STUDIES ON INDIAN MEDICINAL PLANTS

Pharmacological actions & antibacterial activity of *Anisochilus carnosus* [N.O. Labiatæ]

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Anisochilus carnosus belongs to a genus of aromatic herbs distributed in tropical Asia and Africa. Fresh juice of the leaves and the volatile oil of this plant are used in the indigenous system of medicine to relieve intestinal colic, colds and coughs, and fungal affections of the mouth in children.

Investigations conducted on the pharmacological actions and antibacterial activity, indicate that the essential oil of this plant possesses direct muscle relaxant action, musculotropic and neurotropic antispasmodic activity and possibly a slight degree of neuro-muscular blocking effect.

The oil inhibits the growth of *Staphylo aureus*, *E. Coli* and *B. typhosum* and exhibits fungicidal action on *Aspergillus flavus* and *Fusarium moniliforme*.

Anisochilus carnosus

[Syn. Ajapada (Sans.); thick leaved lavender (English); Karpooravalli (Tamil); Doddapatre (Canarese); Panjari-ka-pat (Hindi); Rosachettu (Telugu)].

Habitat: Northern Circars, Mysore and Malabar.

This belongs to a genus of aromatic herbs or under-shrubs comprising 20 species, distributed in tropical Asia and Africa. About 13 species occur in India of which *A. carnosus* Wall (Western Himalayas, Central and South India) is medicinal.[1]

This is commonly grown in the kitchen gardens and the leaves are used for culinary purposes usually mixed with curds in what is known as 'Thambuli'. Fresh juices of leaves mixed with sugar-candy is given to children in coughs; mixed with sugar and gingelly oil it forms a cooling linament for the head. The leaves and stems in infusion are useful in coughs and colds. The plant yields a volatile oil which has stimulant, expectorant and diaphoretic properties. It is usually given in doses of 1 to 3 minims on loaf sugar.[2] Though not specifically mentioned, the leafy juice is considered to be useful and is used as such in fungal affections of the mouth in children, and in relieving intestinal colics.

With a view to assess these therapeutic properties the following investigations were undertaken. The study was confined to the essential oil of the plant which was kindly supplied by Mr. K. V. Harihara Padmanabhan through the courtesy of Dr. B. H. Iyer and Prof. D. K. Banerjee of the Organic Chemistry Department, Indian Institute of Science, Bangalore.

Pharmacology of the Essential Oil

Coughing is a reflex mechanism originating in the pharynx, larynx, trachea and bronchi and innervated by the cholinergic nerves. This can be induced in the experimental animals by stimulating these nerves which by releasing the acetylcholine at the nerve endings cause the spasm or by injecting acetylcholine itself. Spasm can also be induced by the inhalation of histamine and the influence of this chemical is probably the cause of cough in allergic conditions. Very little, however, is known of the interactions between the respiratory centre, the cough reflexes and other respiratory reflexes.

The increased intestinal motility associated with colic is also due to increased activity of the parasympathetic system. Though many other chemicals (like 5-hydroxy-tryptamine and substance P) might produce the spasm of the intestines, acetylcholine produced by parasympathetic stimulation and histamine released by the allergic reactions are probably the chief mediators in these conditions. The spasms may also be due to a direct stimulant action on the musculature and not only through the chemical mediations. Hence for a drug to be efficacious in spasmatic conditions, it should be parasympatholytic, antihistaminic or a direct muscle relaxant. Since the ætiological factors in spasms are different, it necessarily follows that a drug possessing any one of the above properties need not be found useful in all affections; but should a drug possess all these three attributes one would naturally expect it to prove an efficacious remedy.

The antagonistic action of the oil on Histamine, Barium and Acetylcholine induced spasms of guinea pig ileum

These antagonistic effects of a drug could be initially studied by *in vitro* methods on isolated organs.

In Fig. 2 is shown, the contraction of guinea pig ileum to 1 γ of histamine (*a*). The addition of 0·1 ml. of 1/100 dilution of an emulsion of the oil in water to the bath gradually lessens and then completely abolishes the spontaneous activity of the gut and also causes a relaxation of the musculature. Histamine in 1 μg. dose has now no action. After repeated washings, the gut begins to show reactivity to histamine only at the end of 10 mins. (*b*).

The reaction to barium is shown in Fig. 2 (*c*). Barium is a direct muscle stimulant. The oil in the same dilution as above neutralises the action of 1 mg. of barium chloride. The relaxation is not so steep, nor the spontaneous contractions so completely obliterated as with histamine. The recovery time also is shorter.

Acetylcholine, chemical mediator of the parasympathetic nervous system normally produces contraction of the ileum. The effect of 2×10^{-8} is seen in Fig. 2 (*d*). Here also, the oil abolishes the spontaneous movements and causes relaxation of the gut and prevents the contractile action of acetylcholine.

Action on rat ileum and uterus

The antagonistic action of the oil was next tried in another species of animal and in different smooth muscle preparations. The action of acetylcholine was tried on the ileum and uterine musculature. These organs in the rat are not highly sensitive to histamine. Similar result as that of guinea pig is obtained, 0·75 ml. of 1/100 emulsion of the oil inhibiting the action of 1 γ of acetylcholine in respect of ileum (Fig. 3), and 1 ml. of 1/100 completely abolishing the acetylcholine activity with regard to the uterine strip (Fig. 4).

Since the spasmolytic activity exhibited was both musculotropic and neurotropic in character and similar type of result was obtained with the essential oils from *Ocimum* species,[4] the question arose as to whether these reactions were non-specific and common to all oils—fixed or volatile—which by forming a barrier on the surface of the test organ prevented the access of spasmogens.

A series of experiments were conducted with the essential oils extracted from *Ocimum gratissimum* and Goachi-phal (*Psidium guyava*) and the fixed oils of margosa (*Melia qzadirachta*) and Sesame (*Sesamum indicum*). The kymographic recordings Figs. 7 and 8 show the influence of these oils on isolated guinea pig ileum, the spasmogens used being acetylcholine and histamine.

The margosa (Fig. 8 *a*) and sesame oils (Fig. 8 *c*) had no inhibiting effect on histamine contraction while Ach spasm was slightly reduced by sesame oil. The action of *Ocimum* (Fig. 7) and Goachi oils (Fig. 8 *b*) were qualitatively similar to that of *Anisochilus* but differed quantitatively in dosage needed to cause complete spasmolysis.

The spasmolytic activity of the *A. carnosus* oil may be due to a direct depressant effect on the musculature and also neuromuscular blocking action as some essential oils are known to exhibit curariform effects.[5] The isolated phrenic nerve diaphragm preparation of the rat was utilised to study the nature of the reaction. The method was essentially the one described by Bülbring.[6] In Figs. 5 and 6 are shown the influence of the oil on the contracture of the diaphragm by phrenic nerve stimulation 0·1 ml. of 1/10 dilution of the oil causes rapid decrease in extent and complete inhibition to nerve stimulation. The same concentration of the oil causes reduction only but not complete suppression with direct stimulation of the musculature.

The oil is thus shown to possess slight direct muscle depressant action in the dose used. Whether the inhibition through nerve stimulation is due to neuromuscular block or interference in the conductivity of the impulse needs further

elucidation. In higher concentrations (0·5 ml. of 1/10 dilution). The oil caused an irreversible spasm of the musculature (Fig. 6).

The systemic actions of the oil were elicited on anæsthetised dogs (Fig. 1). A fall in blood pressure and a sudden contraction of the intestine on injection of 10 γ histamine is seen in (*a*). After intravenous injection of 0·5 ml. of 1/10 oil, the same dose of histamine causes a little less drop in blood pressure; the immediate intestinal contraction is not seen; a diminution in the tone of the musculature is observed after the initial rise (*b*).

The oil by itself causes a slight fall in blood pressure when injected slowly (c) but a rapid injection causes a sudden drop in pressure, respiratory distress and a diminished intestinal tone (*d*).

Since substances possessing antihistaminic or atropine like actions are likely to be local anæsthetics, the effect of the oil was studied for its local anæsthetic action on rabbits' cornea.

Oil as such when instilled into the eye caused inflammation of the lids and irritation of the cornea which persisted for 24 hours. A 1/10 dilution of the oil caused a very mild irritation and was associated with local anæsthesia lasting for about fifteen minutes.

Antibacterial Action

The antibacterial activity was studied on a few Gram positive and Gram negative organisms. As a comparative study, sandalwood oil, margosa oil, cucurbita seed oil and penicillin were also studied at the same time.

Materials and Methods

The agar cup plate method is not a suitable one for screening the antibacterial activity of water insoluble compounds as they do not freely diffuse into the agar. The filter-paper disc method can be more conveniently adopted for such compounds. The method in brief is as follows:

A 10 mm. filter-paper disc, previously sterilized, was placed at fixed intervals on an agar plate previously seeded with the test organisms. 0·1 ml. of a dilution of the oil in alcohol was placed on the disc and the zone of inhibition was measured after incubation for 24 and 72 hours. The production of a zone of inhibition, complete or partial, maintained till 72 hours was the criterion used in assessing bacteriostatic and bactericidal action. The test was repeated for several dilutions of each substance. Control tests, only with alcohol showed negligible inhibition of growth.

Table I illustrates the results obtained.

0·1 ml. of a 1/10 dilution of the *A. carnosus* oil showed an inhibitory zone of 33 mm. with *Staphylococcus aureus*, 23 mm. with *E. coli* and 28 mm. with

TABLE I

Antibacterial action of oils from A. carnosus, *Sandalwood, Margosa and* C. pepo

	Anisochilus carnosus	Sandalwood	Margosa	*Cucurbita pepo* (Seed Oil)
Staphylo aureus ..	33	13	..	..
E. coli ..	23	13	..	..
B. typhosum ..	28	..	..	..

Figures indicate zone of inhibition in mm.

B. typhosum; the same amount of sandalwood oil showed a zone of 13 mm. against *Staphylo aureus* and *E. coli*; and the penicillin zone (2 units/ml.) was 23 mm. against *Staphylo aureus*. It is thus seen that at this concentration *A. carnosus* oil has a wider spectrum than sandalwood oil. A detailed study of the oil in lower concentrations was carried out.

Staphylo aureus showed a complete inhibitory zone of 22 mm. with 1/50 dilution and a partial one of 20 mm. in 1/100 dilution; *E. coli* at 1/100 was inhibited for 20 mm. while *B. typhosum* at 1/100 was completely inhibited for 20 mm. and partially for another 20 mm.

This action of the oil on typhoid bacteria was further investigated by serial dilution method and compared to that of phenol by the phenol coefficient test. *Staphylo aureus* and *E. coli* were found to be inhibited in 1/200 dilution and *B. typhosum* in 1/500 dilutions. The phenol coefficient of the oil was found to be 7·0.

Antifungal activity

Because of the use of the leaf juice for skin conditions and fungal affections, the influence of the oil on the growth of some fungi was studied.

Fusarium moniliforme, a fungus pathogenic to plants and *Aspergillus flavus* were the organisms tested. The method consisted in suspending the spores grown in Richard's agar medium slopes, in distilled water, adjusting the turbidity to a predetermined level, incorporating a known volume of this in a manner similar to the filter-paper disc method as described under bacteriostatic action. 2% salicylic acid was used for a comparative assay. The oil inhibited the growth of both the fungi. The quantitative data obtained are given in Table II.

The Fig. 9 shows the fungicidal activity of various dilutions of the oil, 2% salicylic acid solution and alcohol control on *Aspergillus flavus*. 1/50 dilution of the oil exhibits activity equivalent to that of 2% salicylic acid on *Asper-*

TABLE II

Antifungal activity of Anisochilus carnosus

Fungus	Concentration of the Oil				Salicylic acid 2%	Alcohol
	Oil	1/10	1/20	1/50		
1. *Aspergillus flavus* ..	54	36	16	14	14	..
2. *Fusarium moniliforme* ..	16	14	..	..	12	..

The figures indicate the diameter of the zone of inhibition in mm.

gillus flavus and an 1/10 dilution of the oil shows greater activity than 2% salicylic on *Fusarium moniliforme.*

Toxicity Studies

An emulsion of the oil 1/10 was prepared and 0·2 c.c. of this was fed orally to a group of rats for a period of a fortnight. Growth, food intake and general activity was similar to the controls and postmortem examination indicated no lesions of the intestines or kidneys.

DISCUSSION

The action of some essential oils is similar in certain respects to that of anæsthetics on animal cells. The first effect of the fat solvents, narcotics and stimulating agents is identical and it may be assumed that they cause reversible lowering of the permeability for water and water-soluble substances[3].

The inhibiting and damaging effect of the oils on many life processes has been the basis for the use of these compounds as bactericidal and fungicidal agents. However, from the diversity of the compounds in essential oils, no general statement on the bactericidal action of these oils can be made. This is clearly shown by the varying effects observed both of a qualitative and quantitative nature[4] by *Anisochilus, Ocimum,* and sandalwood oils. While *B. typhosum* is inhibited by the oil of *Anisochilus* there is hardly any effect by the sandalwood oil.

The effects of some essential oils and oil components are not limited to the organisms to be destroyed but also extend to the host. Excessive use may cause depression of the higher centres followed by convulsions. In a few cases an apparent stimulating effect is noticed. This is the case with the terpene compounds such as camphor and menthol. Camphor may improve the cardiac condition and remove arrythmia when the heart muscle is depressed. These effects seem to be due to the parasympathicolytic effect.

The essential oils probably interfere with delicate mechanism, through their chemical and physical effects either by entering and disturbing colloidal systems or by taking part in certain reactions.

Theoretically because of its antiacetylcholine and antihistaminic actions, the oil from *Anisochilus carnosus* should be effective in cough by relieving the bronchial spasm and diminishing the excessive glandular secretion. A number of volatile, aromatic substances are known to be useful as stimulant expectorants in chronic inflammation of the bronchial passages and by their slightly irritative action cause hyperæmia and healing. They tend to diminish secretion.

The antispasmodic action of the oil should be useful in relieving the colicy pains and its antihistaminic property in alleviating the allergic manifestations. The antibacterial and antifungal properties on the few organisms examined indicate its possible usefulness in certain affection of the skin.

The results obtained *in vitro* and from experimental animals cannot be directly translated on to human beings but only give a lead to the desirability of testing these substances in suitable clinical cases. A proper evaluation, the usefulness and limitations can be obtained only by controlled trials.

SUMMARY

The oil from *A. carnosus* possesses direct muscle relaxant action, anti-acetylcholine and antihistaminic properties. In a dilution of 1/10 slight anæsthetic effect is noticed on the guinea pig cornea. The local irritant action at this dilution was slight.

The oil possesses bacteriostatic and bactericidal properties against *Staphylo aureus*, *E. coli* and *B. typhosum*. The oil is also fungicidal to *Aspergillus flavus* and *Fusarium moniliforme*. At a dose of 0·2 c.c. of 1/10 emulsion the oil is non-toxic to rats.

These properties exhibited by the oil indicate that it might be found useful as an antispasmodic and antifungal remedy in certain types of clinical conditions.

Our thanks are due to Dr. K. P. Menon for helpful suggestions, to Dr. U. G. Nayak for the supply of the essential oil from *Ocimum gratissimum*, to Dr. A. Bhati for *Goachi oil*, to S. K. Sripathi Rao and V. Srinivasan for technical assistance.

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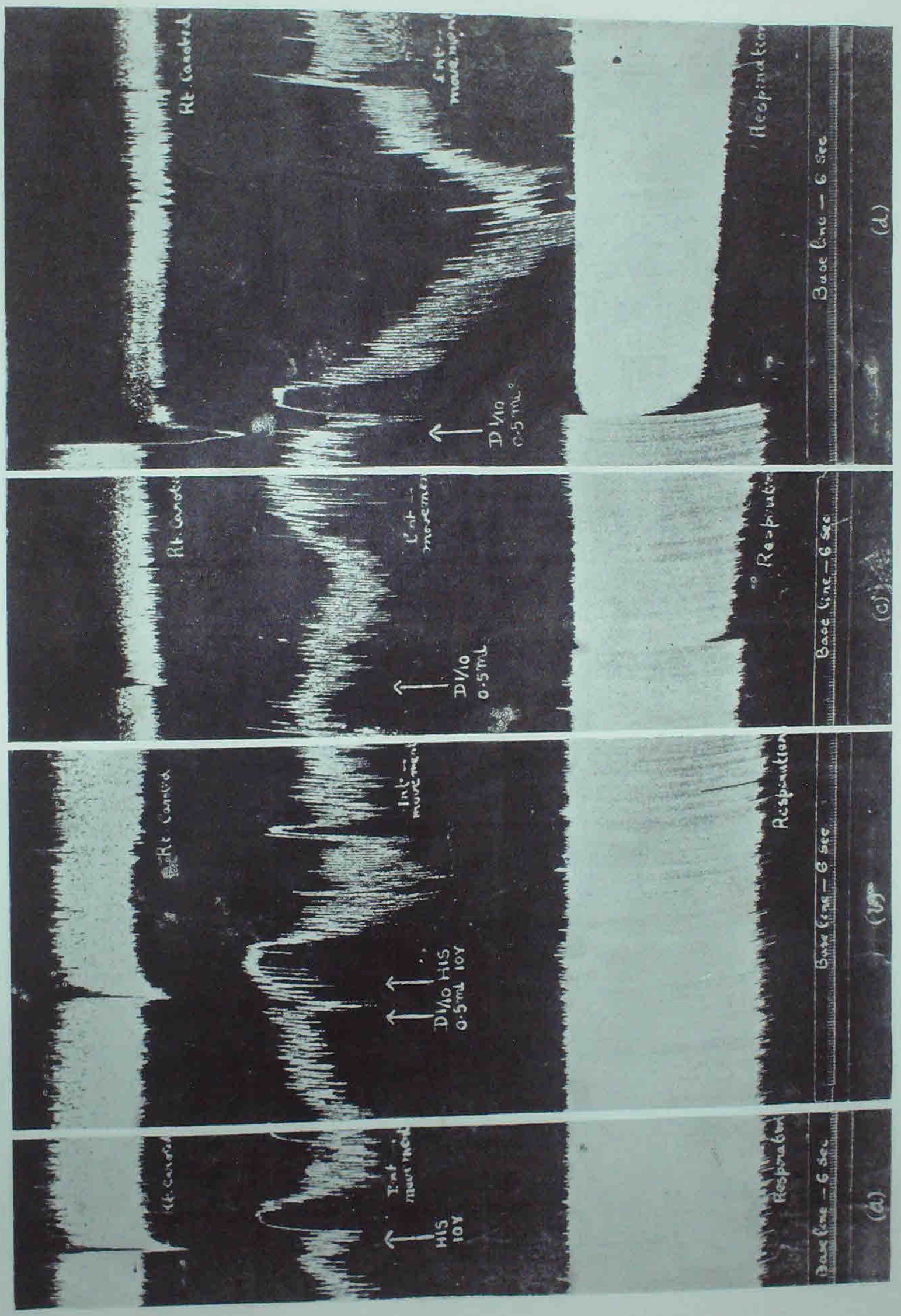

Rt. Carotid
Int. mandibular
D 1/10
0.5 ml.
Respiration
Base line — 6 sec
(d)
Rt. Carotid
Int. mandibular
D1/10
0.5ml
Respiration
Base line — 6 sec
(c)
Rt. Carotid
Int. mandibular
D1/10 HIS
0.5ml 10γ
Respiration
Base line — 6 sec
(b)
Rt. Carotid
Int. mandibular
HIS
10γ
Respiration
Base line — 6 sec
(a)

FIG. 1

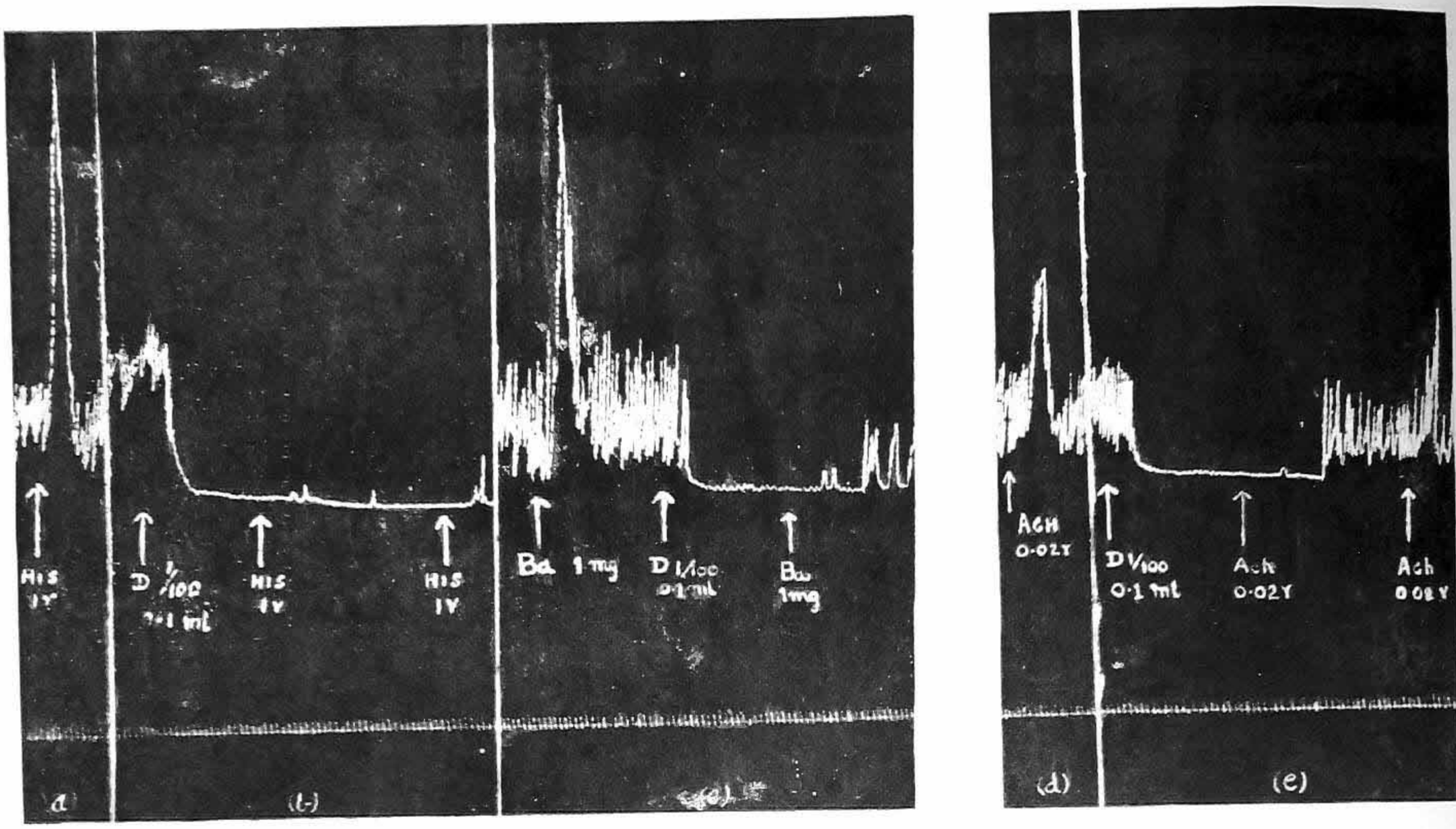
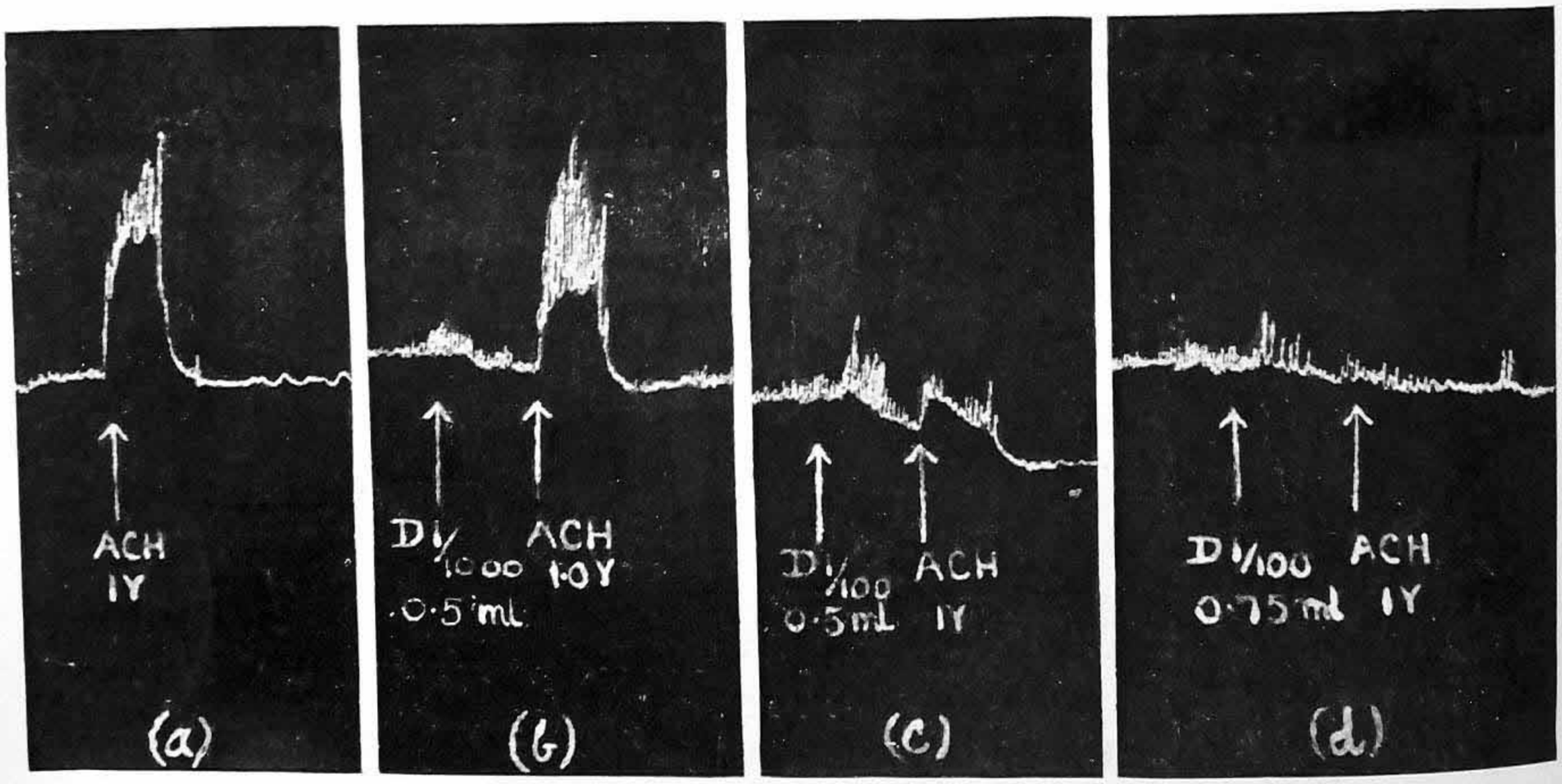

His 1Y
D 1/100 0·1 ml
His 1Y
His 1Y
Ba 1mg
D 1/100 0·1 ml
Ba 1mg
ACH 0·02Y
D 1/100 0·1 ml
Ach 0·02Y
Ach 0·02Y
(a)
(b)
(c)
(d)
(e)

FIG. 2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D 1/1000 0·5 ml
ACH 1·0Y
D 1/100 0·5 ml
ACH 1Y
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ACH 1Y
(a)
(b)
(c)
(d)

FIG. 3

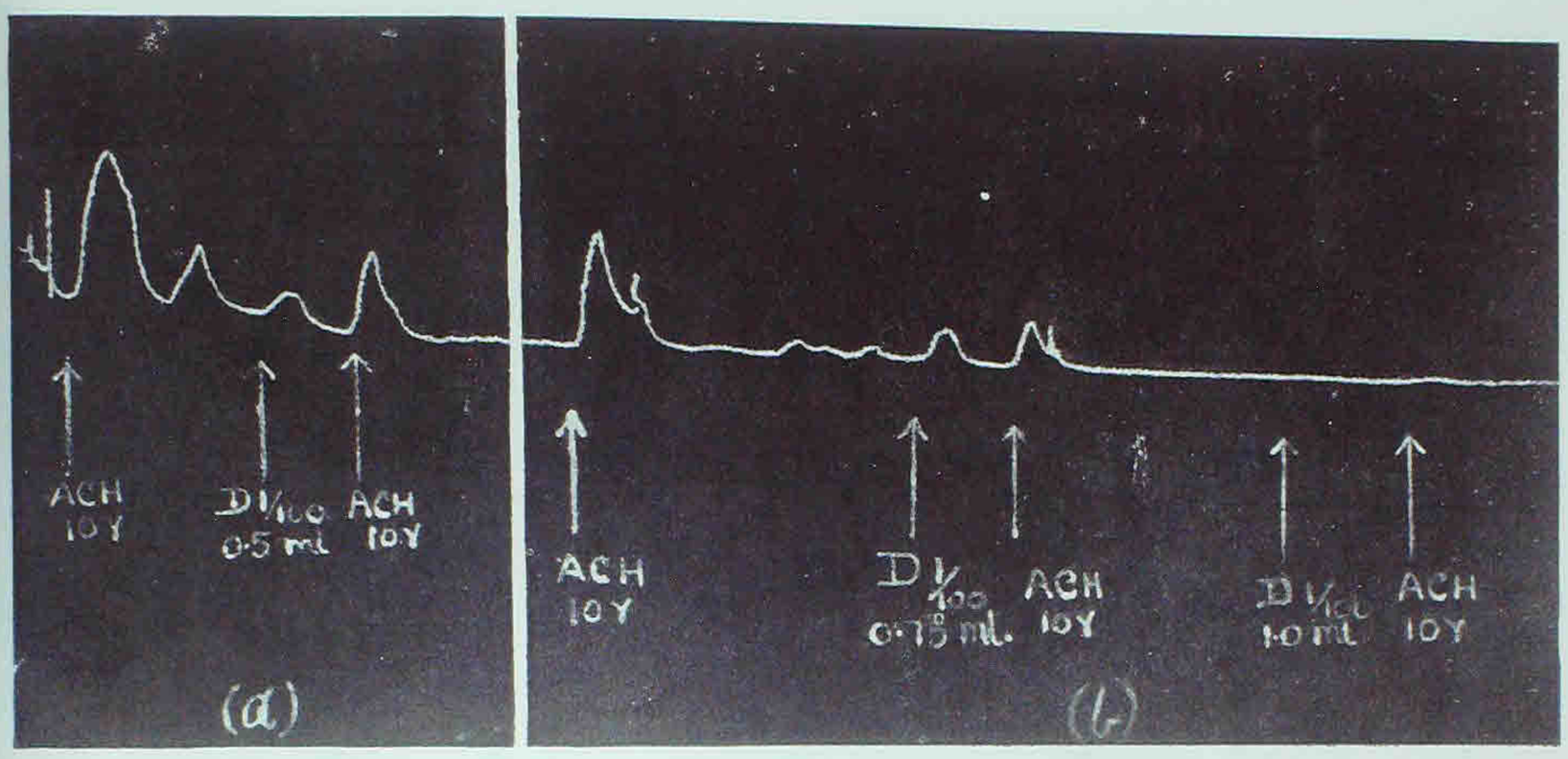
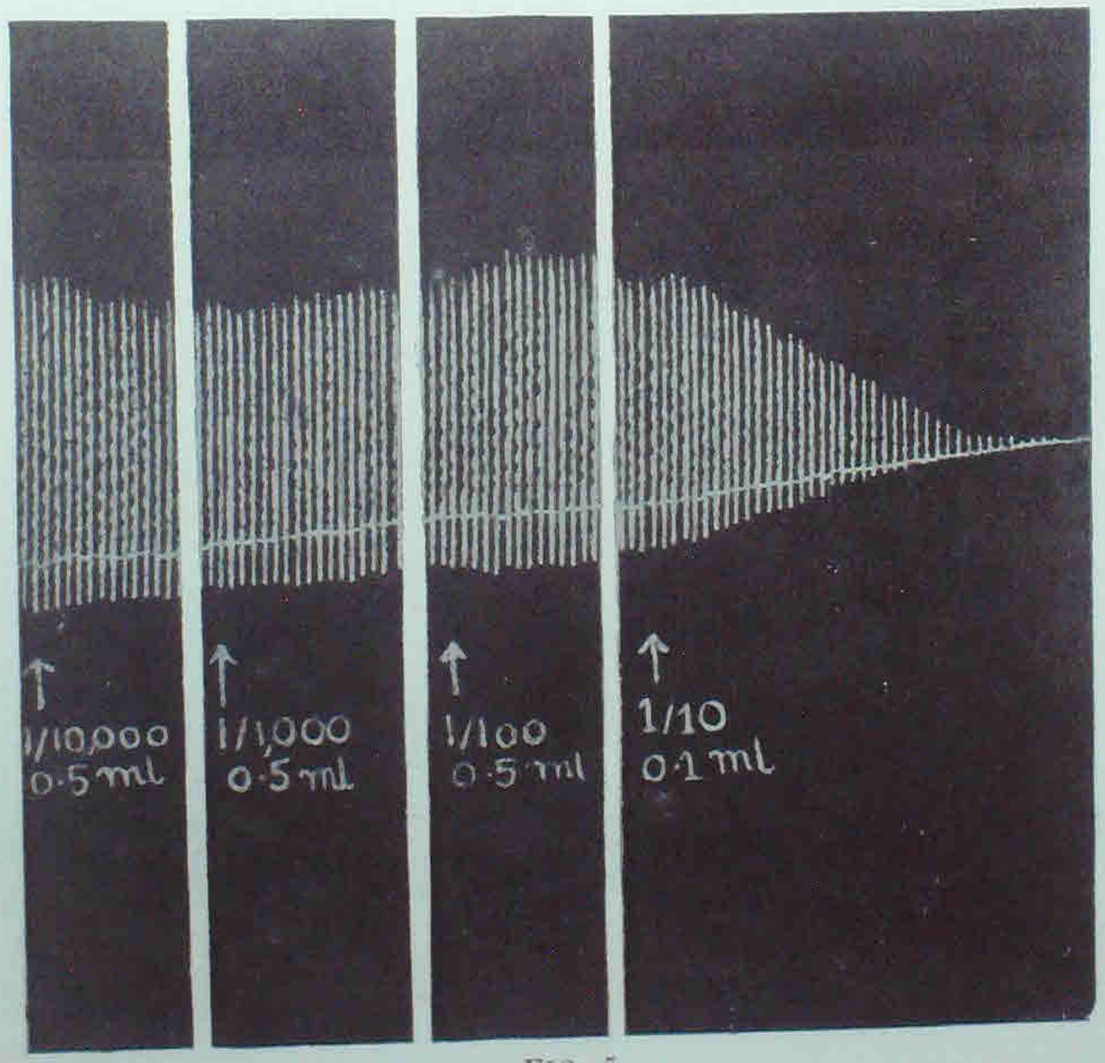

ACH
10 Y
D 1/100
0·5 ml
ACH
10 Y
(a)
ACH
10 Y
D 1/100
0·75 ml.
ACH
10 Y
D 1/100
1·0 ml
ACH
10 Y
(b)

FIG. 4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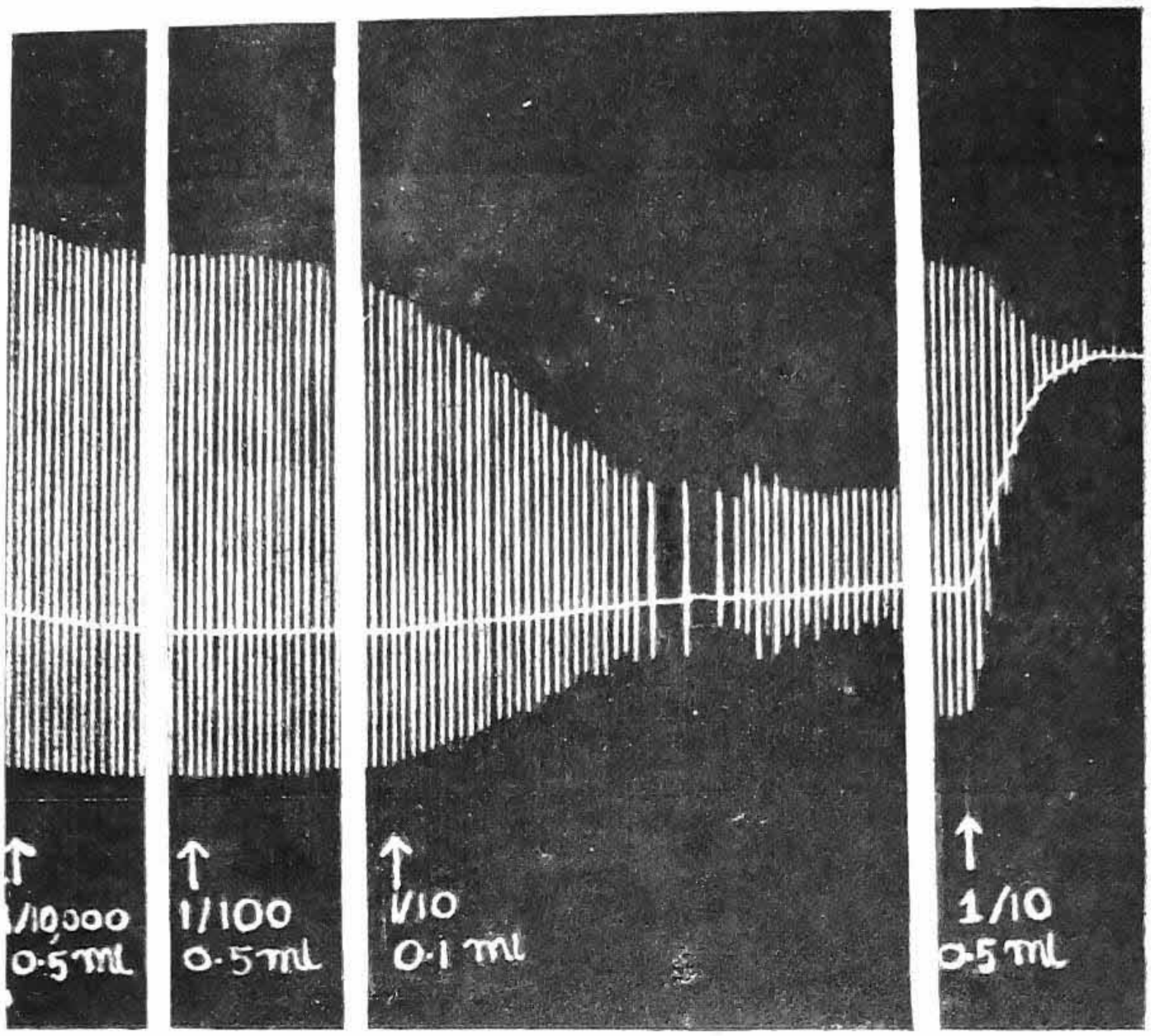
0·5 ml
1/1,000
0·5 ml
1/100
0·5 ml
1/10
0·1 ml

FIG. 5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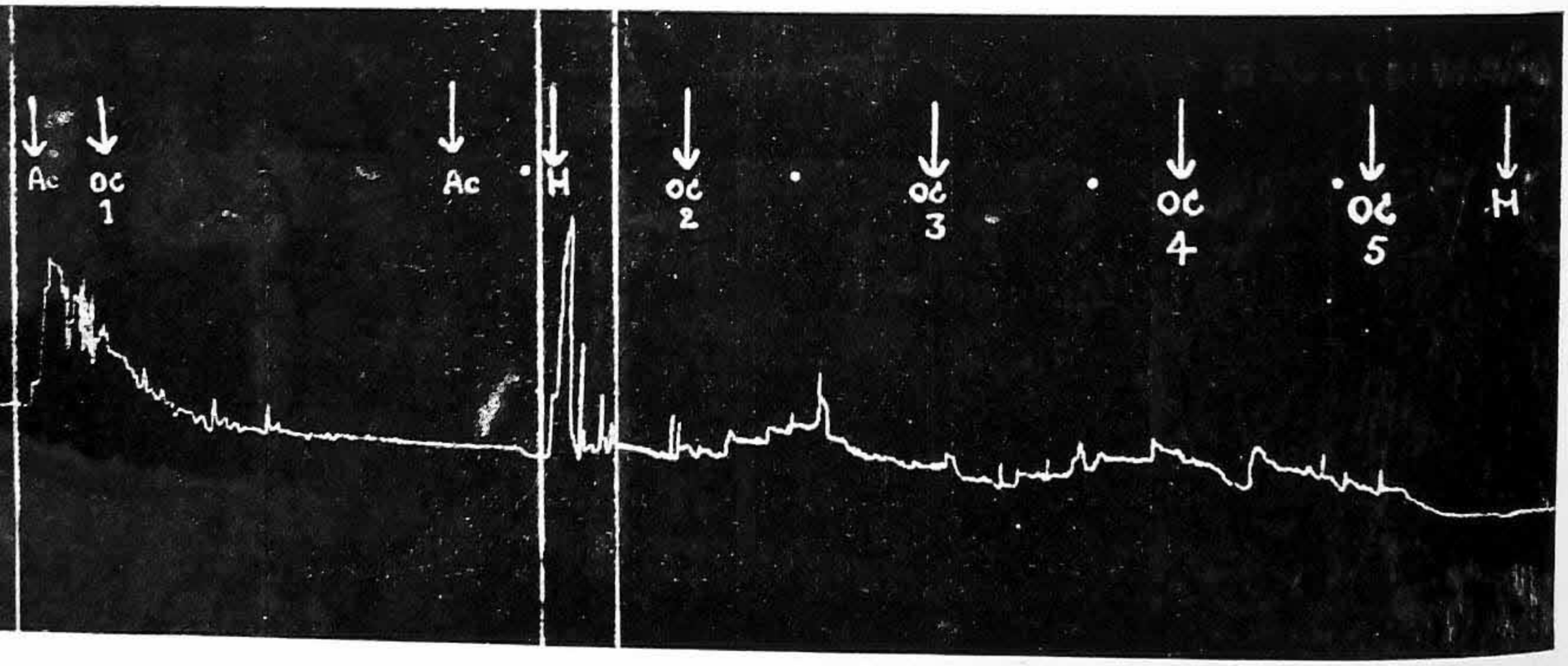
0.5 ml
1/100
0.5 ml
1/10
0.1 ml
1/10
0.5 ml

FIG. 6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H
OC 2
OC 3
OC 4
OC 5
H

FIG. 7

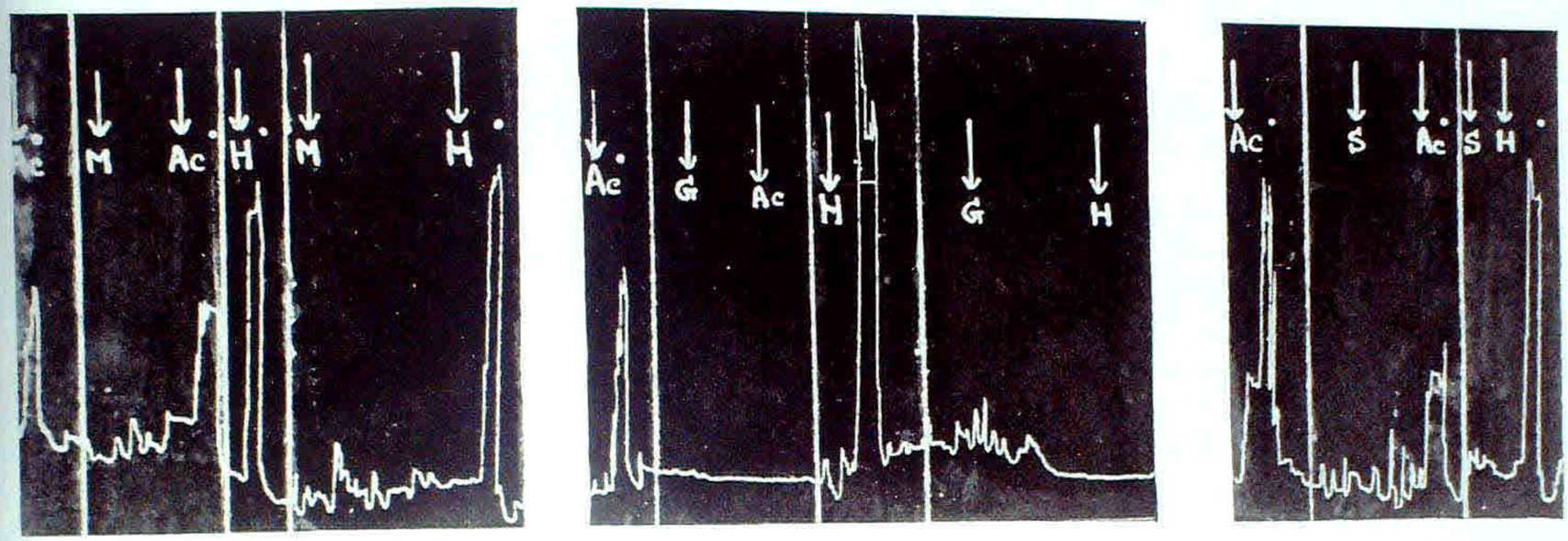

FIG. 8

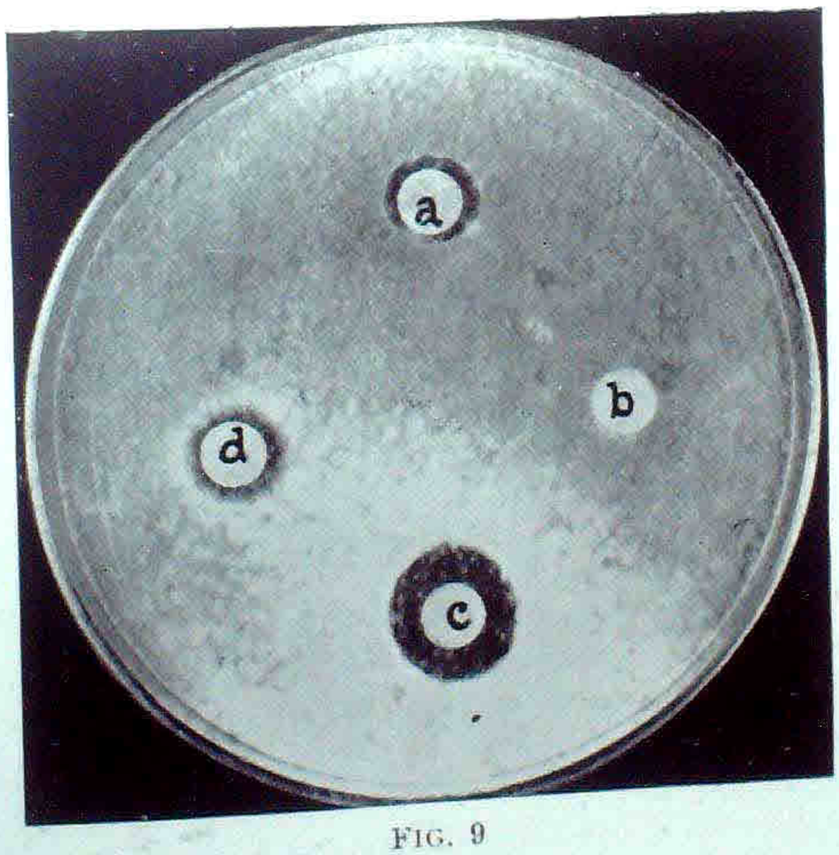

FIG. 9

Explanation of Plates

Fig. 1. Arterial blood pressure, intestinal movement and respiration of 6 kg. female dog in seconal anæsthesia—Time in 6 seconds.

(*a*) Intravenous injection of 10 γ of Histamine; (*b*) Intravenous injection of 0·5 ml. of 1/10 dilution of Anisochilus oil followed after 1 minute by 10 γ Histamine; (*c*) 0·5 ml. of 1/10 dilution of Anisochilus oil alone given slowly; (*d*) 0·5 ml. of 1/10 dilution of Anisochilus oil given rapidly.

Fig. 2. Contraction of guinea pig ileum preparation in 15 ml. Tyrode solution.

(*a*) 1 γ of Histamine; (*b*) 0·1 ml. of 1/100 of Anisochilus oil followed after 1 minute with 1 γ of Histamine; (*c*) (1) Contraction after 1 mg. of Barium (2) 0·1 ml. of 1/100 of Anisochilus oil followed after 1 minute with 1 mg. of Barium; (*d*) 0·02 γ of Acetylcholine; (*e*) 0·1 ml. of 1/100 of Anisochilus oil followed after 1 minute with 0·02 γ of Acetylcholine.

Fig. 3. Contractions of rat's ileum preparation in 15 ml. Tyrode solution.

(*a*) 1 γ of Acetylcholine; (*b*) 0·5 ml. of 1/1000 of Anisochilus oil followed after 1 minute with 1 γ of Acetylcholine; (*c*) 0·5 ml. of 1/100 of Anisochilus oil followed after 1 minute with 1 γ of Ach; (*d*) 0·75 ml. of 1/100 of Anisochilus oil followed after 1 minute with 1 γ of Ach.

Fig. 4. Contraction of rat's uterus preparation in 15 ml. Tyrode solution.

(*a*) 0·5 ml. of 1/100 of Anisochilus oil followed after 1 minute with 10 γ of Acetyl choline; (*b*) 0·75 ml. and 1 ml. of 1/100 of Anisochilus oil followed after 1 minute with 10 γ of Ach.

Fig. 5. Record shows contraction of the isolated rat diaphragm in response to stimulation of the phrenic nerve at 5 min. At the arrows different concentrations of the oil were added to the bath. Note the rapid decrease and complete inhibition in the height of the contraction after 0·1 ml. of 1/10 of the oil was added. Bath Volume: 75 ml. Perfusion fluid: Ringer.

Fig. 6. Record shows contraction of the isolated rat diaphragm in response to direct stimulation of the muscle. At the arrows different concentrations of the oil were added to the bath. Note the gradual decrease in the height of the contraction after the addition of 0·1 ml. of 1/10 oil to the bath (the inhibition of contraction was incomplete) and the tonic contraction of the muscle after 0·5 ml. of 1/10 oil. Bath Volume: 75 ml. Perfusion fluid: Ringer.

Fig. 7. Effect of Ocimum oil on isolated guinea pig ileum.

Bath 15 ml. Tyrode soln. Ach and Histamine contractions elicited by 0·1 γ. OC = Ocimum oil (1) 1/100 dil. 0·5 ml. (2) 1/10,000 dil. 0·1 ml. (3) 1/10,000 dil. 0·5 ml. (4) 1/10,000 dil. 1·0 ml. (5) Same as 1. 0·5 ml. of 1/100 dil. causes relaxation and counteracts the actions of Ach and histamine.

Fig. 8. (*a*) Effect of Margosa oil on isolated guinea pig ileum. 0·5 ml. of 1/100 dil. of the oil has no spasmolytic action on Ach and histamine contracture; (*b*) Effect of Goachi oil on isolated guinea pig ileum 0·5 ml. of the 1/100 dil. of the oil inhibits the actions of Ach and histamine; (*c*) Effect of Sesame oil on isolated guinea pig ileum. 0·5 ml. of 1/100 dil. diminishes Ach contraction but has no effect on histamine.

Fig. 9. Fungicidal Property of *A. carnosus* (*Aspergillus flavus*).

a = 2% salicylic acid; *b* = alcohol; oil in alcohol; *c* = 1/20; *d* = 1/50.