood-pressure and may amount to 25 per cent., but is generally less than this. It begins to pass away before the blood-pressure commences to rise, and has usually completely passed away before the blood-pressure has reached its normal height. It is apparently wholly due to

stimulation of the vagal endings in the heart, since it is unaffected by division of the vagi and is abolished by the injection of sufficient atropine to paralyse these endings. After paralysing the vagal endings by means of atropine, the injection of 1 mg. tetra-methyl-ammonium chloride still causes a fall of blood-pressure, but the fall is more gradual and is usually much less in extent than in non-atropinised animals. Otherwise it runs a similar course. In cats after minimal doses the fall of blood-pressure and of the frequency of the heart is usually less than in rabbits, but otherwise the effects produced are the same.

After larger doses the fall of blood-pressure is more rapid, more marked, and more prolonged, and unless the respiration becomes efficient or artificial respiration is performed, no recovery follows. In some cases even when spontaneous breathing has recommenced or artificial respiration has been performed, the blood-pressure showed no recovery or rose but slightly. In these the blood-pressure remained at a very low level and the animal eventually succumbed.

In decerebrate animals a fall of blood-pressure with slowing of the heart also occurs (figs. 7 and 8), but is almost invariably preceded by a slight transient rise, also associated with a diminution of the heart's frequency. With the fall of blood-pressure the heart for a short period often becomes very slow. The vagal endings are also more sensitive to stimulation than normally—a stimulus which before the injection produced no effect causing after the injection a marked vagal action. The fall of blood-pressure is succeeded by a rise, often considerably above the normal height, which is soon followed by a fall below normal, the rise and fall occupying about twenty seconds. After a varying interval gradual recovery occurs as in anaesthetised animals. When atropine has been given previously, there is usually little or no preliminary fall of blood-pressure after the injection of small doses of tetra-methyl-ammonium chloride, and after a short interval a considerable rise occurs, which is maintained for about twenty seconds, and is followed by a fall to normal or a little below normal (fig. 5). IODLBAUER * also observed a rise of blood-pressure in non-anaesthetised animals after atropine had been given, but this was only followed by a fall below normal when large doses of tetra-methyl-ammonium chloride were injected.

The effect of tetra-methyl-ammonium chloride on the circulation is somewhat complex. IODLBAUER, as a consequence of the experiment just mentioned, concludes that it causes a marked stimulation of the vaso-motor centre, and, since he obtained less slowing of the heart's frequency after than before division of the vagi, that it also stimulates the cardio-inhibitory centre. The variable effects he obtained on the blood-pressure he explains by stating that sometimes stimulation of the vagal mechanism, sometimes stimulation of the vaso-motor centres, is predominant. The fall of blood-pressure, apart from that caused by stimulation of the vagus, he believes is due to paralysis of the vaso-motor centres. In my own experiments the stimulation of the cardio-inhibitory centres was relatively slight; after division of the vagi in decerebrate

* *Loc. cit.*, p. 196.

animals the slowing of the frequency of the heart was only 10 per cent. less than before the vagi were divided. The greater vagal effect seen in decerebrate animals as compared with anæsthetised animals is mainly due to greater stimulation of the vagal terminations.

The fall of blood-pressure in anæsthetised animals is mainly, and the early fall of blood-pressure in decerebrate animals is wholly, vagal in origin, and is due to stimulation of the vagus endings in the heart; but the late fall in decerebrate animals and the fall in anæsthetised animals are partly vascular, and appear, contrary to IODLBAUER'S statement, to be peripheral, and not central, in nature. This was shown by observing the effects of stimulating a splanchnic nerve on the blood-pressure. Thus in a small etherised rabbit (weight 1600 g.) stimulation of the left splanchnic nerve raised the blood-pressure from 52 mm. Hg to 72 mm. Hg. After the injection of 1 mg. tetramethyl-ammonium chloride stimulation of the nerve with the same stimulus produced no effect until the blood-pressure commenced to rise. Two minutes after the injection it raised the blood-pressure from 35 mm. Hg to 37 mm. Hg; four minutes after the injection from 44 mm. Hg to 50 mm. Hg; and six and a half minutes after the injection from 52 mm. Hg to 65 mm. Hg. Whether the effect is due, as TILLIE* maintained for curarin, to a depressant influence on the vaso-constrictor nerve endings or upon the muscle of the vessel walls, has not been determined. The perfusion of the blood-vessels of winter frogs has produced, with concentrations down to 1 in 400,000, only contraction, whereas plethysmographic experiments with the intestines of etherised rabbits have shown only dilatation of the vessels. It seems probable that the dilatation of the blood-vessels is one affecting mainly the splanchnic area and is due to a depressant influence on the terminations of the splanchnic nerves. This is probably the main vascular factor contributing to the fall of blood-pressure, but the diminished resistance associated with the paralysed condition of the voluntary muscles may not be without influence.

The temporary rise in blood-pressure occurring in atropinised decerebrate animals and the rise following the preliminary fall in non-atropinised decerebrate animals are due to stimulation of the vaso-motor centres. The curve of the rise and fall in non-atropinised animals strongly suggests an asphyxial origin, but while there can be no doubt that the increased carbon dioxide tension in the blood caused by the paralysis of the respiration acting upon an excited vaso-motor centre is partly responsible for the rise, it cannot be wholly due to this cause, since it occurred in similar form in animals with an efficient respiration (see fig. 7, in which, however, the rise is relatively small). Moreover, it only followed the earlier injections of an experiment, and was less marked or absent after moderate or large doses had been given. The rise of blood-pressure may therefore be attributed to stimulation of the vaso-motor centres and the subsequent fall to the increasing preponderance of the peripheral action already referred to.

* *Loc. cit.*, p. 29.